CHAPTER 1
General introduction and outline of the thesis
1.1 Diabetes mellitus type 2 and the metabolic syndrome

Type 2 diabetes mellitus has become a significant global health care problem. Worldwide the prevalence has increased substantially over the past decades due to the increasing incidence of overweight and longevity. In addition, more alertness leads to earlier detection. (1) In the Netherlands, approximately 800,000 inhabitants were known to have diabetes in 2011 and estimations have been made that the number of diabetic patients in the Netherlands will increase to 1.3 million (8.4 % of the population) in 2025. (2)

Diabetes mellitus type 2 is a chronic metabolic disease, which is characterized by chronic hyperglycemia caused by insulin resistance and β cell dysfunction (insulin deficiency). (3) Besides chronic hyperglycemia, there is disturbance in protein and fat metabolism. More important, diabetes mellitus is associated with serious long term morbidity (i.e. retinopathy, neuropathy, nephropathy, micro-angiopathy and cardiovascular disease) and increased mortality. (4-6) Although therapies for type 2 diabetes and its complications have been improved, the increasing burden of type 2 diabetes causes a strong need for preventative approaches of the disease.

The clustering of risk factors for diabetes type 2 and cardiovascular disease, which occur together more often than by chance alone, is well known as the insulin resistance syndrome or the metabolic syndrome, i.e. obesity, hypertension, dyslipidemia and hyperglycaemia and was first described by Reaven. (7) Various diagnostic criteria have been proposed by different organizations. (8-10) A harmonized definition of the metabolic syndrome was established by an international committee in 2009 and is displayed in Table 1. The metabolic syndrome is present in case of three or more of the following risk factors: abdominal overweight, high triglycerides concentration, low HDL-concentration, hypertension and high fasting plasma glucose. Insulin resistance and abdominal overweight are independent factors in the clustering of metabolic abnormalities. (11) Diabetes mellitus is defined according to the American Diabetes Association (ADA) as follows: a HbA1c level ≥ 6.5 % (48 mmol/mol), fasting plasma glucose ≥ 7.0 mmol/l, 2-hr glucose during an oral glucose tolerance test of ≥ 11.1 mmol/l, or classic symptoms and a random glucose ≥ 11.1 mmol/l and the first three criteria should be confirmed by repeat testing. (12) Since 2011, the World Health Organization (WHO), also added HbA1c as diagnostic criterium following the ADA in addition to abnormal glucose levels. In the Netherlands however, only abnormal glucose levels are used to diagnose diabetes mellitus currently.
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Table 1. Criteria for Clinical Diagnosis of the Metabolic Syndrome(11)

<table>
<thead>
<tr>
<th>Criteria metabolic syndrome (presence of any 3 of 5 risk factors)</th>
<th>Men</th>
<th>Women</th>
</tr>
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<tbody>
<tr>
<td>Waist circumference (Population- and country-specific definitions)</td>
<td>≥ 94 cm for Middle East/African origin</td>
<td>≥ 80 cm for Middle East/African origin</td>
</tr>
<tr>
<td></td>
<td>≥ 90 cm for Asian origin</td>
<td>≥ 80 cm for Asian origin</td>
</tr>
<tr>
<td></td>
<td>≥ 102 cm or ≥ 94 cm for European origin</td>
<td>≥ 88 cm or ≥ 80 cm for European origin</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>≥ 1.7 mmol/l or medication for high triglycerides</td>
<td>≥ 1.7 mmol/l or medication for high triglycerides</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&lt; 1.0 mmol/l or medication for low HDL cholesterol</td>
<td>&lt; 1.3 mmol/l or medication for low HDL cholesterol</td>
</tr>
<tr>
<td>Hypertension</td>
<td>≥ 130/85 mmHg or antihypertensive medication</td>
<td>≥ 130/85 mmHg or antihypertensive medication</td>
</tr>
<tr>
<td>Glucose</td>
<td>≥ 5.6 mmol/l or glucose lowering medication</td>
<td>≥ 5.6 mmol/l or glucose lowering medication</td>
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</table>

1.2 Non-western immigrants and diabetes

Next to a sedentary lifestyle and obesity, ethnicity and migration are important risk factors for diabetes. (13) It has been demonstrated that African and Asian immigrants in the USA and the United Kingdom have a higher prevalence of diabetes than the indigenous populations.(14;15) In the Netherlands, immigrants from Turkey, Morocco and Surinam are the largest ethnic minority groups. In 2012, the Netherlands comprised around 16.7 million inhabitants, and the absolute numbers of Turkish, Moroccan and Surinam migrants were 392,933, 362,954 and 346,797 respectively. (16) The prevalence rates of diabetes in Turkish and Moroccan inhabitants in the Netherlands, respectively, are two and over three times higher than in the indigenous Dutch population. Moreover diabetes occurs at younger ages in Turkish and Moroccan inhabitants.(17;18) The difference in prevalence can be explained on the one hand by lower socioeconomic status and higher prevalence of obesity and probably on the other hand by genetic susceptibility, (17) other endogenous factors such as hypertension and dyslipidemia and other environmental factors, such as vitamin D deficiency. Vitamin D deficiency is highly prevalent among the different non-western ethnicities living in the Netherlands compared to the indigenous population. Serum 25-hydroxyvitamin D (25[OH]D) levels were lower than 25 nmol/l in 41% of the Turkish and 36% of the Moroccan immigrants. Insufficient exposure to direct sunlight, covering of the skin, a low calcium and vitamin D diet, and high fat mass are contributors to this high prevalence. (19)
1.3 Vitamin D

Vitamin D is a generic term for a group of fat soluble prohormones, including vitamin D3 (cholecalciferol) and vitamin D2 (ergocalciferol). Vitamin D3 is the animal source vitamin and is synthesized in the skin from 7-dehydrocholesterol under the influence of ultraviolet radiation of the sun during the summer months from April to September in the Netherlands. The other source of vitamin D3 is food, mainly fatty fish, such as herring, mackerel and salmon. Vitamin D2, the vegetable form, can be produced industrially through ultraviolet exposure of the plant sterol ergosterol, found in fungi and yeast. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D (25\(\text{OH}\)D) (20) and is the major circulating storage form of vitamin D in the body (Figure 1). After a second hydroxylation, mainly in the kidney, the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25\(\text{OH}\)2D) is generated. (21) This active form of vitamin D acts as a hormone: its formation is stimulated by parathyroid hormone (PTH), with a negative feedback through calcium, phosphate and 1,25(OH)2D itself. The active metabolite 1,25(OH)2D enters the cell and binds to the nuclear vitamin D receptor (VDR), which forms a heterodimer with the vitamin A receptor (RXR). This complex binds to the vitamin D responsive element in the genome, leading to transcription and translation and proteins are formed. More than 300 genes are up- or downregulated by the active vitamin D metabolite.(22) The vitamin D receptor is present in many organs, for example the gut, bone, muscle, parathyroid gland, prostate, mamma, brain and lymphocytes. Furthermore, in extra renal cells and tissues, 25(OH)D can also be hydroxylated into 1,25(OH)2D under the influence of cytokines and this appears to be important for the paracrine regulation of cell differentiation and function. (23)

Severe vitamin D deficiency (serum 25(OH)D < 15 nmol/l) may cause rickets in children, characterized by deficient mineralization at the growth plate, which increases in size. Rickets has become rare in Western countries, although it still exists in populations with low sun exposure and low vitamin D intake, including minorities in the Netherlands. (24) On the other hand sub clinical vitamin D deficiency (or insufficiency, serum 25(OH)D 25-50 nmol/l) is rather common nowadays and has also been described among adolescents (25) and the elderly (26) and may contribute to the development of osteoporosis and increases the risk of falls and fractures in the elderly.(27-29) Moreover, severe longstanding vitamin D deficiency may lead to osteomalacia (“bone weakness”) in adults and older persons.(22) Osteomalacia is characterized by poor mineralization of newly synthesized bone matrix, the osteoid, during bone remodeling.

1.4 Vitamin D deficiency

In the literature there is neither consensus on the diagnosis of vitamin D deficiency, nor on the optimal serum 25(OH)D level. The Dutch Health Council concluded that a serum 25(OH)D level above 30 nmol/l is sufficient for children (based on risk of rickets) and
adults, with a recommended supplementation dose of 400 IU for children, women between 50 and 70 years old and people with dark skin. Furthermore, 800 IU is recommended for the elderly (>70 years), based on research on fracture and fall risk. It also stated that a level above 50 nmol/l is required for the elderly.(30) In 2011, the Institute of Medicine (IOM) (which focused on the general population) concluded that a serum 25(OH)D level of 50 nmol/l or higher was sufficient, <30 nmol/l as deficient and advised 600 IU/day as recommended dietary intake for the age ≤ 70 years and 800 IU/day for ages 71 year and older to obtain the required 25(OH)D level.(31) However, the latest Endocrine Society Guideline (Evaluation, Treatment, and Prevention of Vitamin D Deficiency: an Endocrine Society Clinical Practice Guideline) (focusing on at-risk individuals) defined 25(OH)D < 50 nmol/l as vitamin D deficient and 50-75 nmol/l as vitamin D insufficient and recommended 400 IU/d to all infants aged 0-1 year, 600 IU/d for all persons aged 1-50 year, with the remark that to raise the blood level consistently above 75 nmol/l at least 1500-2000 IU/d is required. For adults aged 50-70 yr and above 70 yr, the recommendation is at least 600-800 IU/d with the same remark as above mentioned. In case of vitamin D deficiency (serum 25(OH)D < 50 nmol/l), 2000 IU/d for infants and children is recommended for at least 6 weeks, followed by a maintenance dose of 600-1000 IU/d. For adults with vitamin D deficiency
50,000 IU once a week for 8 weeks, followed by 1500-2000 IU/d as maintenance therapy is recommended. (32) In Table 2, the main agreements and disagreements between the two recent guidelines are shown, including both differences in recommended intake, and maximum tolerable doses. This leads to confusion among clinicians, researchers and the general population. (33) Based on the existing evidence for classical outcomes as fractures and falls, to my opinion 800 IU/day should be advised to the elderly and patients with osteoporosis. (34) With 600-800 IU/day, most healthy postmenopausal women reached serum 25(OH)D levels of 50 nmol/l, based on a multi-dose study. (35)

### 1.5 Non-skeletal effects of vitamin D

Vitamin D status is the main determinant of active calcium absorption from the gut, which is important for bone mineralization. The precise action of vitamin D and the full spectrum

<table>
<thead>
<tr>
<th>Life stage/group</th>
<th>IOM recommendations</th>
<th>Endocrine Practice Guidelines Committee</th>
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<tr>
<td></td>
<td>Adequate intake</td>
<td>Recommended dietary allowances for general population</td>
</tr>
<tr>
<td>Infants</td>
<td>400 IU</td>
<td>600 IU</td>
</tr>
<tr>
<td>Children (&lt;5 yr)</td>
<td>600 IU</td>
<td>600 IU</td>
</tr>
<tr>
<td>M (6-18 yr)</td>
<td>600 IU</td>
<td>600 IU</td>
</tr>
<tr>
<td>M (&gt;70 yr)</td>
<td>600 IU</td>
<td>4000 IU</td>
</tr>
<tr>
<td>F (0-18 yr)</td>
<td>600 IU</td>
<td>600 IU</td>
</tr>
<tr>
<td>F (&gt;70 yr)</td>
<td>600 IU</td>
<td>4000 IU</td>
</tr>
<tr>
<td>Pregnancy/lactation</td>
<td>600 IU</td>
<td>4000 IU</td>
</tr>
</tbody>
</table>

**Agreements**

- No need to screen the general population routinely vitamin D essential for skeletal health, no convincing evidence for non-skeletal outcome.

**Disagreements**

- Target serum 25(OH)D level
  - Suggested 25(OH)D level 50 nmol/l
  - 25(OH)D < 30 nmol/l
- Definition of deficiency
  - General population, including dark-skinned population, pregnant and lactating women and older adults with history of nontraumatic fracture
- At risk population
  - Special at risk groups: pregnant and lactating women, obese persons, dark-skinned populations, older adults with history of nontraumatic fracture

**Adequate intake:** When the evidence base is insufficient for development of the EAR (estimated average intake=median intake needs of the population)/RDA, an adequate intake (AI) level was estimated instead.

**Recommended dietary allowance (RDA):** Intake that covers the needs of 97.5% of the population, corresponding to a serum 25-hydroxyvitamin D level of at least 20 ng/ml (50 nmol/liter). RDAs for vitamin D were derived based on conditions of minimal sun exposure due to wide variability in vitamin D synthesis from ultraviolet light and the risks of skin cancer.
of activities have been extensively studied in VDR-knockout mice. We know that VDR is widely expressed in mammals and men, that there is extra renal production of 1,25(OH)2D by CYP27B1, and that multiple genes (about 3% of the mouse and human genome) are regulated by VDR all suggesting a more widespread function of vitamin D. For example, VDR-deficient mice develop total alopecia, increased sensitivity to autoimmune diseases, more susceptibility to chemo carcinogenic tumors, high renin hypertension, cardiac hypertrophy and increased thrombogenicity. In humans with vitamin D resistance, generalized alopecia has been observed as well. Moreover, there are many epidemiological links between vitamin D deficiency in humans and colon cancer, breast cancer, prostate cancer, and autoimmune diseases such as type 1 diabetes and multiple sclerosis. Vitamin D deficiency has been associated with an increased cardiovascular risk and the metabolic syndrome as well, but causality has to be proven by intervention studies. (36)

1.6 Vitamin D and type 2 diabetes mellitus

As earlier mentioned, much knowledge about vitamin D metabolism and function has been obtained by animal models. This applies also to the relation between vitamin D and diabetes and the metabolic syndrome. An impaired insulin secretory capacity was found in mice lacking a functional VDR. (37) Treatment with 1,25 dihydroxyvitamin D3 improved de novo insulin biosynthesis in rats.(38) Further evidence on this topic came from a large number of human cross-sectional studies reporting an inverse association between vitamin D status and insulin resistance (39;40), the metabolic syndrome (41;42) and diabetes (43), with similar results from longitudinal observational studies. (44-46) However, intervention studies have shown conflicting results, so causality has not been proven. (47) In chapter 7, the general discussion, the intervention studies will be extensively summarized.

1.7 Vitamin D and physical performance

Many observational studies found a clear link between vitamin D status and muscle strength, and physical performance (48-50) and an inverse association with risk of falls (51), especially in the elderly. In some clinical trials, vitamin D supplementation in older adults with vitamin D deficiency resulted in improvements of muscle performance (52) and reduction of falls (53;54), while other trials showed no effect or an increase of fall incidence in an Australian study with a large supplementation dose once per year.(55;56) The underlying mechanisms can be caused by an indirect effect via calcium and phosphate on muscle strength or directly via activation of the VDR in muscle cells by 1,25(OH)2D (57). VDR activation regulates transcription of genes involved in calcium handling and muscle cell differentiation and proliferation. Also membrane-associated VDR activates intracellular pathways involved in calcium handling, signaling and myogenesis. (58;59)
Chapter 1

1.8 Testosterone and vitamin D

VDRs have been observed in the human male reproductive system. Also vitamin D metabolizing enzymes are expressed in the male reproductive tract. (60) Furthermore VDR is found in the male reproductive system in rats while VDR knockout mice present with gonadal insufficiency. (61;62) In recent literature low serum levels of 25(OH)D have been associated with lower testosterone levels in males. (63;64) One randomized trial found (65) an increase in serum testosterone concentration after vitamin D supplementation with 3332 IU/d for 1 year, but another study did not find such an effect (vitamin D supplementation dose 20,000-40,000 IU/wk for 6 months to 1 year). (66) In conclusion, the main question is whether the positive association between vitamin D and testosterone is causal or not, and randomized trials are needed to answer this question.

1.9 Osteocalcin and type 2 diabetes mellitus

Osteocalcin is a non-collagenous bone matrix protein synthesized by osteoblasts during bone formation. It plays a role during bone mineralization and it is used as a biochemical marker of late phase osteoblastic bone formation. Osteocalcin undergoes vitamin K dependent posttranslational γ-carboxylation (adding three carboxyl groups), which facilitates the binding to hydroxyapatite and retention in the bone. Both carboxylated and un(der)carboxylated forms of osteocalcin are present in the circulation, and are measured as plasma total osteocalcin. (67) Currently, there is some evidence that osteocalcin plays a role in glucose regulation and fat metabolism, linking bone metabolism with energy metabolism. (68;69) It was found in mice that osteocalcin was involved in glucose metabolism by pancreatic β-cell proliferation and increasing insulin secretion and improving insulin sensitivity by up regulating the expression of insulin-sensitizing adipokines in adipocytes. Figure 2 shows a schematic overview. Subsequent human studies showed a negative association between serum osteocalcin and plasma glucose and body fat mass. (70) Furthermore, a positive association between serum osteocalcin and insulin secretion (71), insulin sensitivity (70-72) and serum adiponectin concentration have been described in epidemiological studies. (72) However, the relationship between energy expenditure and bone is still controversial and the data from humans studies are scant. Above all, osteocalcin is an interesting new marker for further research in relation to diabetes and the metabolic syndrome. Further research is needed to answer the question whether un(der)carboxylated osteocalcin is the active form in humans, or the carboxylated form. Animal and in vitro data suggest that only the un(der)carboxylated form of osteocalcin influences glucose homeostasis and energy metabolism. (68;73) In contrast, carboxylated osteocalcin was associated with insulin resistance in humans, not un(der)carboxylated osteocalcin. (72)
Aim and outline of the thesis

Type 2 diabetes has become a significant global health care problem, with increased morbidity and mortality, which requires innovative approaches for prevention. The main object of this thesis was to investigate the role of vitamin D in glucose metabolism, and to investigate if vitamin D supplementation could improve beta cell function and insulin sensitivity, and thereby contribute to the prevention of type 2 diabetes mellitus. Furthermore, the association between the bone marker osteocalcin and the metabolic syndrome was explored.

In Chapter 2 the association between vitamin D and the metabolic syndrome was investigated in a large population study in the Netherlands, the Longitudinal Aging Study Amsterdam (LASA).

The aim of the main study described in this thesis was to test the hypothesis that vitamin D supplementation has a positive effect on insulin sensitivity in non-western immigrants in the Netherlands. To this end, a randomized, placebo-controlled trial was performed in vitamin D deficient, non-western immigrants at high risk for diabetes. The title of the trial was “The effect of vitamin D supplementation on insulin sensitivity in non-western immigrants in the Netherlands”. Non-western immigrants, with a serum 25(OH)D concentration below 50 nmol/l, an impaired fasting or random glucose and a body mass index (BMI) above 27 kg/m² were randomly assigned to receive either cholecalciferol 1200 IU and calcium 500 mg once daily or placebo combined with calcium 500 mg once daily during 16 weeks. Before and after the intervention an oral glucose tolerance test was performed to investigate β-cell function and insulin sensitivity. This study is described in Chapter 3. The secondary outcomes regarding physical activity and physical performance in this study are described in Chapter 4.

In Chapter 5 the relation between vitamin D and testosterone was explored to gain insight in possible effects of vitamin D on male fertility.

Chapter 6 describes the association between the bone marker osteocalcin and the metabolic syndrome in the LASA study.

In Chapter 7 the results are summarized and discussed and placed in context of the existing literature.

Figure 2. Interactions between bone and adipocytes, pancreas and muscle mediated by undercarboxylated osteocalcin (adapted from (74))
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