Chapter 10

General Discussion

This thesis had three different aims. The first aim was to better define the determinants of vitamin D deficiency in the older population. The association between medication use in general and vitamin D status was examined. In addition, several specific medication groups were studied regarding their relation to serum 25(OH)D concentration and whether sex modified these relationships. Furthermore, two prediction models, based on easy assessable factors, were developed to estimate the risk of being vitamin D deficient. The second aim of this thesis was to better define the consequences of vitamin D deficiency. The relationship between vitamin D status and muscle function was further explored, by using a subjective and a more objective measure, both cross-sectionally and longitudinally. In addition, the association of vitamin D status with bone health was examined including the modifying effect of body mass index, sex, age and physical activity. Last, the association between vitamin D status and early signs of cardiovascular disease was studied, and again several effect modifiers were taken into account. The third aim of this thesis was to estimate the optimal levels for serum 25(OH)D with respect to different, mainly non-skeletal, outcomes and for different subgroups. Lastly, a strategy for a better implementation of the advice of the Dutch Health Council for vitamin D supplementation was proposed.

In this chapter, the main findings will be summarized and discussed. In addition, several methodological issues will be discussed and implications for clinical practice and suggestions for future research will be given.

Summary of main findings

Defining the determinants of serum 25(OH)D

In chapter 2, the cross-sectional association between medication use in general and serum 25(OH)D concentration was examined in two population based samples of older Dutch individuals. In addition, it was studied whether users and non-users of several medication groups had different serum 25(OH)D concentrations and whether sex modified these associations. In both samples, the number of medicines used was associated with lower serum 25(OH)D concentrations. It is challenging to suggest a mechanism behind these associations, because of the diversity of medicines used by the participants. In addition, several specific medication groups were associated with lower vitamin D status, i.e., loop diuretics, inhaled corticosteroids (only in men), oral antidiabetics, calcium-channel blockers and ACE-inhibitors. We could not confirm the previously described association of statin use and higher vitamin D status [1-3]. The associations found could be explained by direct effects of medicines themselves, or the presence of (chronic) diseases for which the medicines were used. Alternatively, the (chronic) diseases might be a consequence of

vitamin D deficiency. In the literature, medication use has also been considered as a marker of frailty [4], which could also be an explanation for the relationship between medication use in general and the lower serum 25(OH)D concentrations. Sex modified some of the observed associations, but no clear overall difference between the two sexes was found.

In chapter 3, two prediction models for the risk of being vitamin D deficient were developed. The models were developed using a cohort of a representative sample of the older Dutch population, consisting of 1509 participants. Two models were created; one for the prediction of serum 25(OH)D concentrations of < 30 nmol/l and one for the prediction of serum 25(OH)D concentrations < 50 nmol/l, based on the current Dutch advice for adults and older persons respectively [5]. Both models were developed using a set of potential predictors which can easily be assessed by for example general practitioners or by the individuals themselves. The model < 30 nmol/l consisted of 10 parameters: age, smoking habits, alcohol use, season, vitamin tablets use, bicycling, gardening, medication use, limitations in transport use, and problems with remembering the current year. The model <50 nmol/l consisted of 13 parameters: age, female sex, body mass index (BMI), smoking habits, alcohol use, season, vitamin tablets use, bicycling, sporting, gardening, medication use, presence of appetite, and partner status. Both models were able to predict vitamin D deficiency accurately and the model < 50 nmol/l was successfully validated externally in an independent cohort of Dutch older individuals. The first model could not be validated externally, due to limited numbers of participants with serum 25(OH)D < 30 nmol/l in the validation cohort.

Defining the consequences of vitamin D deficiency

Vitamin D receptors have been found in muscle cells [6] and therefore, in chapters 4 and 5, the association of vitamin D status with physical functioning was studied, both cross-sectionally and longitudinally. In addition, the modifying effect of age, sex and physical activity was examined. In chapter 4, we found that vitamin D status was cross-sectionally associated with a total physical performance score in three independent cohorts. Physical performance was objectively measured by three tests: the walking test, the chair stand test and the tandem stand test. Longitudinally, a decline in physical performance after three and six years of follow-up in the age group of 55-65 years old individuals could not be predicted. This is in contrast to previous research from our institution, within the first cohort of LASA, showing that low vitamin D status was predictive of a decline in physical performance in older individuals aged 65 years and older [7].

In chapter 5, the cross-sectional and longitudinal associations between vitamin D status and the more subjective measure of physical functioning, functional limitations,

were studied. Functional limitations were assessed by asking questions on the ability to perform six activities of daily life. We found that low vitamin D status was associated with the presence of any functional limitation and with a higher number of limitations in two independent cohorts. Longitudinally, low vitamin D status was associated with an increase in the number of limitations (at least 2 more limitations) over three years follow-up in individuals of 65 years and older and over six years in individuals of 55-65 years. This difference may be explained by the fact that only a few individuals of 55-65 years showed an increase in number of limitations in three years as compared to the older age group. There were no significant effect modifiers in these associations.

In chapter 6, the cross-sectional associations between vitamin D status and bone health were examined in three independent cohorts. In addition, effect modification by age, sex, physical activity, and body mass index was studied. Bone health was estimated using quantitative ultrasound measurements (QUS) and dual X-ray absorptiometry (DXA). In two out of three cohorts (aged 65 years and older), BMI modified the associations: vitamin D status was only associated with lower broadband ultrasound attenuation (BUA) in persons with a low-to-normal BMI (< 25 kg/m^2). This modifying effect of BMI was also found in the analyses on bone mineral density measured by DXA in one of the cohorts. In the third, younger (55-65 years), cohort no associations were found at all. An explanation for the importance of BMI is that mechanical loading may be more important for bone health than an adequate vitamin D status. In addition, because vitamin D is fat-soluble and the bone marrow also contains fat, the concentration of 25(OH)D in the serum may not be a good reflection of the concentration in the bone [8].

In chapter 7, the relationship of vitamin D status and pre-clinical vascular disease and the potential modifying effect of age, calcium and/or vitamin D tablets use, sex, hypercholesterolemia, diabetes and cardiovascular disease (CVD) was studied. Pre-clinical stages of CVD were measured by indices of arterial stiffness and atherosclerosis, such as pulse wave velocity (PWV) and carotid intima media thickness (IMT). We found that the relationship between vitamin D status and IMT and PWV was not linear and especially in persons with high levels of 25(OH)D (≥ 50 nmol/l), higher serum 25(OH)D was associated with higher IMT. Age significantly modified the association of serum 25(OH)D with IMT; only in participants under the age of 80 years, there was a significant association. The association could be explained by the possible increased calcium absorption from the gut under influence of higher 25(OH)D levels and the inactivation of metalloproteinases, which can contribute to calcium deposition in the vessel wall [9]. No clinically relevant results were found for the analyses on PWV.

The estimation of the optimal concentrations of serum 25(OH)D and the promotion of better vitamin D status in the population

In chapter 8, the thresholds for serum 25(OH)D were estimated for several, mainly non-skeletal, outcomes. The outcomes that were considered were: falling, hypertension, cardiovascular disease, functional limitations, physical performance, parathyroid hormone (PTH), blood pressure, grip strength, BMI, mortality, and fractures. In addition, the analyses were performed separately for different age groups, men and women, and different BMI groups. Thresholds for the whole population ranged from 46 (PTH) to 68 (hypertension) nmol/l. On average, women, the oldest old (> 75 years) and people with high BMI (> 25 kg/m^2) had lower thresholds of serum 25(OH)D as compared to men, the younger old and individuals with low-to-normal BMI.

In chapter 9, the results of a national expert meeting on the implementation of the current Dutch advice on vitamin D supplementation were reported. The main recommendations are the use of several existing contact moments with health care professionals for dissemination of the advice; the children's health clinic can be used to inform the whole family; and in nursing homes vitamin D supplementation should be marked as an indicator of quality.

Methodological considerations

The results presented in this thesis were based on two independent cohorts of the Longitudinal Aging Study Amsterdam (LASA) and the baseline measurement of the B-vitamins for the PRevention Of Osteoporotic Fractures (B-PROOF) study. This section will discuss the methodological strengths and weaknesses in order to facilitate an optimal interpretation of the results.

Methodological issues on study design: a cohort study and the baseline measurement of a clinical trial

LASA

The LASA study is an ongoing cohort study on the four domains of aging (emotional, cognitive, physical and social aging). Because LASA focuses on a wide scope of aging, all studies in this thesis could rely on a large set of variables. This enables us to include almost all necessary confounders in our models and we were able to test several effect modifiers to see whether there are any differences between the subgroups. Despite the large amount of available variables, the LASA study did not include any detailed information on vitamin D supplement use, and therefore we could not adjust for this in our analyses. However, because within LASA information on over-the-counter (multi-)vitamins use is

available, we performed sensitivity analyses and the results did not change materially. In addition, especially for the development of the prediction models (chapter 3), the large diversity in variables available within LASA was very useful. While previous studies could usually only rely on clinical variables, such as BMI, age, and gender [10-12], we were able to include more detailed information on physical activity and some social characteristics, such as partner status. On the other hand, as LASA was not originally designed for research on vitamin D status, some more specific questions were missing, such as sunshine exposure and the consumption of fatty fish. It is likely that the performance of the prediction models would have been better if it was possible to include these variables.

In addition to the large dataset, LASA is an ongoing longitudinal study, with follow-up measurements at roughly three year intervals with most of the parameters measured at every wave using the same instrument. Therefore, it was possible to reliably analyse changes in, for example physical functioning, correctly. Furthermore, LASA is based on a representative sample of Dutch older individuals, which enables us to extrapolate our results to the Dutch older population. However, one should note that the subsample used in this thesis, defined by a serum 25(OH)D measurement, may be slightly healthier as compared to the general population, because the participants sometimes had to visit the hospitals for blood collection. Nevertheless, it is likely that this rather led to an underestimation than an overestimation of the effects.

Another main limitation is that no inference can be made on causality. It remains therefore unclear whether vitamin D is the cause of worse physical functioning, poor bone health and cardiovascular damage or whether these conditions themselves contribute to low vitamin D status, for example by a restriction in sunshine exposure. Because vitamin D receptors have been found in cells of many organs [13], it may be suggested that a low serum 25(OH)D levels is the cause and not the effect. In addition, regarding physical functioning, we were able to show that low vitamin D status is also associated with a decline in physical functioning over a period of three or six years (chapter 5), indicating that vitamin D deficiency precedes the worsening of physical functioning. This temporality is one of the important criteria in the epidemiological criteria for causality [14]. However, the effect of prevention strategies is still questionable: systematic reviews and meta-analyses on the effect of vitamin D supplementation on physical functioning are somewhat disappointing and not conclusive [15-17].

Furthermore, attrition and exclusion is unavoidable in cohort studies. LASA is no exception in this. However, attrition does not automatically lead to bias [18], but it becomes a problem when it occurs selectively. Loss to follow-up in LASA is mainly due to death and to lesser extent to refusal or frailty [19;20]. As LASA was designed as a representative random sample of Dutch older individuals, it is likely that, because death is a

natural phenomenon, the remaining participants of the cohort still reflect the whole population [21]. It was found that attrition in the whole LASA sample was more often in relatively older participants, in participants with more chronic diseases and with cognitive impairment [20]. Although, the influence of this on the results of our studies remains unclear, it is more likely to result in an underestimation than a overestimation of the effects.

B-PROOF

As B-PROOF was designed as a randomized clinical trial on the prevention of osteoporotic fractures in older persons and all participants received vitamin D supplementation during the trial, it was not possible to conduct longitudinal analyses within B-PROOF. Therefore, the remarks regarding causality for the analyses in LASA, also apply to B-PROOF. In addition, it was not a population based sample, and some comments should be made regarding the generalizability of the results. On the one hand, the participants may be somewhat healthier than the average Dutch older population, due to the willingness to participate in a clinical trial for two years. It is likely that frail older individuals will not agree to participate in a study with two years of intervention. In addition, the people interested in nutrition and health, with subsequently healthier lifestyle, might be more willing to participate in a study on vitamins, while bedridden and wheelchair-bound persons were excluded [22]. On the other hand, participants were only included if they had elevated homocysteine levels (≥ 12 µmol/l). An elevated homocysteine level (≥ 15 µmol/l) is present in 30-50% of the individuals of 60 years and older. Hyperhomocysteinemia is caused by multiple factors, such as age, sex, lifestyle, environmental and genetic factors [23] and it is associated with frailty and all-cause mortality [24].

Furthermore, there might be an interaction between vitamin D and homocysteine. One study found that $1,25(OH)_2D_3$ has a small homocysteine lowering effect [25]. This may have led to the inclusion of participants with relatively low vitamin D status. However, the prevalence of vitamin D deficiency in B-PROOF is in the same range as in the LASA study (about 50%, see for example chapter 4). In addition, it remains unclear whether the relatively high homocysteine levels themselves influenced the results presented in this thesis.

Serum 25(OH)D measurements

The measurement of serum 25(OH)D is challenging. The results vary according to the method used, which may be caused by several factors. First, serum 25(OH)D has a lipophilic character and is strongly bound to the vitamin D binding protein. Second, several vitamin D

metabolites can be detected in the circulation. And third, direct sunlight will degrade serum 25(OH)D and therefore the blood samples have to be stored in the absence of direct sunlight [26;27].

In the studies reported in this thesis, the serum 25(OH)D concentrations were measured by three different assays, hampering direct comparison of the results. In the 1995/1996-blood samples of the LASA participants, serum 25(OH)D was measured by using a competitive protein binding assay, which was developed in the 1970s. The main advantage of this method is that both serum 25(OH)D_2 and 25(OH)D_3 are measured. However, other metabolites of vitamin D, such as 24,25-dihydroxyvitamin D are also detected by this assay [28]. The 2001/2002- and 2008/2009-samples of the LASA participants were measured by a radioimmunoassay (RIA) and comparable to the competitive binding protein assay. Both forms of serum 25(OH)D were taken into account and the main problem was that also other metabolites were additionally recognized [28]. In conclusion, both RIA and the competitive protein binding assay appear to overestimate the serum 25(OH)D concentration. The RIA and competitive protein binding assay used in LASA were compared by measuring 117 samples (41 LASA participants, and 76 patient samples, measured for clinical purposes) with both methods (range from <5 - 123 nmol/l). This comparison showed that levels of 25, 50, and 75 nmol/l measured with the competitive protein binding assay equalled 26.0, 48.2, and 70.4 nmol/l, respectively, when measured with the RIA assay. The correlation coefficient was r=0.94. It was also shown that the level of vitamin D binding protein in the serum is inversely associated with the results of the serum 25(OH)D measurement depending on the method used [29].

Within B-PROOF, serum 25(OH)D was measured using a liquid chromatography – tandem mass spectrometry (LC-MS/MS) method, which is, at this moment, considered as the gold standard method [29]. By using this method, it is possible to distinguish between the several forms (D_2 and D_3) and metabolites of vitamin D and thus there will be no such overestimation of the true serum 25(OH)D concentrations [27]. However, LC-MS/MS has limitations as well, such as the cross-reactivity with 3-epi-25(OH)D, thereby also somewhat overestimating the concentration of 25(OH)D [27].

Furthermore, it is well known that seasonal variation exists in serum 25(OH)D concentrations, especially in high latitude regions, also in the Netherlands [30-32]. In all studies described in this thesis, serum 25(OH)D was measured only once per participant. This means that the measured concentration was dependent on the season in which the blood sample was drawn and the results may be influenced by seasonal variation [33]. Therefore, more ideal, blood samples should be drawn at least twice, in summer and winter [34]. However, in a large cohort as LASA and in the large trial B-PROOF, this was practically not feasible. Most of the data was collected throughout the year and we

accounted for seasonal variation in our analyses by adjusting for season of blood collection. Another method is to use season specific vitamin D categories. One previous study showed that both methods reduced the bias away from the null as compared to analyses without any adjustment. However, it is suggested that the second method may be preferable, due to the ability to better reduce the bias [33].

Measurement of determinants and consequences

In chapter 2, the use of medication was related to vitamin D status. Medication use was assessed by asking the participants to show their medication containers to the interviewers in a face-to-face interview. In order to create groups of medication, all medicines were coded by using the Anatomical Therapeutic Chemical classification system (ATC) of the World Health Organization [35]. Alternatively to the self-reported medication use, data could also be obtained from pharmacist databases or general practitioners. The main advantage of self-reported data is that participants are likely to report only the drugs they actually use, because a doctor's prescription does not automatically mean that the drug is picked up at the pharmacy and is taken by the patient. Over-report of use is thus more likely by using general practitioner or pharmacist databases, whereas underreport is more likely to occur in self-report [36]. On the other side, we now only have information on medication use at the time of the interview. By using pharmacist databases, the duration of use could have been taken into account. In all, self-report and pharmacist data seem to complement each other. Previous studies, however, showed that the agreement of both methods is fairly good, especially for people with higher household income, married individuals with good health [36-38]. Lastly, it is known that the prescription of medication changes over time. The studies described in chapter 2 relied on data from 1995/1996 and 2001/2002. However, it is not likely that this influenced the results of the studies because the mechanisms of action will be the same for the specific drugs, regardless of the time point of the measurements.

In chapter 4 and 5, the relationship of vitamin D status with physical functioning was studied. Both an objective (physical performance tests) and a subjective measure (questions on functional limitations) were used. The physical performance tests are objective, but give little information on real functioning in daily life [39]. Functional limitations are hypothetical questions on the presumed abilities [39]. The correlation between the objective tests and the subjective question is moderate: 0.48 [40]. Therefore, it is likely that other aspects will influence both measures of physical functioning [41]. In an epidemiological study, physical performance tests were associated with age and physiological aspects, whereas self-reported functional limitations were also associated

with psychosocial factors [42]. While different aspects were associated with both the objective and subjective physical functioning measures, one study showed that both physical performance tests and self-reported functional limitations are a predictor of future mobility disorder [43]. Thus, although both are a predictor of future problems, it seems that both complement each other.

In chapter 6, two types of measurements of bone health were performed, i.e., QUS and DXA measurements. The current diagnostic criteria for osteoporosis according to the World Health Organization are based on DXA measurements [44]. In the last decades, QUS measurements were introduced, but there is much uncertainty about the use of QUS measurements in clinical practice [44]. Correlations between QUS and DXA parameters are between 0.28 and 0.68 [45;46]. QUS measurements rely on the property that ultrasound is transmitted by bone in different patterns according to the quality of bone [47]. Two types of parameters are reported during the measurement (Speed of Sound (SOS) and Broadband Ultrasound Attenuation (BUA)), but the underlying biophysics are not completely known [47]. In this thesis only associations of vitamin D status with BUA were found, indicating differences between BUA and SOS. This is in line with the suggestion that BUA may reflect the changes in bone tissue better than SOS [48]. In addition, there is also uncertainty about the meaning of the absolute values of SOS and BUA, partly because of lack of standardization between the values of different manufacturers [49]. While QUS reflects the quality of bone, DXA measurements reflect the bone mineral density. In contrast to QUS, much information exists in the literature on the validity of DXA measurements and it is the current “gold standard” for assessing bone density for the diagnosis of osteoporosis [44]. However, it is relatively time consuming, it uses ionizing radiation and is only available in clinics [50;51]. In addition it provides, little information on bone architecture [52]. Recently, an updated meta-analysis showed that QUS (all parameters) predicts fracture incidence partly independently of BMD [44]. An Italian study showed that fractures can be predicted by QUS as well as DXA with comparable accuracy [53]. In the future, more research is needed to conclude whether QUS can be used in clinical practice for example in fracture prevention strategies or that a combination of QUS and DXA, or DXA alone is more preferable.

In chapter 7, measurements on preclinical stages of vascular disease were studied by using measurements of pulse wave velocity (PWV) and intima media thickness (IMT). PWV was measured by using applanation tonometry of the right femoral and carotid artery. Studies have shown that PWV is highly associated with arterial stiffness, which predicts cardiovascular risk [54]. Nowadays, PWV is considered as the ‘gold standard’ for arterial stiffness measurement [54]. In the study described in this thesis, PWV was only measured at one set of two places (right femoral and carotid artery), but it may be better

to determine PWV at more sites to have a better indication of the vascular stiffness of the whole body [55]. Previous studies showed that PWV is a good marker of atherosclerosis, indicating that this measure is indeed a method to determine preclinical stages of vascular disease [56;57]. In addition, the evidence for the predictive value of PWV to predict mortality is growing, but large longitudinal studies are necessary before this type of measurement can be used in clinical practice [55]. IMT of the carotid arteries is often considered as a surrogate marker for coronary atherosclerosis [58]. Although it is associated with some atherosclerotic risk factors and the prevalence of coronary artery disease [59], relatively poor correlations were found for IMT of the carotids and coronary angiography [59]. Post-mortem studies showed higher correlations between the carotid and coronary arteries and a recent study also found that IMT correlates better with coronary atherosclerosis [59].

Statistical methods

Several remarks can be made on the statistical methods used in this thesis. In chapter 3, prediction models were developed using the data of the participants of the medical interview of the second measurement cycle of LASA. However, only for 1320 of the 1509 participants serum 25(OH)D measurements were available and therefore we decided to use multiple imputation in order to increase the statistical power. Although, currently more applied, multiple imputation still is a matter of debate in medical research [60]. Opponents of multiple imputation feel that investigators are making up data, which is scientifically unethical. However, missing values are predicted based on other known variables in the datasets [60]. Missing values can be classified into three main groups: missing completely at random (MCAR), missing at random (MAR), and missing not at random (MNAR). MCAR is present when the underlying reason for missing values is independent of known and unknown characteristics; this is the only type of missing value that is not likely to influence the results. Within MAR, missing values are related to known characteristics and within MNAR missing values are related to unknown characteristics [60;61]. Because multiple imputation uses the other variables in the dataset, an assumption is that missing values are related to other variables (MAR). Usually, and also in most studies described in this thesis, complete case analyses were performed. The main limitation of this method is that results may be biased since missing values are hardly ever missing completely at random (MCAR) [60]. However, it is likely that this causes rather a underestimation than a overestimation of the effects, because it will be the most frail individuals who had missing values.

As the goal of chapter 3 was to develop prediction models, which have to be practical in clinical practice and in other populations, we needed to reduce imprecision as much as possible. This was reached by using multiple imputation, which reduces the imprecision by increasing statistical power through pooling the results of the different imputed datasets [60;61]. External validation of the developed model was performed in the second cohort of LASA. Although this is a totally independent cohort, it was based on the same sampling frame as the original cohort. Therefore, validation in a totally different population may result in a somewhat lower performance.

In chapter 8, thresholds for sufficient serum 25(OH)D were determined by using spline curves and the best model fit, expressed as R^2 for linear models and the C-index for Cox and logistic models. The proposed thresholds were based on relative risks within the population. One may argue that the observed thresholds rely on the distribution of serum 25(OH)D in our sample and that higher thresholds a priori could not be found because of the relatively low levels of serum 25(OH)D in our sample. However, we used a four-step strategy to determine the thresholds and by using this, the shape of the curve was also taken into account. This means that if the above mentioned comment is true, then one would expect an increasing line until the highest levels of serum 25(OH)D. Because this was not the case for most of the variables, this is not likely to be a limitation of our approach. Previous studies on determining thresholds of 25(OH)D levels are mostly based on the optimal sensitivity-specificity ratio, expressed as the Area Under the Curve, which is close to the C-index used in our models [62]. The main difference with our four-step approach is that we first determined whether there was a clinically relevant difference between the lowest and highest risks or absolute values and second, that we used the visually determined thresholds in the statistical analyses. We did thus not only rely on the best statistical fit. This enables us to confirm that the found threshold is of clinical relevance.

Implications for clinical practice

In short, in this thesis we showed that the use of medication was associated with lower serum 25(OH)D levels, that vitamin D deficiency could be predicted by asking the patient simple questions, that low vitamin D status was associated with worse physical functioning, with worse bone health in individuals with low-to-normal BMI and with higher IMT in persons with high vitamin D levels and in the younger old. We revealed that the thresholds of sufficient serum 25(OH)D levels ranged from 46-68 nmol/l according to different outcomes, and varied between different subgroups. In the following paragraph, we will discuss these findings within the scope of clinical practice.

Determinants of serum 25(OH)D

Various drugs are metabolized by or may induce or inhibit the activity of the CYP3A4 enzyme, which is the main enzyme for the 25-hydroxylation in the liver [63]. Therefore, it is likely that some of these drugs influence the concentration of serum 25(OH)D. Drug-drug interactions are common and a relatively highly prevalent cause of health deterioration especially in the elderly [64]. One study showed that 5-10% of the hospitalizations were related to adverse drug reactions [65]. Although, clinicians are aware of these adverse drug-drug interactions, much less attention is paid to drug-nutrient interactions [66;67]. Our study showed that the use of several drugs, such as loop diuretics, inhaled corticosteroids, oral antidiabetics, calcium-channel blockers and ACE-inhibitors are related to lower serum 25(OH)D and that the higher the number of drugs used, the lower the serum 25(OH)D concentration was. This suggests that there indeed may be a direct effect of medication on vitamin D status. Clinicians should therefore be aware of the risk of low vitamin D status when their patients use one or more of these specific drugs or when the patients use a high number of drugs. The study described in this thesis does not make any inference on causality because of the cross-sectional design. In particular, polypharmacy could be an indicator of frailty [4]. Frailty may be associated with low vitamin D status and therefore, although maybe not causal, the number of medications may be an indication for assessing serum 25(OH)D in the blood.

The models to predict vitamin D deficiency developed in chapter 3, may be useful in clinical practice. All people above 50 years (women) or 70 years (men) are advised by the Dutch Health Council to take vitamin D supplements every day [5]. However, only about 50% of the general older population has vitamin D deficiency (serum 25(OH)D < 50 nmol/l). One can imagine that not all these older individuals feel that it is necessary to take the vitamin D supplements, especially when they have a healthy lifestyle. These models can be used as a discussion tool by health care professionals to convince people to take vitamin D supplements in case the model predicts a high risk for vitamin D deficiency. In addition, the model could also be used as a self-test, for example hosted on the internet. Previous research revealed that most individuals take action after a negative test result of a self-test [68]. The main advantage of transforming the developed models into a self-test is that people will be more aware of this public health problem and of the current advice of the Dutch Health Council.

Consequences of vitamin D deficiency

The second part of this thesis was on the elaboration of the consequences of vitamin D deficiency, especially physical functioning, bone health and vascular disease. Low vitamin D status was associated with all these three outcomes. However, subgroup analyses were not

consistent across the outcomes. Most of the associations were found in the older age groups (the first cohort of LASA and the B-PROOF-study). This may implicate that clinical guidelines on vitamin D supplementation should aim at the older age groups, and that the urgency for vitamin D supplementation is less in the younger age group. However, in the longitudinal analyses on functional limitations, we found that also in the younger age group (55-65 years) low vitamin D status was predictive of an increase in functional limitations over six years. Although it seems that the older age group is more at risk of vitamin D deficiency with respect to physical functioning, this has to be confirmed in further research.

With respect to bone health, vitamin D supplementation guidelines may be aimed at people with low BMI (< 25 kg/m^2), as this was the subgroup in which we found significant associations. Because further research is also necessary to confirm this finding, clinicians should at least pay more attention to persons with low BMI and with a high fracture risk, by for example determining their vitamin D status or advising vitamin D supplements. Studies on the effect of vitamin D supplementation have revealed that supplementation with at least 800 IU in institutionalized and independently living older individuals reduced the risk for fractures with 15% and 10-20%, respectively. But the influence of BMI was not taken into account [69].

Fitting the pieces together: towards an optimal vitamin D status in the older population

Nowadays, about 50% of the older individuals in the Netherlands has a suboptimal vitamin D status (serum 25 (OH)D < 50 nmol/l). To decrease this percentage, the first step is to ascertain the definition of deficiency. Both the advice of the Dutch Health Council and the statement of the IOM are based on concentrations for optimal bone health and both committees advise minimal concentrations of 30-50 nmol/l [5;70]. In chapter 8, we determined the thresholds with respect to different, also non-skeletal, outcomes. For some of these outcomes, the association with vitamin D status was also presented in this thesis (physical performance, functional limitations, and (preclinical) cardiovascular disease). For the other outcomes, i.e. falling, hypertension, mortality, grip strength, PTH, and fractures, previous studies already showed an association with vitamin D status [71-77]. Apart from falling, fractures and muscle function [69;78;79], it has not been proven that vitamin D supplementation will improve these extraskeletal outcomes. Based on the observational research presented in this thesis, thresholds for serum 25(OH)D levels for the population are between 46 and 68 nmol/l according to different outcomes. These results are very close to the recommendations of the current guidelines. On the other hand, to meet the requirements of almost the whole population (RDA: recommended dietary allowance, the requirement of 97.5% of the population), the advised concentrations may have to be

higher as the results of our study are based on population means and not on 97.5% of the population. Therefore, the range of thresholds of 46-68 nmol/l more reflects the estimated average requirement (EAR, the median requirement of the population). The EAR mentioned in the IOM statement is 40 nmol/l; based on our results this is on the low side. When considering outcomes with sufficient evidence from vitamin D supplementation trials (falls, fractures, and muscle function), a range of thresholds of 48-60 nmol/l was found and this will not change materially the above mentioned deliberations.

In chapter 8, we also studied whether there was a difference in thresholds between different subgroups. On average, but not for all outcomes, thresholds were lower in women, older individuals and individuals with high BMI. This may indicate that these subgroups may need less vitamin D supplementation as compared to the other subgroups. However, in the current Dutch advice, women were advised to take vitamin D supplements earlier in their lives (from 50 years of age, as compared to 70 years of age for men) based on a higher fracture risk. Furthermore, older individuals are also advised to take a higher dose compared to younger people. In the future, the guidelines and advices may have to consider different BMI-groups and the current advises for different age groups and gender may need to be reconsidered. However, the results of our study first need to be validated in other prospective cohort studies.

After defining the thresholds, the second step to optimize the vitamin D status of the population is to determine the supplementation dose needed. Several studies on the dose-response relationships were performed previously. A recent meta-regression analysis of 33 clinical trials, found a dose-dependent increase in serum 25(OH)D up to 800 IU per day, with only a slight increase with higher doses. With a dose of 800 IU per day, the serum 25(OH)D concentration of 97.5% of the participants (RDA) was higher than 50 nmol/l after 6-12 months [80]. Based on the results of our analyses on thresholds, which were between 46 and 68 nmol/l, 800-1000 IU per day will be sufficient to reach an optimal vitamin D status in the older population.

After defining the serum 25(OH)D concentration which should be aimed for and the supplementation dose needed, the final step to a better vitamin D status is to implement the current advice. In chapter 9, a strategy was developed to facilitate the implementation of the guidelines. By using the strategy proposed in that chapter, the awareness of the advice and of the need of vitamin D supplements will grow. These will help to achieve an optimal vitamin D status in the older population.

Implications for future research

This thesis supported a better definition of the determinants and consequences of vitamin D deficiency in order to facilitate an optimal vitamin D status in the older population, but one important question remains unanswered: does vitamin D supplementation improve non-skeletal outcomes, such as cardiovascular disease, cancer, respiratory infections, and mortality? As described previously in this thesis, because of the observational design, no inference on causality can be made. To answer the question on the effect of vitamin D supplementation, large clinical trials, with adequate doses of vitamin D and long follow-up times, are needed. Several well-designed trials in older adults are currently undertaken. These are, for example, ViDA [81], VITAL [82], TIPS3 [83], FIND [84], and D-Health [85]. All these trials aim to include thousands of individuals and the dose of vitamin D given ranges from 2000 IU per day to 100.000 IU per month, equivalent to 3300 IU per day. The results of these trials will become available in the next years. More details of these trials are shown in Table 1. Because of the large amount of participants in these trials, it will be possible to stratify for different subgroups and for different baseline serum 25(OH)D concentrations. If these large trials show positive clinically relevant effects of vitamin D supplementation on non-skeletal outcomes, the current guidelines may be updated. Because of the high dosages of vitamin D used in the current large trials, the main change may then be a higher recommended dose.

Table 1. Overview of the trials currently undertaken in older individuals

Study name	N	Age (years)	Outcomes	Dosage of vitamin D	Duration of follow-up	Results expected in
ViDA	> 5000	50-84	CVD, respiratory infections, falls, fractures	100,000 IU/month	4 years	2016
VITAL	> 25,000	> 50	Cancer, CVD, stroke	2000 IU/day	5 years	2017
TIPS3	5000	> 55	CVD, fractures	60,000 IU/month	5 years	2019
FIND	18,000	> 60	CVD, cancer	1600/3200 IU/day	5 years	2019
D-Health	25,000	60-85	Cancer, mortality	60,000 IU/month	5 years	2020

CVD: cardiovascular disease

Furthermore, this thesis showed some interesting associations between medication use and lower vitamin D status. However, the chronic diseases for which the drugs are prescribed could also cause the lower serum 25(OH)D levels. In addition, immobility, caused by chronic diseases, may also lead to lower vitamin D status through lower sunshine exposition. To distinguish between these three causes, the research design will be placebo controlled clinical trials on the effect of the drugs on serum 25(OH)D levels. However, in practice, this will be challenging, as it may not be ethical for most of the chronic diseases to give a placebo instead of, for example, antidiabetics. However, there might be frozen blood samples of registration trials of specific drugs, which can be used. Another option is to perform longitudinal observational studies with shorter intervals of blood measurements and of the interviews. A continuous monitoring of chronic disease status and medication use by pharmacist databases offer an alternative approach.

In chapter 3, we developed prediction models for a current vitamin D deficiency. It will be interesting to develop such a model for predicting the risk of vitamin D deficiency in the future. To develop this, repeated measurements of serum 25(OH)D are necessary, as well as detailed information on lifestyle, food consumption, supplement use and several other potential predictors.

Conclusion

This thesis aimed to better define the determinants and consequences of vitamin D deficiency in order to facilitate an optimal vitamin D status in the older population. First, it was shown that the use of (specific) medication was associated with lower vitamin D status. Further research is needed to explore the underlying mechanisms, but clinicians should be aware of low vitamin D status, in patients with chronic diseases, especially in case of polypharmacy, regardless of the underlying mechanisms. Second, models to predict current vitamin D deficiency were developed. These models can be used, for example, by general practitioners or can be used as a self-test on the internet, in order to make individuals aware of a potential vitamin D deficiency.

Third, it was found that low vitamin D status was related to worse physical functioning, cross-sectionally as well as longitudinally, measured by both objective and subjective measures. Fourth, the association of vitamin D status with bone health was modified by BMI. Only in individuals with low-to-normal BMI, low vitamin D status was associated with low BMD and low BUA values. Fifth, the association of vitamin D status with IMT was examined and a non-linear relationship was found. In addition, only in individuals with high levels of serum 25(OH)D, serum 25(OH)D was associated with IMT. We did not find any association with PWV. To draw definite conclusions on the

relationships of vitamin D with non-skeletal outcomes, well designed clinical trials have to be performed. Several of such clinical trials are currently underway.

Sixth, we found that thresholds of serum 25(OH)D sufficiency ranged between 46 and 68 nmol/l and lower thresholds were found for women, the older age group and individuals with high BMI, contrary to expectation. These results have to be validated in longitudinal studies, ideally by clinical trials, before including it in the guidelines on vitamin D supplementation. Lastly, a strategy for a better implementation of the current advice on vitamin D supplementation may include the use of existing contact moments with health care professionals as was suggested by an expert meeting.

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