Summary, discussion and future perspectives
Summary

This thesis contains an array of studies addressing the role of vitamin D in non-skeletal health, i.e. the metabolic syndrome, glucose tolerance, physical activity, testosterone, and also provides a search towards a possible prevention strategy for diabetes mellitus in the future. The rationale for the studies was the fact that diabetes mellitus type 2 has become a serious global health problem with the expectation that without effective prevention strategies, the burden will continue to increase globally. We focused primarily on the elderly and non-western immigrants in the Netherlands, because the prevalence of diabetes and the metabolic syndrome is high among these populations.

Recently, much attention is being given to the relation of vitamin D status to diabetes mellitus. Several epidemiological and clinical studies suggest an increased risk of type 2 diabetes mellitus (DM2) among persons with vitamin D deficiency. At present, a debate on the possible causal relation between vitamin D and diabetes is going on.

In chapter 2 we assessed the association between vitamin D status and the metabolic syndrome in the elderly (> 65 year) in the Longitudinal Aging Study Amsterdam (LASA) study, an ongoing cohort study in a representative sample of Dutch older persons. A total of 1286 subjects participated in the study. The metabolic syndrome was observed in more than one third of the study participants, and the average serum 25(OH)D level was 53.3 ± 24.1 nmol/l. A serum 25(OH)D level below 50 nmol/l was associated with an increased risk of the metabolic syndrome, compared to serum 25(OH)D levels above 50 nmol/l, after adjustment for confounders. The adjusted odds ratio was 1.29 (95% CI 1.00-1.68).

In chapter 3 the results of a randomized, placebo-controlled trial in non-western immigrants, with vitamin D deficiency and at high risk for diabetes (n=130) were reported. In this trial, the effect of vitamin D supplementation on insulin sensitivity was investigated. We hypothesized that vitamin D supplementation could have a positive effect on insulin resistance and β-cell function in people with vitamin D deficiency and at risk for diabetes. The subjects included in this trial had fasting glucose levels > 5.5 mmol/l or random glucose levels of 7.8-11.1 mmol/l and 25(OH)D < 50 nmol/l. Furthermore, the participants had a BMI > 27 kg/m2 and the mean serum 25(OH)D level at baseline was 23.4 nmol/l ± 10.7. Daily supplementation with 1200 IU cholecalciferol in combination with 500 mg calcium did not have an effect on glycaemia and insulin sensitivity parameters, measured with oral glucose tolerance testing after 4 months of intervention, compared to the control, who received placebo and 500 mg calcium. The lack of effect was not in line with our initial hypothesis. However, in a post-hoc analysis, when patients with diabetes at baseline were excluded, a significant increase in insulinogenic index was observed in participants who obtained a 25(OH)D concentration of ≥ 60 nmol/l after intervention. (P=0.040) (27 subjects in the intervention group, 54 subjects in the placebo group)

In chapter 4 the influence of vitamin D supplementation on physical activity and per-
performance in the same randomized-placebo controlled clinical trial is described. The percentage of participants meeting the international physical activity guidelines in this trial was extremely low, less than 10%. There was no effect of 1200 IU cholecalciferol on the different physical performance and activity scores. These scores included the physical performance score (calculated from the tandem test, the chair stand test and walking test), exercise capacity (six minutes walk test, 6-MWT) and daily physical activity (questionnaire and accelerometer). In a post-hoc analysis restricted to participants reaching a serum 25(OH)D level of > 60 nmol/l after intervention, there was a non-significant effect (P=0.053) of 19 meters increase of the walking distance measured with 6-MWT in the intervention group. The clinical importance of this increase has not been validated in this specific population. In postoperative subjects, a clinical meaningful difference of 19 meter between groups has been reported earlier.(1)

In chapter 5 the association between vitamin D status and testosterone levels in men is presented on the basis of data from three earlier vitamin D trials, including data of men from our randomized trial. In addition, the effect of vitamin D supplementation on testosterone levels is presented in this chapter. Study 1 consisted of 92 men with heart failure who received 2000 IU of vitamin D or placebo for 6 weeks. In study 2, 49 vitamin D deficient men received either 600 IU of vitamin D or placebo for 4 months. In study 3 (our randomized trial), 43 vitamin D deficient non-western immigrant men received 1200 IU of vitamin D or placebo for 16 weeks. There was an association between vitamin D status and serum testosterone at baseline in agreement with the existing literature, but vitamin D supplementation did not alter testosterone levels in the three different studies.

In chapter 6 we investigated the association between the bone formation marker osteocalcin and the metabolic syndrome in the LASA cohort study. Our hypothesis was that low plasma osteocalcin levels are associated with a higher risk for the metabolic syndrome. Plasma osteocalcin was clearly, inversely associated with the metabolic syndrome with an adjusted odds ratio of 3.69 (95% CI 2.53-5.34) for the lowest osteocalcin quartile compared to the subjects in the highest osteocalcin quartile, which is in line with our hypothesis and is in accordance with recent literature.

In conclusion, we found a clear association between vitamin D status and the metabolic syndrome, but no clear effect of vitamin D supplementation on insulin sensitivity parameters, except a possible small effect on insulin secretion in subjects with the most explicit increase in 25-hydroxyvitamin D levels. Further research is needed to prove a possible causal relationship. Furthermore, we found an association between the bone formation marker osteocalcin and the metabolic syndrome, which is a base for new research strategies. Next to this, we explored the effects of vitamin D supplementation on physical activity and testosterone levels in male subjects, which revealed minor effects on 6-MWT and no effect on testosterone levels.
General discussion

Vitamin D and insulin sensitivity and secretion

Vitamin D plays a crucial role in the development and maintenance of the skeleton throughout life. Besides this, in the past years, vitamin D has been associated with many non-skeletal outcomes, i.e. autoimmune diseases, some types of cancer, type 2 diabetes mellitus, cardiovascular disease, infectious diseases and depression. (2) A potential role of vitamin D in diabetes was suggested by Campbell et al. (3) by observing a seasonal variation in glycaemic control in young men in the Antarctic with glycaemic control being worse in winter. Additional evidence for a role of vitamin D in diabetes mellitus type 2 came from animal models (4;5) and a large number of cross-sectional studies, most studies showing a consistent association between vitamin D status and insulin sensitivity (6-9) and variable associations between vitamin D status and insulin secretion. (6-8;10;11) In line with this, we found a clear association in the elderly between serum 25(OH)D and the metabolic syndrome in the LASA cohort, described in chapter 2. Moreover, longitudinal cohort studies also showed a relation between vitamin D status and incident metabolic syndrome (12) and diabetes (13;14). To prove causality, randomized controlled intervention trials have been set up.

The earliest intervention studies examining the effect of vitamin D supplementation on glycaemic control were small and not randomized and showed varying results from no effect on insulin secretion and sensitivity to improved insulin secretion and/or sensitivity. (10;15-20) Some studies (18;21) using the active metabolite 1,25(OH)2D instead of cholecalciferol supplementation, did not show any effect on insulin secretion. The pancreas has the ability to produce 1,25(OH)2D by 1α-hydroxylation in the beta cells which needs 25(OH)D as a substrate, and possibly this pathway could be more important. (22) Vitamin D might be preventative in prediabetes, but not curative when diabetes already is present. The recently diagnosed DM2 subjects in the trial of Orwoll et al. did show a tendency towards a better insulin secretion response (21), supporting the conception that vitamin D supplementation will only be useful in prediabetes and early diabetes, when the β-cell is not yet exhausted. In accordance with this, Jorde et al. did not find any effect of high dose vitamin D supplementation on insulin secretion and insulin sensitivity in established DM2 subjects, (23) similar to Witham et al. (24) and two other studies. (25;26) Pittas et al. showed in a post-hoc analysis in a bone-related trial improvements in HOMA and fasting glucose only in the subjects with impaired glucose tolerance and not in normal glucose tolerant subjects. (27) In line with this, trials including normal glucose tolerant participants did not show changes after vitamin D supplementation. (28-31) In conclusion, it appears that vitamin D supplementation may only improve glucose metabolism in prediabetes and glucose intolerant subjects. The question remains which dosage of vitamin D supplementation and at which serum 25(OH)D level any effect may be suspected.
The earlier randomized placebo-controlled trials did not show any effect on insulin sensitivity. (32-34) More recently, a few randomized trials (23-31;35-39) on this topic have been performed, but these were heterogeneous in study population, study duration, varying from 4 weeks to 6 years, in doses of vitamin D supplementation varying from 400 IU/day to 88863 IU/week to large infrequent doses of vitamin D (23;24;26;36). Two trials have reported beneficial effects on insulin sensitivity (35;36), but most other trials did not report any effect. The trial from New Zealand, found an effect on insulin sensitivity in insulin resistant subjects, most explicitly in the women reaching a 25(OH)D level of 80 nmol/l after 6 months of vitamin D supplementation, but not after 3 months.(35) In contrast, Harris et al. (38) found a significant decrease in insulin sensitivity in the vitamin D supplemented group. Insulin secretion was not affected by vitamin D supplementation in the majority of studies, increased in a minority of studies (37;38;40), and decreased in one study (41). In agreement with most previous trials, the latest trial from Australia did not observe improvements in insulin sensitivity and secretion, but in a post-hoc analysis restricted to prediabetic subjects, there was a beneficial effect on insulin sensitivity with 2000-6000 IU/day.(42) However, the results needs to be interpreted with caution given the post hoc nature of the analysis.

Next to heterogeneity in study population, duration and dosing, some trials combined vitamin D with calcium supplementation (27-29;37), while others did not. In addition, there is heterogeneity in outcome parameters. Studies (27;28;31), using fasting insulin and glucose (which reflects hepatic insulin sensitivity) generally did not reveal any effect of vitamin D supplementation. However, the study of Nagpal et al studied peripheral insulin sensitivity and showed significant improvements in 3-hour OGTT-derived insulin sensitivity. (36) Finally, there are quite a few studies (23;27;29;37;39) investigating populations without vitamin D deficiency. Vitamin D supplementation of vitamin D sufficient subjects might not be useful. In conclusion, the randomized trials are difficult to compare and heterogeneous and therefore, it is difficult to perform a meta-analysis. (43) However, in the past two years, some systematic reviews and meta-analyses have been reported. (43-46) Mitri et al. concluded some beneficial effects in patient with glucose intolerance, Seida et al. did not find any effect of vitamin D3 supplementation on glucose homeostasis or diabetes prevention, but in accordance with the above mentioned limitations, definite conclusions may be limited.

Another variable that could possibly explain the diverging results in the studies, could be the presence of vitamin D receptor (VDR) polymorphisms and polymorphisms in genes affecting vitamin D and glucose metabolism. In recent literature, certain VDR polymorphisms have been associated with low response to vitamin D supplementation in terms of improvement in serum 25(OH)D concentrations, insulin sensitivity and inflammation. (47;48) Other genetic variants affecting glucose homeostasis (i.e IRS-1) and vitamin D status (affecting vitamin D binding protein concentration and vitamin D metabolite catabolism) could also alter response to treatment. (49)
If one wants to formulate an overall conclusion, then it could be concluded that most studies showed no effect of vitamin D supplementation on insulin sensitivity. In Table 1 and Table 2, an overview of most intervention studies is displayed. Overall, the randomised trials did not show beneficial effects on glucose tolerance in normal glucose tolerant subjects or diabetic subjects and some small inconsistent effects in glucose intolerant subjects.\(^{(43)}\) While the results of cross-sectional reports were very promising, the results of the randomized trials investigating the effect of vitamin D supplementation on glucose tolerance are disappointing to date.

Our trial, described in chapter 3 did not reveal any effect on glucose, insulin and insulin sensitivity outcomes as well. However, in a post-hoc analysis, some effect on β-cell function was observed in subjects reaching a serum 25(OH)D level of 60 nmol/l or higher after intervention and after exclusion of diabetic subjects. It was of particular interest, because in contrast to most other trials, we investigated vitamin D deficient subjects. At the moment, there is evidence from literature, including our own research for a clear association between vitamin D and diabetes. However, the effects of vitamin D supplementation are not convincing. There are many possible reasons for the discrepancy between the positive association studies and the negative randomized controlled trials. When considering the results of our trial in particular, some remarks can be made. The lack of effect of the vitamin D supplementation could be due to an insufficient supplementation dose. To date, the vitamin D experts worldwide do neither agree on the supplementation dose, nor on the optimal 25(OH)D levels, as was explained in the general introduction. Many experts advice to supplement with higher vitamin D supplementation doses for various reasons. However, Davidson et al. investigated 117 prediabetes subjects with a mean weekly dose of 88,865 IU for 1 year and did not find significant effects on insulin secretion and insulin sensitivity, but in this study the average baseline 25(OH)D level of 20 ng/ml (50 nmol/l) was relatively high. Furthermore, the follow-up time in our trial could be too short, since in the study of von Hurst the effects on insulin sensitivity did not occur until 6 months. However, there are trials with a longer follow-up (29;31;39), that did not reveal any effects, arguing against it. Then, in our trial the subjects should have been glucose intolerant, but after inclusion, several participants appeared diabetic or normal glucose tolerant. This is inherent to screening with random glucose measurements alone, what was demonstrated in a study by Herdzik et al.(50), who showed that a fasting plasma glucose correctly classifies subjects to the impaired glucose tolerant group in only 29% of the cases. Moreover, our study group of non-western immigrants was challenging as is expressed by the large number of screened subjects. It proved difficult to recruit non-western immigrants and perform valid measurements and it was not feasible to perform oral glucose tolerance tests prior to inclusion. Furthermore, euglycaemic clamp procedures are well established for assessing β-cell function and insulin sensitivity, although Stumvoll et al. showed a good correlation \(r=0.6-0.8\) between oral glucose tolerance testing and the
clamp procedure. (51) The clamp procedure was not feasible in this specific study population and oral glucose tolerance testing was the maximum that could be obtained. Another reason for the lack of effect could be the lack of power. We observed a wide variability in outcome parameters within the groups. Finally, the reason could be that there is no causal relationship between vitamin D and diabetes, and the association originates from other common lifestyle factors, such as sedentary and dietary lifestyle. Vitamin D deficiency could be a marker of frailty, as vitamin D sufficiency is associated with younger age, lower body weight and healthy lifestyle. (52;53) Otherwise, when there is an effect, this effect is probably rather small; this fact has already been proven by the trials performed before. Although there are limitations in all trials, concerning study population (NGT/IGT/DM2), baseline serum 25(OH)D levels, vitamin D dose, duration, sample size, the effect probably is not major; otherwise the results would have been more convincing at this time. So for the future, it will be important to perform the ‘perfect trial’ with a sufficient number of participants, a high supplementation dose, around 2000 IU/d (54), 6 months to a year follow-up, real vitamin D deficient subjects (< 25 nmol/l at baseline) with glucose intolerance (proven with oral glucose tolerance test), using a euglycaemic clamp procedure, to prove whether vitamin D supplementation will be useful to prevent diabetes. In case of a positive effect, it would be worthwhile to investigate new possibly more pancreas-specific vitamin D agents (i.e. paracalcitrol), which could be more potent in relation to diabetes. Nevertheless, vitamin D will be of great importance in optimizing bone health and in preventing fractures in future, but whether vitamin D will play a major role in diabetes prevention in future appears somewhat unlikely. In Table 3, large upcoming trials regarding this subject are displayed. Some of them have a promising design.

Osteocalcin and glucose metabolism

As the results of vitamin D supplementation in diabetes prevention are disappointing, more attention should be focused on other new pathways. In 2011, Clemens and Karsenty discussed the role of the osteoblast targeting glucose homeostasis. (55) The osteoblast produces osteocalcin and osteocalcin is subject to posttranslational (vitamin K dependent) carboxylation, which gives osteocalcin more affinity to hydroxyapatite. The less carboxylated (undercarboxylated) osteocalcin enters the circulation more easily and in mice models, this undercarboxylated osteocalcin acts as a hormone in glucose homeostasis and energy expenditure, which can alter pancreatic β-cell proliferation, insulin secretion and insulin sensitivity. Osteocalcin knockout mice, which have been studied in the context of bone formation before, were obese and showed hyperglycaemia, low insulin sensitivity and low energy expenditure. More recently, intervention with intermittent injections of osteocalcin improved glucose metabolism in mice. (56) This gives possibilities for new research directions, also for clinical purposes, to discover a possible new endocrine axis involving bone and glucose metabolism.
First of all, the role of osteocalcin in humans should be examined. The role of undercarboxylated osteocalcin in humans has still not been clarified, because most studies measured total osteocalcin (which comprises undercarboxylated osteocalcin and carboxylated osteocalcin), or intact osteocalcin instead of undercarboxylated osteocalcin. In humans, total osteocalcin is inversely associated with body fat, dysmetabolic markers (57), diabetes, glucose (58) and the metabolic syndrome as we observed in the LASA study population reported in chapter 6. This is in concordance with the findings in the mice models. Among diabetic patients, higher total osteocalcin levels correlated with better glycaemic control.(59) Moreover, weight loss can induce higher total osteocalcin levels. (60) Remarkably, Shea et al. did not find in a study of 348 non-diabetic subjects aged 68 years, an association between HOMA-IR and circulating undercarboxylated osteocalcin, but they did observe an association with total osteocalcin. (61) On the contrary, Hwang et al. studied 199 men aged 47 years and showed a positive association between β-cell function and undercarboxylated osteocalcin.(62) Kanazawa et al. reported that both forms of osteocalcin were inversely related to blood glucose level in type 2 diabetes.(63) At the moment, it is unclear whether the lack of a robust assay for undercarboxylated osteocalcin has contributed to the conflicting results and this needs to be clarified with a robust assay in future. When undercarboxylated osteocalcin is the active hormone as well in humans as in mice, the underlying (patho)physiology has to be better clarified.

Furthermore, older humans are at risk of diabetes and cardiovascular disease and have lower bone density and as a consequence higher risk of fractures. The role of osteocalcin is of particular interest in this setting, leading to some questions. Can osteocalcin possibly play a role in treatment of osteoporosis and diabetes in future? What are the effects of existing therapies, for example antiresorptive therapies? The effects of antiresorptive therapies in humans are interesting, because of their widespread use. It was expected that a decrease in undercarboxylated osteocalcin caused by bisphosphonate treatment would have a detrimental effect on glucose homeostasis. From literature it is known that bisphosphonates and denosumab did indeed reduce total OC (64-66) and alendronate reduced undercarboxylated OC by 56%. (67) However, more recently three trials of antiresorptive therapies did not show any effects on glucose metabolism, body weight or diabetes incidence (OC and uOC were not measured in these specific trials), so the clinical importance has not been demonstrated. (68) One important difference between osteocalcin knockout mice and human studies with antiresorptives, is the difference in suppression of osteocalcin levels. In human studies there is a marked reduction in osteocalcin levels with antiresorptive therapy, in contrast to the absence of osteocalcin in osteocalcin knockout mouse models.

As it is known that vitamin K is essential for the carboxylation of glutamic acid residues in osteocalcin, vitamin K metabolism is also of particular interest in investigating the relation between osteocalcin and glucose metabolism. Vitamin K deficiency increases un-
dercarboxylated osteocalcin concentration, while supplementation of vitamin K showed a decline in undercarboxylated osteocalcin levels. (69) It was expected that supplementation of vitamin K could have negative effects on glucose metabolism and that coumarine-derivatives (inhibitor of vitamin K dependent carboxylase) possibly could have positive effects on glucose metabolism. Several randomized controlled trials demonstrated that vitamin K supplementation decreased undercarboxylated osteocalcin, but the effects on glucose metabolism were diverse, from positive effects in younger men (70) and older men (71), to no effects in women.(71;72)

Vitamin D and osteocalcin
To gain more insight in the protective effects of osteocalcin on metabolic parameters, we performed an additional analysis in our randomized controlled trial. As it is known from literature and from the studies presented in this thesis that both lower serum osteocalcin levels and lower serum 25(OH)D levels are associated with a higher risk for the metabolic syndrome, we hypothesized that vitamin D could possibly have its effects on the beta-cell and insulin sensitivity by influencing osteocalcin levels, besides the direct effect of vitamin D on the β-cell and insulin sensitivity. The active vitamin D metabolite is known to increase osteocalcin by increasing osteocalcin gene expression. (73) In addition, this active vitamin D metabolite promotes osteoblast maturation, and osteocalcin is produced by the mature osteoblast. (74) Higher osteocalcin levels could have a protective effect for diabetes. In a posthoc analysis using data of our randomized trial, however, we observed decreasing osteocalcin levels in both the vitamin D and the placebo group (data not shown). This was also observed in the recent trial in Australia. (42) Total osteocalcin levels were measured, as well as undercarboxylated osteocalcin. We observed a similar decrease for both parameters in both study groups, which is not in line with our initial hypothesis. For the moment, it is more likely that vitamin D lowers serum osteocalcin levels by its effects on bone turnover.
### Table 1. Effects of vitamin D supplementation on insulin secretion. To convert nmol/l to ng/ml, divide by 2.496. Randomized-placebo controlled trials are depicted in bold.

<table>
<thead>
<tr>
<th>Author, Year (Ref)</th>
<th>Population</th>
<th>25(OH)D level nmol/l Mean or median</th>
<th>Vitamin D dose &amp; duration</th>
<th>Determinant of glucose/insulin metabolism</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gedik et al. 1986 (16)</td>
<td>4 women with osteomalacia (Turkey)</td>
<td>Mean age: 33 yr Mean BMI: 23 kg/m²</td>
<td>Oral, 2000 IU/d cholecalciferol 6 months</td>
<td>OGTT</td>
<td>Increased insulin area under the curve (AUC) and insulinogenic index</td>
</tr>
<tr>
<td>Inomata et al. 1986 (17)</td>
<td>14 Japanese DM2</td>
<td>Mean age: 54 yr</td>
<td>2 μg/d alphacalcidol versus control (n=7) 3 weeks</td>
<td>OGTT</td>
<td>Improved insulin secretion (AUC)</td>
</tr>
<tr>
<td>Zofkova and Stolba 1990 (18)</td>
<td>13 vitamin D-sufficient adults in Czechoslovakia</td>
<td>Mean age: 33 yr</td>
<td>Oral, 3 μg/d 1,25(OH)₂D₃ 4 days</td>
<td>IVGTT</td>
<td>No change in insulin secretion</td>
</tr>
<tr>
<td>Orwoll et al. 1994 (21)</td>
<td>20 DM2 (US)</td>
<td>Mean age: 61 yr Mean BMI: 30 kg/m²</td>
<td>1 μg/d 1,25(OH)₂D₃ versus placebo 4 days</td>
<td>Meal challenge</td>
<td>No change (tendency towards better insulin secretion in recently diagnosed subjects)</td>
</tr>
<tr>
<td>Boucher et al. 1995 (10)</td>
<td>22 glucose-intolerants East London Asians</td>
<td>Mean age: 45 yr Mean BMI: 26 kg/m²</td>
<td>9.0±4.5 → 33.7±18.5 Intravenously 100,000 IU (once) cholecalciferol 8-12 weeks follow-up</td>
<td>OGTT</td>
<td>Increase in postchallenge insulin and C-peptide</td>
</tr>
<tr>
<td>Borissova 2003 (19)</td>
<td>10 Bulgarian women DM2</td>
<td>Mean age: 54 yr Mean BMI: 31 kg/m²</td>
<td>Oral, 1332 IU/d cholecalciferol 1 month</td>
<td>IVGTT</td>
<td>Increased first-phase insulin secretion</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Weekly Vitamin D Dose</td>
<td>Comparator</td>
<td>Study Duration</td>
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<tr>
<td>Jorde et al. 2009</td>
<td>32 Norwegian adults, DM2 with metformin and insulin</td>
<td>Age: 21-75 yr; Mean BMI: 32 kg/m²</td>
<td>40,000 IU/wk cholecalciferol</td>
<td>placebo</td>
<td>6 months</td>
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<tr>
<td>Nagpal et al. 2009</td>
<td>71 Asian-Indian (India) centrally-obese men</td>
<td>Age≥35 yr; Mean BMI: 27 kg/m²</td>
<td>Oral, 3 doses of 120,000 IU cholecalciferol</td>
<td>versus placebo</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Tarcin et al. 2009</td>
<td>23 young healthy volunteers in Turkey</td>
<td></td>
<td>IM, 300,000 IU/mth</td>
<td>versus placebo</td>
<td>3 months</td>
</tr>
<tr>
<td>Von Hurst et al. 2010</td>
<td>77 Insulin resistant South Asian women in New Zealand</td>
<td>Age 21-40yr; Mean BMI: 27.5 kg/m²</td>
<td>Cholecalciferol versus placebo</td>
<td>HOMA-β</td>
<td>6 months</td>
</tr>
<tr>
<td>Parekh et al. 2010</td>
<td>28 Asian Indian (India) DM2, diet or oral glucose lowering agents</td>
<td>Age 37→103.7 yr; Mean BMI: 27 kg/m²</td>
<td>Cholecalciferol versus placebo</td>
<td>HOMA-β</td>
<td>6 weeks</td>
</tr>
<tr>
<td>Grimnes et al. 2011</td>
<td>94 NGT (Norway)</td>
<td>Age 39.2±12.1 yr; Mean BMI: 27 kg/m²</td>
<td>Oral, 20,000 IU 2/wk cholecalciferol</td>
<td>Versus placebo</td>
<td>6 months</td>
</tr>
<tr>
<td>Study</td>
<td>Patients Description</td>
<td>Mean Age</td>
<td>Mean BMI</td>
<td>Treatment Duration</td>
<td>Treatment Details</td>
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<tr>
<td>Mitri et al. 2011 (37)</td>
<td>88 IFG/IGT or DM2 on diet (US)</td>
<td>57 yr</td>
<td>32 kg/m²</td>
<td>4 months</td>
<td>Cholecalciferol 2000 IU/d, calcium 800 mg/d (VitD/Calcium, Vit D/ Plac, Calcium/Plac/plac)</td>
</tr>
<tr>
<td>Nazarian et al. 2011 (41)</td>
<td>8 IFG (US)</td>
<td>51.4 → 111.8</td>
<td></td>
<td>1 month</td>
<td>10,000 IU/d</td>
</tr>
<tr>
<td>Harris et al. 2012 (38)</td>
<td>89 overweight African Americans</td>
<td>40 → 81</td>
<td></td>
<td>12 weeks</td>
<td>Cholecalciferol</td>
</tr>
<tr>
<td>Davidson et al. 2013 (39)</td>
<td>109 prediabetes</td>
<td>55 → 175</td>
<td></td>
<td>12 months</td>
<td>Mean dose: 88,865 IU/wk</td>
</tr>
<tr>
<td>Oosterwerff et al. 2013 (40)</td>
<td>110 prediabetes</td>
<td>22 → 23</td>
<td></td>
<td>16 weeks</td>
<td>Oral, 1200 IU/d</td>
</tr>
<tr>
<td>Gagnon et al. (42)</td>
<td>80 multi-ethnic</td>
<td>47 → 95</td>
<td></td>
<td>6,000 IU</td>
<td>Cholecalciferol 2,000-6,000 IU</td>
</tr>
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</table>
Table 2. Effects of vitamin D supplementation on insulin sensitivity. To convert nmol/l to ng/ml, divide by 2.496. Randomized-placebo controlled trials are depicted in bold.

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<th>Determinant of glucose/insulin metabolism</th>
<th>Results</th>
</tr>
</thead>
</table>
| Nilas et al. 1984 (32) | 151 Danish healthy postmenopausal women  
Age: 45-54 yr  
BMI:- | - | Oral, 2000 IU/d cholecalciferol versus 0.25 μg/d alfacalcidol versus placebo  
All received calcium 500 mg/d, 2 years | Blood glucose | No change |
| Nyomba et al. 1986 (15) | 10 Belgian subjects with epilepsy and 15 elderly  
Mean age: 56 yr  
Mean age: 78 yr | 17±3→36±6  
19±3→35±3 | Oral, 200 μg loading + 10 μg/d  
2 weeks | OGGTT | Decrease in fasting insulin and postchallenge insulin in subjects with epilepsy |
| Ljunghall et al. 1987 (33) | 65 vitamin D sufficient, Caucasian men with IGT  
Age: 61-65 yr  
Mean BMI: 28 kg/m2 | 92.4±23.5→104.8±20.7 (treatment)  
97.3±72.4→134.8±119.8 (placebo) | Oral, 0.75 μg/d alfacalcidol versus placebo  
3 months | IVGTT | No change in insulin sensitivity |
| Lind et al. 1989 (20) | 14 normal-weight, Danish men with IGT  
Age: 60-63 yr | - →78±43 | Oral, 2 μg/d alfacalcidol  
18 months | IVGTT | No change in insulin sensitivity |
| Fliser et al. 1997 (34) | 18 healthy German men  
Mean age: 26 yr  
Mean BMI: 22 kg/m2 | - | Oral, 1.5 μg/d  
1,25(OH)D versus placebo  
7 days | Euglycemic clamp | No change in insulin sensitivity |
<table>
<thead>
<tr>
<th>Study</th>
<th>Sample Description</th>
<th>Study Design</th>
<th>Treatment</th>
<th>HOMA-IR</th>
<th>Comments</th>
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<td>Borissova 2003 (19)</td>
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<td>Oral, 1332 IU/d cholecalciferol 1 month</td>
<td>35.3±15.1→63.3±31</td>
<td>Non-significant decrease in HOMA-IR</td>
<td></td>
</tr>
<tr>
<td>Pittas et al. 2007 (27)</td>
<td>314 Caucasian American adults</td>
<td>Oral, 700 IU/d cholecalciferol +500 mg calcium versus placebo 1 month</td>
<td>71±5→102 (NFG)</td>
<td>Improved HOMA and FPG in IFG subjects</td>
<td></td>
</tr>
<tr>
<td>Boer et al. 2008 (28)</td>
<td>33,951 Postmenopausal women of different ethnicities</td>
<td>Oral, 400 IU cholecalciferol+1000 mg calcium versus placebo 7 yr</td>
<td>43.7→ - (NFG)</td>
<td>No change in HOMA-IR, secretion, or diabetes risk</td>
<td></td>
</tr>
<tr>
<td>Sugden et al 2008 (26)</td>
<td>34 DM2</td>
<td>Oral, once D2 100,000 IU versus placebo 8 weeks</td>
<td>38.3→61.2</td>
<td>No change in Hba1c, HOMA-IR. Significant improvement HOMA-IR if 25(OH)D rise&gt;11nmol/l</td>
<td></td>
</tr>
<tr>
<td>Tai et al. 2008 (76)</td>
<td>33 primarily Caucasian adults</td>
<td>Oral, 2 doses of 100,000 IU cholecalciferol 1 month</td>
<td>39.9±8.6→90.3±4.3</td>
<td>No change in HOMA-IR, Quicki, Avignon’s insulin sensitivity</td>
<td></td>
</tr>
<tr>
<td>Tarcin et al. 2009 (75)</td>
<td>23 young healthy volunteers</td>
<td>Oral, once IM, 300,000 IU/mth 3 months</td>
<td></td>
<td>No change in HOMA-IR, Matsuda’s modelling of OGTT</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Intervention</th>
<th>Follow-up</th>
<th>Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorde et al. 2009 (23)</td>
<td>32 Norwegian adults, DM2 with metformin and insulin</td>
<td>60±14 →118 (treatment)</td>
<td>40,000 IU/wk cholecalciferol versus placebo 6 months</td>
<td>No change in HOMA-IR, HbA1c</td>
</tr>
<tr>
<td>Age: 21-75 yr Mean BMI: 32 kg/m2</td>
<td>59±21 →57 (placebo)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Nagpal et al 2009 (36)</td>
<td>100 Asian-Indian centrally-obese men</td>
<td>36.5±14.6→72</td>
<td>Oral, 3 doses of 120,000 IU cholecalciferol versus placebo 6 weeks</td>
<td>Increased insulin sensitivity (3-hour oral glucose insulin sensitivity index) by Mari’ modelling of OGTT</td>
</tr>
<tr>
<td>Age: 35 yr Mean BMI: 27 kg/m2</td>
<td>30±12.5→31</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Zitterman et al. 2009 (31)</td>
<td>200 Healthy obese 33 % men Mean age: 48 BMI &gt; 27 kg/m2</td>
<td>30±12</td>
<td>Oral, 3332 IU/d versus placebo. All received weight reduction program 12 months</td>
<td>No change HbA1c, FPG</td>
</tr>
<tr>
<td>Jorde et al. 2010 (29)</td>
<td>330 overweight participants (Norway) 36%M</td>
<td>59→140 (40,000)</td>
<td>Cholecalciferol 40,000 IU/wk versus 20,000 IU/wk versus placebo</td>
<td>No change HbA1c, HOMA-IR, Quicki, FPG</td>
</tr>
<tr>
<td>Age: 49 yr Mean BMI: 34.5 kg/m2</td>
<td>57→101 (20,000)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age: 49 yr Mean BMI: 34.5 kg/m2</td>
<td>59→59 (placebo)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Witham et al. 2010 (24)</td>
<td>61 DM2 (UK)</td>
<td>45</td>
<td>Oral, cholecalciferol 100,000 IU once versus 200,000 IU once versus placebo, 16 weeks</td>
<td>No change in insulin sensitivity</td>
</tr>
<tr>
<td>Mean age: 65 Mean BMI: 31</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
### Table 2. Continued

<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Intervention</th>
<th>Vitamin D dosage</th>
<th>Comparison</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Von Hurst et al 2010 (35)</td>
<td>77 South Asian women in New Zealand</td>
<td>Cholecalciferol versus placebo</td>
<td>4,000 IU/d</td>
<td>6 months</td>
<td>HOMA-IR</td>
</tr>
<tr>
<td>Parekh et al. 2010 (25)</td>
<td>28 Asian Indian (India) DM2, diet or oral glucose lowering agents</td>
<td>Cholecalciferol versus placebo</td>
<td>4,000 IU/d</td>
<td>6 months</td>
<td>HOMA-IR</td>
</tr>
<tr>
<td>Grimnes et al. 2011 (30)</td>
<td>94 NGT (Norway)</td>
<td>Oral, 20,000 IU 2/wk versus placebo</td>
<td>6 months</td>
<td>Hyperglycaemic clamp</td>
<td>No change in insulin sensitivity</td>
</tr>
<tr>
<td>Mitri et al. 2011 (37)</td>
<td>88 IFG/IGT or DM2 on diet (US)</td>
<td>Cholecalciferol 2000 IU/d, calcium</td>
<td>61</td>
<td>FSIVGTT</td>
<td>No change in insulin sensitivity</td>
</tr>
<tr>
<td>Nazarian et al. 2011 (41)</td>
<td>8 IFG (US)</td>
<td>Oral, 10,000 IU/d</td>
<td>1 month</td>
<td>FSIVGTT</td>
<td>Increased insulin sensitivity</td>
</tr>
<tr>
<td>Harris et al. 2012 (38)</td>
<td>89 overweight African Americans</td>
<td>Cholecalciferol 12 weeks</td>
<td>Dose based on body weight and vitamin D level. Mean dose: 88,865 IU/wk</td>
<td>OGGT</td>
<td>Increased insulin sensitivity in placebo group</td>
</tr>
<tr>
<td>Davidson et al. 2013 (39)</td>
<td>109 prediabetes</td>
<td>Cholecalciferol 12 weeks</td>
<td>OGGT</td>
<td>No change in insulin sensitivity</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Intervention</td>
<td>Outcomes</td>
<td></td>
<td></td>
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<tr>
<td>---------------------</td>
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<td></td>
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<tr>
<td>Salehpour et al.</td>
<td>77 women BMI &gt; 25 kg/m²</td>
<td>25 μg/day (1000 IU) versus placebo</td>
<td>No change in insulin sensitivity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oosterwerff et al.</td>
<td>110 prediabetes</td>
<td>Oral, 1200 IU/d Cholecalciferol versus placebo</td>
<td>No change in insulin sensitivity</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Gagnon et al.       | 80 multi-ethnic 25(OH)D < 55 nmol/l Ausdrisk ≥ 15 | Cholecalciferol 2,000-6,000 IU (25(OH)D > 75 nmol/l) versus placebo | No change in insulin sensitivity  
In post-hoc analysis restricted to prediabetes better insulin sensitivity |
Future perspectives

Future studies may be focused on the following subjects: First a randomized placebo-controlled trial, with sufficient power in a vitamin D deficient study population with impaired glucose tolerance or impaired fasting glucose with a vitamin D supplementation dose of at least 2000 IU/d and at least 6 months follow-up is needed to answer the question whether vitamin D supplementation will help to prevent diabetes in future. To date, no studies with this study design have been conducted, but in a few years the results of several clinical trials are expected. In Table 3, there is an overview of upcoming vitamin D studies on the prevention of DM2.

Next to this, studies using new active vitamin D analogues would be interesting. Finally, more studies are needed to clarify the effect of osteocalcin and undercarboxylated osteocalcin on glucose metabolism in humans. It is unclear, whether the genetically modified mouse model can be extrapolated directly to humans. In future, the effect of (recombinant) osteocalcin administration on glucose metabolism in humans in an intervention study might be very interesting, and this has not been reported to date. Another approach to study the osteocalcin effect on glucose metabolism comes from studies intervening in bone metabolism with different agents. Finally, study designs regarding vitamin K metabolism could be an alternative approach to study the osteocalcin effects in humans. Next to this, it is important to investigate additional bone hormones to clarify whether other hormones could influence glucose metabolism next to osteocalcin.
### Table 3. Upcoming intervention trials regarding vitamin D supplementation and diabetes and glucose metabolism, with study size > 150 participants

<table>
<thead>
<tr>
<th>Study center</th>
<th>Population</th>
<th>Intervention &amp; Follow-up</th>
<th>Outcome</th>
<th>Trialregister</th>
<th>Estimated Study Completion date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toronto, Canada</td>
<td>Fasting glucose 5.6-6.9 mmol/l HbA1c 5.4-6.4%</td>
<td>Vitamin D enriched cheese (cholecalciferol 28,000 IU/wk)</td>
<td>OGTT, 2-hr glucose</td>
<td>NCT01726777</td>
<td>2015</td>
</tr>
<tr>
<td></td>
<td>25(OH)D ≤ 65 nmol/l 18-75 yr 160 participants</td>
<td>Versus normal cheese 24 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tromso, Norway</td>
<td>21-80 years</td>
<td>IGT 600 participants</td>
<td>20,000 IU/wk Versus placebo 5 years</td>
<td>NCT00685594</td>
<td>2016</td>
</tr>
<tr>
<td>Tufts medical Center The United States D2D study</td>
<td>Fasting glucose 5.6-6.9 mmol/l 2-hr gluc. 7.8-11.0 mmol/l HbA1c 5.7-6.4%</td>
<td>4000 IU/d Versus placebo 4 years</td>
<td>Time to development of diabetes</td>
<td>NCT01942694</td>
<td>2017</td>
</tr>
<tr>
<td></td>
<td>2382 participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beiroet Lebanon GEHF-VitD</td>
<td>65-95 yr</td>
<td>BMI ≥ 25 kg/m2 258 participants</td>
<td>1000 mg/d calcium, 500 IU/d cholecalciferol and 20,000 IU/wk for one year versus 1000 mg/d calcium, 500 IU/d cholecalciferol and placebo 1 year</td>
<td>The McAuley index, HOMA, Quicki</td>
<td>NCT01315366</td>
</tr>
<tr>
<td>Location</td>
<td>Age</td>
<td>Diagnosis</td>
<td>25(OH)D Level</td>
<td>Participants</td>
<td>Intervention Comparison</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>------------------------</td>
</tr>
<tr>
<td>Saudi Arabia</td>
<td>18-60 yr</td>
<td>IFG/IGT</td>
<td>10-30 nmol/l</td>
<td>500</td>
<td>Cholecalciferol 5000 IU/d Versus placebo</td>
</tr>
<tr>
<td>Boston, The United States</td>
<td>50 years</td>
<td>No diabetes history</td>
<td>25875 participants</td>
<td></td>
<td>2000 IU cholecalciferol and Omega-3 fatty acid versus Plac/Omea Plac/Omea Plac/Omea Plac</td>
</tr>
<tr>
<td>Chicago, The United States</td>
<td>35-85 years</td>
<td>M African American</td>
<td>BMI 28-39.9 kg/m2</td>
<td>205</td>
<td>Cholecalciferol 400 IU +placebo versus 400 IU cholecalciferol and D2 50 K</td>
</tr>
</tbody>
</table>
Reference List


Summary, discussion and future perspectives


Summary, discussion and future perspectives


Summary, discussion and future perspectives


