# Vitamin D in insulin sensitivity and obesity

# SUMMARY

Currently, the global prevalence of obesity has doubled over decades across socio-economic and demographic status. At the same time, vitamin D insufficiency and deficiency are major public health issue worldwide, where the prevalence of vitamin D deficiency was estimated high in obesity regardless of ethnicity, age and sex. Evidence from observational studies suggests a negative correlation between body mass index (BMI) and circulating vitamin D 25(OH)$D_3$ levels. Up to now, the vitamin D status in the general population is based on circulating vitamin D 25(OH)$D_3$ (inactive metabolite) concentrations. Furthermore, vitamin D deficiency has also been reported to relate to whole-body insulin resistance. Of note, the development of insulin resistance is caused by a complex inter-organ crosstalk, including several insulin sensitive tissues such as the liver, the skeletal muscle, and the adipose tissue (AT). In this thesis, we aimed to investigate the link between vitamin D status and (tissue-specific) insulin sensitivity in human obesity.

**Chapter 2** provides an extensive literature review of studies that have examined the effects of vitamin D on glucose and lipid metabolism, as well as inflammation in insulin sensitive tissues such as the liver, skeletal muscle, AT, pancreas, and the gastro-intestinal tract. The majority of the data about the effects of vitamin D in these tissues were derived from animal or *in vitro* studies, with often inconsistent findings. From human observational studies, more evidence supports the association between vitamin D and obesity-related insulin resistance. However, from human randomized clinical trials (RCTs), evidence for a causal role is debatable. Most of RCTs used surrogate markers such as Homeostatic model assessment of insulin resistance (HOMA-IR) to define insulin resistance and did not take into account ethnic differences. Based on our extensive literature review we concluded that vitamin D deficiency may be associated with obesity through several mechanisms including sequestration in the AT, an impaired vitamin D related metabolism within AT, and a blunted release of vitamin D from AT. However, the link between vitamin D with (tissue-specific) insulin sensitivity warrants further investigation, using more standardized (state-of-the-art) methodologies such as hyperinsulinemic-euglycemic clamps or insulin sensitivity indices derived from an OGTT.

Sequestration of vitamin D metabolites in the excessive amount of AT in conditions of increased adiposity is one of the mechanisms that may explain the low circulating vitamin D 25(OH)$D_3$ levels in human obesity. Vitamin D is a fat-soluble vitamin, and *ex vivo evidence* from obese AT donors suggests that vitamin D release from the AT obese is blunted following lipolytic stimulation with adrenaline. Therefore, we hypothesized that in human obesity the often observed blunted catecholamine-mediated lipolysis coincides with a blunted release of vitamin D metabolites *in vivo*. Therefore, in **chapter 3**, we analyzed vitamin D (inactive) 25(OH)$D_3$ and (active)1.25(OH)$_2D_3$ fluxes across the abdominal subcutaneous adipose tissue (SAT) of obese compared to lean men, in relation to changes in circulating vitamin D levels and local AT lipolysis. We observed that both net glycerol release (marker of lipolysis) and net release of the active vitamin D metabolite 1,25(OH)$_2D_3$ across abdominal SAT during ß-adrenergic stimulation were significantly reduced in obese as compared to lean men, suggesting a blunted vitamin D 1,25(OH)$_2D_3$ release and an impaired lipolysis across abdominal SAT in obese men *in vivo*. In contrast, no significant release of the inactive vitamin D metabolite 25(OH)$D_3$ across SAT was observed in lean or obese men following an

overnight fast or during ß-adrenergic stimulation, possibly indicating an impaired AT vitamin D metabolism (hydroxylation of 25(OH)$D_3$) in human obesity. Total adipose tissue mass may be an important determinant of circulating vitamin D levels as it has been proposed that a sequestration of vitamin D in the expanded adipose tissue mass may be a responsible factor for the vitamin D deficiency in obesity. Additionally, an altered vitamin D metabolism in AT may also contribute to vitamin D deficiency and insulin resistance.

In **chapter 4**, we investigated (1) whether obesity (BMI) is related to circulating vitamin D levels (active, inactive and ratio), and whether alterations in plasma vitamin D metabolites may be mediated by an altered AT expression of VDR or vitamin D-metabolizing (hydroxylation) enzymes; (2) whether plasma vitamin D and AT expression of VDR relates to tissue-specific (adipose tissue, liver and skeletal muscle) insulin sensitivity determined by a 2–step hyperinsulinemic-euglycemic clamp with a [6,6-$^2H_2$]-glucose tracer; (3) Finally, we investigated whether an altered AT vitamin D metabolism may relate to AT insulin sensitivity. We demonstrated that (1) BMI was negatively associated with of plasma 25(OH)$D_3$ but not with plasma 1,25$(OH)_2D_3$, nor its ratio; (2) Plasma vitamin D 25(OH)$D_3$ deficiency was neither related to changes in SAT vitamin D-metabolizing enzymes nor SAT VDR gene expression; (3) plasma 25(OH)$D_3$, 1,25$(OH)_2D_3$, and the active/inactive metabolite ratio were not significantly associated with hepatic, peripheral or AT insulin sensitivity; (4) Interestingly, SAT VDR gene expression was negatively associated with AT insulin sensitivity (as indicated by % suppression of systemic FFA). Therefore, future studies are needed to unravel the molecular mechanisms by which nuclear and membrane-bound VDR interacts with insulin action and lipolysis in abdominal SAT.

Twin and familial studies from past decades have demonstrated a nontrivial heritability of both obesity and circulating vitamin D concentrations. Moreover, several large-scale genome-wide association studies (GWAS) have discovered associations with key vitamin D enzymes and serum levels of vitamin D. Therefore, we hypothesized that VDR genetic variants may be associated with adiposity, whole body insulin resistance and the development of T2D. However, whether these VDR variants may also affect AT vitamin D metabolism and human SAT at the transcriptional level is unknown. In **chapter 5**, we tested the hypothesis that VDR genetic variants are associated with obesity phenotypes, tissue-specific insulin resistance and changes in the SAT transcriptome. Therefore, we included 553 overweight/obesity men and women from DiOGenes study, a multi-center, randomized double-blind controlled dietary intervention trial in 8 European countries. We estimated hepatic insulin resistance (HIRI) and muscle insulin sensitivity (MISI) using insulin and glucose concentrations from a 5 time-points oral glucose tolerance test (OGTT) and adipose tissue insulin resistance (Adipo-IR) from fasting insulin and free fatty acids levels. We found that TaqI and ApaI genetic variants were associated with markers of adiposity including BMI, WC, and Fat Mass. However, the VDR genetic variants were not associated with HIRI or MISI. Variants in FokI VDR were associated with Adipo-IR as well as elevated circulating FFA. However, cis and trans eQTL analysis demonstrated no major effects of these VDR polymorphisms on the SAT transcriptome, indicating that the putative mechanisms of action remain to be determined. The VDR polymorphisms did not relate to changes in body weight and insulin resistance as result of the dietary intervention.

Several plausible mechanisms to explain a potential role of vitamin D in improving insulin sensitivity have been described **in chapter 2,** including its effects on skeletal muscle substrate metabolism, insulin sensitivity and lipid composition. Importantly, the skeletal

muscle is recognized as key organ in peripheral insulin sensitivity as it affects 70-90% of total glucose disposal under postprandial conditions (non-fasting conditions). Based on the conclusions derived from our review in **chapter 2**, we conducted a systematic review and meta-analysis on the effect of vitamin D supplementation on insulin sensitivity in individuals with or at risk of insulin resistance (Chapter 6). We systematically searched and performed a meta-analysis from studies (published between 1980 and 2018) that met the PICOS (Patients/participants, Intervention, Comparison/control group, Outcome, and Study Design) criteria: (1) study was a randomized controlled trial; (2) study population consisted of individuals with elevated (risk for) insulin resistance (overweight, obesity, prediabetes, polycystic ovary syndrome (PCOS), and type 2 diabetes without complications); (3) participants were ≥ 18 years; (4) interventions were vitamin D supplementation vs the appropriate placebo; (5) vitamin D supplementation dose was daily, weekly, or monthly; (6) trial length was ≥ 2 months; (7) serum 25(OH)D level was measured; (8) insulin sensitivity was measured by Matsuda index derived from an OGTT and/or insulin sensitivity index derived from IVGTT, or by a hyperinsulinemic-euglycemic clamp at the beginning and at the end of the trial. This systematic review (**chapter 6**) provides no evidence that supplementation with vitamin D has a beneficial effect on peripheral insulin sensitivity, as determined by the hyperinsulinemic-euglycemic clamp, the Matsuda or insulin sensitivity index, and postprandial glucose concentrations after an OGTT in people with, or at risk for insulin resistance.

In conclusion, we found that BMI but not insulin sensitivity (in the liver, muscle, or adipose tissue) is the main determinant of circulating vitamin D 25(OH)$D_3$ concentration. However, the VDR gene expression in abdominal SAT is associated with adipose tissue insulin sensitivity, Whether this is a primary defect or more a secondary phenomenon of the obese insulin resistant state (e.g. chronic low-grade inflammation) needs to be investigated in future research. Furthermore, a blunted vitamin D 1,25$(OH)_2D_3$ release and an impaired lipolysis across abdominal SAT in obese men was observed *in vivo* following ß-adrenergic stimulation. Further studies are still needed to investigate whether this impaired release of vitamin D 1,25$(OH)_2D_3$ might be linked with changes in the VDR expression in abdominal SAT of obese individuals or possibly due to an impaired AT vitamin D metabolism (i.e. hydroxylation) in human obesity. In addition, The VDR polymorphisms are unlikely to play a primary role in tissue-specific insulin resistance. Finally, our meta-analysis showed no effect of vitamin D supplementation on insulin sensitivity or postprandial glucose metabolism in individuals with or at risk of insulin resistance. Nevertheless, it is possible that vitamin D supplementation may have a beneficial effect on other cardio-metabolic risk factor like chronic low-grade inflammation and may have beneficial effects on gut microbiota composition/diversity and intestinal health. Further studies with more specific approaches by taking into account genetic variations of the VDR and combining vitamin D supplementation with other modes of intervention might provide new strategies towards a more personalized approaches for treatment and prevention of insulin resistance in humans.