Vitamin D: emerging new roles in insulin sensitivity

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The growing incidence of prediabetes and clinical type 2 diabetes, in part characterised by insulin resistance, is a critical health problem with consequent devastating personal and health-care costs. Vitamin D status, assessed by serum 25-hydroxyvitamin D levels, is inversely associated with diabetes in epidemiological studies. Several clinical intervention studies also support that vitamin D, or its active metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D), improves insulin sensitivity, even in subjects with glucose metabolism parameters classified within normal ranges. The mechanisms proposed which may underlie this effect include potential relationships with improvements in lean mass, regulation of insulin release, altered insulin receptor expression and specific effects on insulin action. These actions may be mediated by systemic or local production of 1,25(OH)2D or by suppression of parathyroid hormone, which may function to negatively affect insulin sensitivity. Thus, substantial evidence supports a relationship between vitamin D status and insulin sensitivity; however, the underlying mechanisms require further exploration.

Vitamin D: Diabetes: Insulin sensitivity: Insulin resistance

The reported incidence of diabetes is increasing at an alarming rate. The WHO estimates that more than 180 million individuals worldwide have diabetes and that 1.1 million died from diabetes in 2005(1). Further, the WHO estimates that this number is likely to more than double by 2030(2). The rate of change in incidence of insulin resistance and diabetes cannot be accounted for by shifts in population demographics, which suggests that lifestyle choices, rather than differences in genetics, are a primary contributor. Unfortunately, the dramatic rise in the prevalence of diabetes in this decade is likely to continue given the number of Americans with prediabetes and given that current recommendations for prevention are either ineffective or are not implemented sufficiently.

Several lifestyle factors may play a role in this rapid increase in prediabetes and progression to clinical diabetes. An increase in diabetes has occurred concurrently with an increase in obesity, as the latter is a strong risk factor for diabetes. This relationship may be rooted in the general relationship between energy balance, obesity and diabetes. However, the presence, or absence, of specific dietary factors may also play a role in these diseases. Therefore it is critical to identify factors that influence body weight, factors that are independent of weight that will contribute to the prevention of abnormal glucose homeostasis and insulin resistance to reduce the incidence of diabetes beyond the difficult process of weight loss. It has been proposed that vitamin D may play an important role in the development of insulin resistance and diabetes(2–4). Although low vitamin D status is also implicated in the development of type 1 diabetes (or insulin-dependent diabetes)(5), the present review will focus on the relationship of vitamin D status with insulin sensitivity and the development of type 2 diabetes.

Discussion

Classical role of vitamin D in metabolism and prevalence of deficiencies in US populations

It is well established that vitamin D functions to regulate Ca homeostasis. Studies on the hormonal response to dietary Ca deprivation have identified the vitamin D metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) and parathyroid hormone (PTH) as major hormonal regulators of Ca homeostasis. Low serum Ca is sensed at the level of the parathyroid gland through a Ca-sensing receptor(6). The Ca-sensing receptor relays a signal that leads to the increased production and release of PTH into the circulation. Finally, PTH is a strong stimulator of the renal enzyme 1α-hydroxylase that catalyses the conversion of 25-hydroxyvitamin D (25(OH)D) to 1,25(OH)2D, the

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone; VDR, vitamin D receptor.

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hormonally active form of vitamin D\(^{(7)}\). The 1,25(OH)\(_2\)D produced by the kidney releases into the serum acts on intestine, bone and kidney to regulate Ca homeostasis. Thus, a dietary Ca load results in an acute lowering of serum PTH\(^{(8)}\), whereas higher vitamin D status as well as overall better Ca status leads to a lower serum PTH even in the fasting state\(^{(9,10)}\).

The primary dietary sources of vitamin D are fortified dairy products; however, the availability of vitamin D for individuals is heavily influenced by the exposure to sunlight, as vitamin D is also produced in the skin. With trends towards reduction in milk intake and less sun exposure, much of the population of the USA is considered functionally vitamin D deficient\(^{(11)}\); however, the prevalence of vitamin D deficiency is highly dependent on the working definition of deficiency. When vitamin D deficiency is defined as \(\leq 37.5\, \text{nmol} \cdot \text{L}^{-1} \) 25(OH)D\(^{(12)}\), a marker for vitamin D status, there are 42 % Caucasians and 42 % African-Americans that can be considered vitamin D deficient. Other definitions used indicate that serum levels of at least 80 nmol-25(OH)D are necessary for individuals to be considered adequate for vitamin D status. The latter greatly elevates the estimates of vitamin D inadequacy\(^{(13,14)}\). The net effect of the latter may be dietary recommendations to promote vitamin D intakes that promote maximal bone health\(^{(11)}\) but may not reflect vitamin D requirements for overall health. In order to establish appropriate recommendations for vitamin D it is critical to consider the plethora of effects of vitamin D as a metabolic regulator that extend far beyond the well-recognised role of this nutrient in maintaining bone. Of particular importance to the discussions presented in this review are the roles of vitamin D in modulating body composition, energy homeostasis, insulin sensitivity and the development of insulin resistance, a predisposing factor for type 2 diabetes.

### Roles of vitamin D status, calcium and diet as risk factors for diabetes

In discussing the role of vitamin D in the development of insulin resistance and diabetes, it is important to also consider both the level of vitamin D supplied and the dietary source. Because vitamin D is derived, at least in part, through the same dietary source of dairy products as Ca, it is difficult in epidemiological studies to separate the impact of dietary Ca from dietary vitamin D in the regulation of body composition. Assessing the impact of vitamin D from the diet is also particularly difficult since vitamin D produced by UV exposure is a much greater influence on vitamin D status than dietary vitamin D.

Inadequate vitamin D status is proposed to play a role in insulin resistance\(^{(2,4,15–17)}\). The primary source of dietary vitamin D is fortified dairy products. The relationship of dietary intake with risk for type 2 diabetes was explored in a prospective analysis utilising the Health Professionals Follow-up Study (n 41 254 male participants; Table 1)\(^{(18)}\). The results of this study show that in 12 years of follow-up, the relative risk for type 2 diabetes in men in the top quintile of dairy intake was 0.77 (95 % CI 0.62, 0.95; \(P\) for trend = 0.003) compared with those in the lowest quintile. Thus, each serving per d increase in total dairy intake was associated with a 9 % lower risk for type 2 diabetes, independent of BMI\(^{(18)}\). However, there are many components of dairy foods, in addition to vitamin D, that may influence the risk for diabetes. As a consequence, there is considerable confounding interpretation of experimental outcomes.

### Body composition, vitamin D and diabetes

Obesity is a major risk factor for the development of type 2 diabetes. A number of studies have been conducted to study the effects of dietary Ca on body fat mass\(^{(19–22)}\). Many studies, both epidemiological and intervention studies, support an inverse relationship between higher Ca intakes, enhanced by dairy product intake, and body fat mass\(^{(19)}\). However, other intervention studies\(^{(23–25)}\) do not support that Ca or dairy products will be effective in every situation but instead alternatively suggest that another dietary factor, probably vitamin D, may be responsible for inducing changes that improve body composition. Evidence that supports a link between vitamin D status and an increase in energy expended from a meal\(^{(26)}\) provides an explanation of the role of vitamin D to reduce adiposity. In the study of 250 overweight and obese adults of different ethnicities, serum 25(OH)D was shown to be inversely related to weight \((r = -0.21)\), BMI \((r = -0.18)\) and waist circumference \((r = -0.14)\), but not fat mass\(^{(27)}\). These results suggest that the relationship of vitamin D status was specific to waist circumference, an independent risk factor for disease, and not to fat mass.

Muscle mass is an important determinant of overall body insulin sensitivity and vitamin D status clearly has effects on muscle and physical activity. Changes in gait, difficulties in rising from a chair, inability to ascend stairs and diffuse muscle pain are classic symptoms of vitamin D deficiency\(^{(28)}\). Results of epidemiological studies support a need for diet and lifestyle factors conducive to higher vitamin D status, assessed by 25(OH)D and greater muscle function\(^{(28–34)}\). Several intervention studies also support the conclusion that improved vitamin D status improves muscle function\(^{(35–37)}\). In a meta-analysis which included five double-blind randomised, controlled trials in elderly populations (mean age 60 years; \(n = 1237)\), vitamin D supplementation reduced the corrected OR of falling by 22 % compared with patients receiving Ca or placebo, independent of Ca supplementation\(^{(35)}\). A randomised controlled trial of patients (ninety-six women) with post-stroke hemiplegia receiving vitamin D supplementation of 1000 IU (25 \(\mu\)g)/d for 2 years reduced injurious falls by 59 %\(^{(37)}\).

The efficacy of vitamin D to promote muscle growth is supported by laboratory experiments. Rodents receiving diets containing high levels of vitamin D for 12 weeks had 8 % greater muscle mass compared with animals receiving suboptimal vitamin D levels\(^{(38)}\). In support of an important physiological role for 1,25(OH)\(_2\)D on muscle, vitamin D receptor (VDR) mice experience myopathy characterised by smaller muscle fibres\(^{(39)}\).

The purported impact of vitamin D on insulin sensitivity may be, at least in part, through an increase in muscle mass, which will improve overall body insulin sensitivity. Therefore, the role of vitamin D in potentially reducing
Table 1. Epidemiological studies investigating relationship of vitamin D and diabetes or insulin resistance endpoints

<table>
<thead>
<tr>
<th>Study</th>
<th>Study type</th>
<th>Indicator</th>
<th>Sex</th>
<th>Baseline</th>
<th>Subjects (n)</th>
<th>Length</th>
<th>Endpoint</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi et al. (2005)</td>
<td>Prospective</td>
<td>Diet, dairy intake</td>
<td>Male</td>
<td>Self-report no diabetes</td>
<td>41254</td>
<td>12 years</td>
<td>Type 2 diabetes</td>
<td>Yes</td>
</tr>
<tr>
<td>Liu et al. (2005)</td>
<td>Cross-sectional</td>
<td>Diet, dairy intake</td>
<td>Female</td>
<td>Age &gt; 45 years, self-report no diabetes</td>
<td>10066</td>
<td>20 years</td>
<td>Metabolic syndrome</td>
<td>Depending on Ca intake</td>
</tr>
<tr>
<td>Pittas et al. (2006)</td>
<td>Prospective</td>
<td>Diet</td>
<td>Female</td>
<td>Age 30–55 years, self-report no diabetes</td>
<td>83779</td>
<td>20 years</td>
<td>Type 2 diabetes</td>
<td>Yes</td>
</tr>
<tr>
<td>Scragg et al. (2004)</td>
<td>Cross-sectional</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>Age ≥ 20 years</td>
<td>6228</td>
<td>20 years</td>
<td>Blood glucose, OGTT, HOMA-IR</td>
<td>Yes</td>
</tr>
<tr>
<td>Martins et al. (2007)</td>
<td>Cross-sectional</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>Age ≥ 20 years</td>
<td>15088</td>
<td>20 years</td>
<td>Blood glucose, type 2 diabetes</td>
<td>Yes</td>
</tr>
<tr>
<td>Reis et al. (2007)</td>
<td>Cross-sectional</td>
<td>25(OH)D and PTH</td>
<td>Female and male</td>
<td>Age 44–96 years</td>
<td>1070</td>
<td>20 years</td>
<td>Blood glucose, metabolic syndrome</td>
<td>None for 25(OH)D, for PTH, men only</td>
</tr>
<tr>
<td>Ford et al. (2005)</td>
<td>Cross-sectional</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>Age ≥ 20 years</td>
<td>8421</td>
<td>20 years</td>
<td>Fasting glucose</td>
<td>Yes</td>
</tr>
<tr>
<td>Baynes et al. (1997)</td>
<td>Cross-sectional</td>
<td>25(OH)D and male</td>
<td>Male</td>
<td>Age 70–88 years</td>
<td>142</td>
<td>20</td>
<td>OGTT</td>
<td>Yes</td>
</tr>
<tr>
<td>Need et al. (2005)</td>
<td>Cross-sectional</td>
<td>25(OH)D and female</td>
<td>Male</td>
<td>Post-menopausal, no diabetes</td>
<td>753</td>
<td>20</td>
<td>Fasting glucose</td>
<td>Yes</td>
</tr>
<tr>
<td>Targher et al. (2006)</td>
<td>Case–control</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>Type 2 diabetes or controls</td>
<td>780</td>
<td>20 years</td>
<td>Hypovitaminosis D</td>
<td>Yes</td>
</tr>
<tr>
<td>Scragg et al. (1995)</td>
<td>Case–control</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>Newly diagnosed type 2 diabetes or controls, age 40–64 years</td>
<td>476</td>
<td>20 years</td>
<td>Hypovitaminosis D</td>
<td>Yes</td>
</tr>
<tr>
<td>Chiu et al. (2004)</td>
<td>Cross-sectional</td>
<td>25(OH)D and male</td>
<td>Male</td>
<td>Normal glucose tolerance</td>
<td>126</td>
<td>20</td>
<td>Hyperglycaemic clamp</td>
<td>Yes</td>
</tr>
<tr>
<td>Boucher et al. (1995)</td>
<td>Case–control and cross-sectional</td>
<td>25(OH)D and male</td>
<td>Female and male</td>
<td>High fasting glucose or controls</td>
<td>59</td>
<td>20</td>
<td>OGTT, insulin, C-peptide</td>
<td>Yes</td>
</tr>
</tbody>
</table>

25(OH)D, 25-hydroxyvitamin D; OGTT, oral glucose tolerance test; HOMA-IR, homeostasis model assessment of insulin resistance; PTH, parathyroid hormone.
adiposity, improving insulin sensitivity indirectly through improving muscle mass, and the reduction in vitamin D status with increased adiposity (Fig. 1) are factors that need to be carefully considered when reviewing the epidemiological literature on the relationship of vitamin D with diabetes or insulin sensitivity.

**Epidemiological evidence linking vitamin D status to diabetes**

Several epidemiological analyses of large datasets support that dietary vitamin D is associated with abnormal glucose homeostasis (Table 1). Cross-sectional results from the Women’s Health Study show that in women (n 10 066; aged > 45 years) dietary vitamin D is inversely associated with the prevalence of the metabolic syndrome, but this relationship is not independent of total Ca intake (40). Women (n 83 779) participating in the Nurses’ Health Study with no history of diabetes were followed for approximately 20 years (41). The results suggest that although vitamin D intake had a minor influence on the risk of developing diabetes, a combined daily intake of > 1200 mg Ca and > 800 IU (20 μg) vitamin D was associated with a 33 % lower risk of type 2 diabetes with a relative risk of 0·67 (95 % CI 0·49–0·90) compared with an intake of < 600 mg and 400 IU (10 μg) Ca and vitamin D, respectively. These results suggest that dietary Ca may enhance the impact of dietary vitamin D (41). A primary shortcoming of linking vitamin D intake to health outcomes is a potential confounding of vitamin D source and adequacy of supply through the diet and sunlight exposure. For the study described above the dietary vitamin D may not adequately predict vitamin D status, but may instead be an indicator of a healthier diet that includes dairy products or may suggest greater physical activity which may involve increased exposure to the sun.

Serum 25(OH)D concentration, a preferred indicator of vitamin D status, has been correlated with improved glucose homeostasis and increased insulin sensitivity (Table 1). In cross-sectional analyses, serum 25(OH)D (using quartiles) of participants (n 6228) in the Third National Health and Nutrition Examination Survey (NHANES) was negatively related to high fasting glucose levels in non-Hispanic whites (OR 0·25) and Mexican blacks (OR 0·17) after adjusting for sex, age, BMI, leisure activity and time of year (42). For the study described above the dietary vitamin D may not adequately predict vitamin D status, but may instead be an indicator of a healthier diet that includes dairy products or may suggest greater physical activity which may involve increased exposure to the sun.

In addition, serum 25(OH)D concentration was inversely associated with fasting and 2 h glucose, fasting insulin and the homeostasis model assessment of insulin resistance in Mexican Americans, with a trend towards significance for non-Hispanic whites (P = 0·06) (42). However, no relationship between 25(OH)D concentration and measures of glucose homeostasis was observed in non-Hispanic blacks (42). These contradictory results suggest a potentially race-specific inverse relationship of vitamin D status with serum glucose and possibly insulin resistance.

Analysis of the third NHANES database shows that serum 25(OH)D levels were lower in participants with diabetes (OR 1·98 lowest compared with highest quartile) (43). In a secondary analysis of the results from the Rancho Bernardo Study (1070 men and women aged 44–96 years), PTH, but not serum 25(OH)D, was inversely associated with hyperglycaemia in men, but not women (44). Finally, the results of a study which included over 8000 adult (aged ≥ 20 years) men and women showed serum 25(OH)D concentration, in quintiles, was negatively related to fasting hyperglycaemia (45). In a cross-sectional analysis including 142 men (aged 70–88 years), controlling for age, BMI, physical activity, month of sampling, cigarette smoking and alcohol, serum 25(OH)D concentration was inversely correlated with glucose levels at 1 h and area under the curve of an oral glucose tolerance test (r = −0·23 (P < 0·01) and r = −0·26 (P < 0·01), respectively) (46). In postmenopausal women (n 753), serum 25(OH)D level, but not PTH or 1,25(OH)2D, was inversely related to fasting serum glucose, when controlled for BMI, weight and age (47). Thus, there is substantial epidemiological data to support an inverse relationship between vitamin D status and abnormal glucose homeostasis.

Several case–control studies also support a relationship between vitamin D status and risk of diabetes or glucose intolerance (Table 1). For example, the prevalence of hypovitaminosis D (25(OH)D ≤ 37·5 nmol/l) was higher in diabetic patients (24 %; 16 %; P < 0·001) than in controls (390 subjects per group) (48). In addition, serum 25(OH)D concentration was lower in newly diagnosed diabetics and those with impaired glucose tolerance compared with controls (total n 5677, aged 40–64 years) (49). However, epidemiological studies cannot demonstrate cause and effect.

Several smaller epidemiological studies explored the relationship of vitamin D status with other indices of glucose homeostasis (Table 1). Serum 25(OH)D levels correlated positively with insulin sensitivity index and negatively with first- and second-phase insulin response in normal healthy glucose-tolerant subjects in a cross-sectional study (49), suggesting a better insulin sensitivity with higher vitamin D status in healthy young individuals independent of changes in weight. In addition, in another study 95 % of at-risk (defined by glucose levels) and 80 % of low-risk subjects were vitamin D deficient (serum 25(OH)D < 11 ng/ml) and 30 min blood glucose during a glucose tolerance test, serum insulin and C-peptide levels were correlated with serum 25(OH)D concentrations in at-risk subjects (n 44) and even in the ‘not-at-risk’ subjects (n 15) (51). These results support that lower vitamin D status may be a significant risk factor for glucose intolerance.

**Fig. 1.** Potential mechanisms underlying the putative relationship between vitamin D and insulin sensitivity or diabetes. 25(OH)D, 25-hydroxyvitamin D; 1,25(OH)2D, 1,25-dihydroxyvitamin D; PTH, parathyroid hormone.
Increased serum PTH may contribute to the risk for diabetes due to its relationship with obesity. Increased levels of fasting PTH have been hypothesised to influence increased levels of body fat mass (56). In a meta-analysis of studies investigating the relationship of primary hyperparathyroidism with indices of body weight, in thirteen studies, patients with primary hyperparathyroidism were 3.34 (95% CI 1.97, 4.71) kg heavier ($P < 0.00001$) than age-matched controls or had an increased BMI of 1.13 (95% CI -0.29, 2.55) kg/m$^2$ ($P = 0.12$) compared with controls (53). Studies in young adults with PTH in normal limits show that serum PTH levels are higher in the obese than in the non-obese (54, 55). Thus, serum PTH may be involved in regulating adiposity, a major risk factor for diabetes.

**Vitamin D status, diabetes and controlled interventions**

Several intervention studies support that vitamin D supplementation may affect glucose homeostasis or insulin resistance (Table 2). Non-diabetic adults (aged ≥ 65 years; $n = 314$) received Ca (500 mg/d) and vitamin D (700 IU; 17.5 μg) for 3 years in a double-blind, randomised, controlled trial which was designed for bone-related outcomes (56). In a post hoc analysis, there was no effect of the intervention in participants when fasting glucose concentrations were within normal limits at the initiation of the study ($< 5.6$ mmol/l; $n = 222$). However, non-diabetic subjects with fasting glucose above normal ($5.6–6.9$ mmol/l; $n = 92$) displayed a reduced change in fasting plasma glucose at 3 years compared with those on placebo (0.02 mmol/l (4 mg/l) vs. 0.34 mmol/l (61 mg/l), respectively; $P = 0.042$) and a lower increase in homeostasis model assessment of insulin resistance (0.05 vs. 0.91; $P = 0.031$) (56). Therefore supplementation with Ca and vitamin D may attenuate the development of insulin resistance that occurs over time and rescue the progression of abnormal glucose metabolism toward insulin resistance and type 2 diabetes.

The largest intervention study to examine the influence of dietary vitamin D and Ca on health risks is the Women’s Health Initiative, and diabetes risk was examined in secondary analysis from this trial (57). In this study, postmenopausal women ($n = 33951$) without diabetes at baseline were assigned to received either 1000 mg Ca/d plus 4001U (10 μg) vitamin D or placebo. The women were followed for a median of 7 years and there was no change in the risk for developing diabetes with the treatment. Unfortunately, these study results need to be interpreted with caution, as the level of vitamin D supplementation may have been too low to achieve a significant change in vitamin D status. Thus, recent studies demonstrate that a higher level of intake may be required for optimal health (58).

Shorter-term intervention studies also demonstrate a positive impact of vitamin D or its active metabolites on insulin resistance. Treatment of type 2 diabetic females with oral vitamin D for 1 month led to an anticipated increase in 25(OH)D but also led to a reduced first-phase insulin secretion and caused a substantial reduction in insulin resistance of 21.4% (58). Treatment of uraemic patients with vitamin D and Ca (59) or treatment with intravenous 1.25(OH)$_2$D (60) also improved insulin response. In contrast, in a double-blinded, placebo-controlled, cross-over trial in diabetics, acute administration of 1,25(OH)$_2$D did not affect fasting or stimulated glucose, insulin or C-peptide (61). On the other hand, a single dose of vitamin D (300 000 IU (7500 μg) intramuscular injection) in type 2 diabetics resulted in improvements in the oral glucose tolerance and glucose-stimulated increase in serum insulin levels in 4 weeks compared with placebo controls (62). However, a single oral dose up to 450 000 IU (11 250 μg) vitamin D did not alter these parameters, even up to 12 weeks following the dose (63), suggesting that higher doses of vitamin D may be required orally to achieve improvements in insulin sensitivity compared with intramuscular injections. The results of these intervention studies provide support that vitamin D specifically can improve insulin sensitivity.

**Genotypic links to vitamin D action and insulin resistance**

Support for a relationship of vitamin D in mediating improved insulin resistance is found in assessment of responsiveness of gene transcripts whose protein products regulate vitamin D metabolism. There are two known polymorphisms in exon 11 of the vitamin D-binding protein (DBP) gene that result in amino acid variants: at codons 416 GAT - - > GAG (Asp- - > Glu) and 420 ACG - - > AAG (Thr- - > Lys) which are the genetic basis for the three common electrophoretic variants of DBP (Gc1F, Gc1S and Gc2). These variants of DBP, the serum carrier of vitamin D metabolites, are associated with higher risk for type 2 diabetes or prediabetic phenotypes in several populations (64–68) but not others (69, 70). While the precise association of variant subtypes with predisposition to insulin resistance and type 2 diabetes is not completely understood, the relationship between variant expression and predisposition to insulin resistance provides further evidence of the importance of vitamin D and maintenance of glucose homeostasis. An important mediator of vitamin D action is the VDR which functions as a transcription factor when bound to 1,25(OH)$_2$D. There is evidence that VDR genotype may affect insulin resistance, both in regards to insulin secretion (the Apa1 VDR polymorphism) and insulin resistance (the BsmI VDR polymorphism) (71). The association of the FokI, Apal, BsmI and TaqI polymorphisms of the VDR gene with type 2 diabetes was explored in a case–control design (308 type 2 diabetic patients and 240 control cases). In this study, there was no association of the four VDR polymorphisms examined with type 2 diabetes (72). In another study, the influence of BsmI VDR genotype in young males with low ($n = 752$) and high ($n = 787$) physical activity was investigated. Those with the BsmI VDR BB genotype had significantly higher levels of fasting glucose ($n = 137$; 5.61 (SD 0.49) mmol/l) than gene carriers with the genotype Bb ($n = 370$; 5.44 (SD 0.44) mmol/l) or bb ($n = 245$; 5.38 (SD 0.44) mmol/l). Of the BB gene carriers, 47% had fasting glucose levels > 5.55 mmol/l compared with 36% of Bb gene carriers and 34% of bb gene carriers ($P = 0.02$) (73). In another study, the distribution of alleles and genotypes of the four single nucleotide polymorphisms in intron 8 (Bsm1, Tru9I, Apal) and exon 9 (TaqI) of the VDR gene was similar in type 2 diabetics ($n = 309$) and controls ($n = 143$) (74).
Table 2. Intervention studies investigating the relationship of vitamin D and insulin resistance or diabetes

<table>
<thead>
<tr>
<th>Study</th>
<th>Sex</th>
<th>Baseline</th>
<th>Subjects (n)</th>
<th>Metabolite</th>
<th>Dose (IU)*</th>
<th>Length</th>
<th>Endpoint</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittas et al. (2007)</td>
<td>Female</td>
<td>Age &gt; 65 years</td>
<td>314</td>
<td>Vitamin D</td>
<td>700/d</td>
<td>3 years</td>
<td>Fasting glucose, HOMA-IR</td>
<td>Yes</td>
</tr>
<tr>
<td>De Boer et al. (2008)</td>
<td>Female</td>
<td>Self-report no diabetes</td>
<td>33951</td>
<td>Vitamin D or placebo</td>
<td>400/d</td>
<td>7 years</td>
<td>Type 2 diabetes, fasting glucose, insulin, HOMA-IR</td>
<td>None</td>
</tr>
<tr>
<td>Borissova et al. (2003)</td>
<td>Female</td>
<td>Post-menopausal, type 2 diabetes</td>
<td>10</td>
<td>Ca or placebo Vitamin D</td>
<td>1000 mg/d</td>
<td>1 month</td>
<td>Intravenous GTT, HOMA-IR</td>
<td>Yes</td>
</tr>
<tr>
<td>Allegra et al. (1994)</td>
<td>Female and male</td>
<td>Uraemia</td>
<td>17</td>
<td>Vitamin D Ca</td>
<td>0–100/d</td>
<td>21 d</td>
<td>Intravenous GTT</td>
<td>Yes</td>
</tr>
<tr>
<td>Mak (1998)</td>
<td>Female and male</td>
<td>Uraemia</td>
<td>16 patients, 7 controls</td>
<td>1,25(OH)2D or dihydrotachysterol</td>
<td>500 mg/d</td>
<td>4 weeks</td>
<td>Fasting glucose, OGTT, insulin, euglycaemic clamp Glucose, insulin, C-peptide OGTT, insulin</td>
<td>Yes</td>
</tr>
<tr>
<td>Orwoll et al. (1994)</td>
<td>Female and male</td>
<td>Type 2 diabetes</td>
<td>20</td>
<td>1,25(OH)2D</td>
<td>200/d</td>
<td>4 d cross-over</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>Raghuramulu et al. (1992)</td>
<td>Female and male</td>
<td>Type 2 diabetes</td>
<td>42</td>
<td>Vitamin D</td>
<td>300 000 intramuscular placebo or oral</td>
<td>4 weeks</td>
<td>Glucose, insulin, C-peptide OGTT, insulin</td>
<td>Yes</td>
</tr>
<tr>
<td>Ayesha et al. (1998)</td>
<td>Female and male</td>
<td>Type 2 diabetes</td>
<td>32 (eight per group)</td>
<td>Vitamin D</td>
<td>150 000, 300 000, 450 000</td>
<td>12 weeks</td>
<td>OGTT, insulin</td>
<td>None</td>
</tr>
</tbody>
</table>

HOMA-IR, homeostasis model assessment of insulin resistance; GTT, glucose tolerance test; 1,25(OH)2D, 1,25-dihydroxyvitamin D; OGTT, oral glucose tolerance test.* 1 IU = 0.025 µg vitamin D.
Thus, the evidence supporting an association of VDR genotypes with risk for diabetes is conflicting.

Another critical protein in the regulation of vitamin D action is the vitamin D 1α-hydroxylase gene, which converts 25(OH)D to the active vitamin D metabolite 1,25(OH)2D. The prevalence of two variants in the 1α-hydroxylase was determined in type 2 diabetic patients (n = 291) and controls (n = 231). There were no differences in the distribution of genotypes, haplotypes and haplotype combinations between the groups. However, the T-C/T-T heterozygous haplotype combination was more prevalent in the subgroup of obese type 2 diabetics (BMI ≥ 30 kg/m2) than in the controls (41.5 vs. 28.6%; P = 0.01), suggesting an association with the risk factor for diabetes, obesity75.

### Tissue, cellular and molecular actions of vitamin D to alter glucose homeostasis

Several mechanisms have been proposed to explain the impact of vitamin D on insulin sensitivity and glucose homeostasis (Fig. 1). Because dietary vitamin D and elevated serum 25(OH)D are well known as regulators of PTH and 1,25(OH)2D, these are likely candidates to mediate systemic changes in glucose metabolism. The regulation of serum Ca via PTH and 1,25(OH)2D following changes in dietary Ca has been proposed to mediate the effects of vitamin D, at least in part, on insulin resistance. Vitamin D and PTH have also been associated with a variety of other actions beyond their classical actions. In addition to its rapid signalling pathways in a variety of tissues including adipocytes and muscle cells$,^{22,76,77}$ in both hereditary and renal 1α-hydroxylase deficiencies, the gene locus of vitamin D$^{79}$, regulates these effects by vitamin D, at least in part, on insulin resistance. Vitamin D and PTH have also been associated with a variety of other actions beyond their classical functions (Fig. 2). Both hormones have been shown to increase levels of intracellular Ca and other rapid signalling pathways in a variety of tissues including adipocytes and muscle cells$,^{22,76,77}$. In addition to its rapid actions in cells, 1,25(OH)2D also mediates genomic regulation through the VDR, a member of the steroid hormone receptor family$.^{78}$ Thus, both hormones have the capability of regulating a variety of processes far beyond their classical actions in mediating Ca homeostasis.

It is well established that PTH regulates the activity of the renal 1α-hydroxylase to convert 25(OH)D to 1,25(OH)2D; however, extra-renal 1α-hydroxylase enzymes have also been identified in a variety of tissues which may lead to the local production of 1,25(OH)2D under conditions of high vitamin D status$^{79}$. The evidence suggests that these enzymes are not regulated by PTH$^{80}$. When vitamin D status is improved (as evidenced by higher 25(OH)D levels), PTH levels are reduced. Total serum concentrations of 1,25(OH)2D are subsequently reduced by the lower PTH levels. However, the increased 25(OH)D may lead to an increase in 1,25(OH)2D locally at the tissues even with decreased fasting PTH. Thus, while the dogma exists that 1,25(OH)2D is positively related to 25(OH)D, and 1,25(OH)2D levels are coordinated, this remains a controversial issue in normal physiology. Many tissues have been shown to express the 1α-hydroxylase$^{79}$, including muscle and adipocytes$^{81}$.

There is also evidence to support an effect of 1,25(OH)2D at multiple levels of insulin release and action. Insulin release is low in vitamin D-deficient rats$^{82,83}$ and is enhanced by treatment with 1,25(OH)2D$^{84}$, potentially via synthesis of proteins and increased conversion of pro-insulin to insulin$^{85}$. Results of studies suggest that the increase in insulin release mediated by 1,25(OH)2D may involve increases in intracellular Ca through the phosphoinositide/protein kinase C pathway and facilitating Ca entry by Ca channels$^{86}$ and that activation of the cyclic AMP-mediated pathway restores insulin release in vitamin D deficiency$^{87}$. Further, the 1α-hydroxylase is expressed in pancreatic islet cells$^{88}$. Consistent with the ability of vitamin D to enhance insulin secretion, following a single intramuscular injection of 100 000 IU (2500 μg) vitamin D, insulin and C-peptide concentrations at 30 min of an oral glucose tolerance test increased 8–12 weeks later in patients with elevated glucose concentrations, but not in normal subjects$^{51}$. Therefore, vitamin D is important for insulin secretion.

The active metabolite of vitamin D, 1,25(OH)2D, also may be directly involved in altering insulin action. These actions may be either direct at the level of the adipocyte to alter insulin action or at the level of the pancreatic B cell to alter insulin release. Supporting data indicate that when adipocytes are incubated with 1,25(OH)2D there is an observed decrease in insulin-stimulated glucose uptake$^{89}$. Likewise there is a vitamin D response element sequence in the insulin receptor gene promoter$^{90}$ and cellular tests indicate increased transcription and protein expression of the insulin receptor induced by 1,25(OH)2D$^{91}$. Therefore an adequate vitamin D status or adequate Ca intake will act to promote higher levels of 25(OH)D and reduced levels of PTH, and reduce 1,25(OH)2D levels. An increase in 25(OH)D, the substrate for 1α-hydroxylase, could drive a local increase in 1,25(OH)2D, which may affect the insulin receptor $\alpha$-subunit$^{91}$. Therefore the evidence suggests that the increase in 1,25(OH)2D$^{92}$ and 25(OH)D$^{93}$ may affect insulin resistance by enhancing insulin; however, the effect of improved vitamin D status alone on insulin receptor expression has not been explored.

PTH levels are also regulated by vitamin D status. Serum levels of 25(OH)D, a marker for vitamin D status, are inversely correlated with fasting levels of serum PTH$^{94,95,99}$ and fasting serum PTH levels, contrary to the model that only PTH controls the rate of conversion of 25(OH)D to 1,25(OH)2D to regulate serum concentration. Overall, Ca homeostasis mediated by higher vitamin D status limits levels of fasting serum PTH. Therefore vitamin D may improve insulin action is by its ability to reduce PTH levels$^{10}$.

There is evidence that increased blood PTH is associated with insulin resistance or glucose intolerance$^{94,95}$. It is well established that there is an increased prevalence of type 2 diabetes mellitus (8 %) and glucose intolerance (40 %) in patients with primary hyperparathyroidism$^{96}$. In addition, fasting PTH levels were shown to be inversely correlated with insulin sensitivity index in fifty-two normotensive, healthy subjects, even after adjustment for potentially confounding factors. Therefore vitamin D is important for insulin secretion.

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PTH levels are also regulated by vitamin D status. Serum levels of 25(OH)D, a marker for vitamin D status, are inversely correlated with fasting levels of serum PTH$^{94,95,99}$. Studies also support a negative relationship between serum 25(OH)D levels and PTH in healthy young women$^{93}$. It is intriguing that this study also demonstrates that 1,25(OH)2D is positively related to 25(OH)D, and 1,25(OH)2D shows no relationship with fasting serum PTH levels, contrary to the model that only PTH controls the rate of conversion of 25(OH)D to 1,25(OH)2D to regulate serum concentration. Overall, Ca homeostasis mediated by higher vitamin D status limits levels of fasting serum PTH. Therefore vitamin D may improve insulin action is by its ability to reduce PTH levels$^{10}$.

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factors (97), supporting the hypothesis that higher fasting serum PTH may mediate a reduction in glucose tolerance. These results support a role of physiological levels of PTH and 1,25(OH)₂D hormones in insulin action.

Likewise, PTH may mediate insulin resistance by reducing glucose uptake by liver, muscle and adipose cells. PTH treatment (16 h) decreased insulin-stimulated glucose transport (98) in an osteoblast-like cell type. GLUT1 mRNA was reduced in osteogenic sarcoma cells following the 16 h PTH treatment (98), suggesting a mechanism for the PTH-mediated reduction in glucose transport. In addition, PTH decreased insulin-stimulated glucose uptake in rat adipocytes (99). These studies suggest that PTH may elicit insulin resistance by reducing the number of glucose transporters (both GLUT1 and GLUT4) available in the membrane to promote glucose uptake. On the other hand, PTH has been shown to suppress insulin release (100) and to promote insulin resistance in adipocytes (101). Therefore, cumulatively, the results of these studies suggest that PTH may negatively affect insulin sensitivity through altering body composition and inhibiting insulin signalling.

Vitamin D, inflammation and emerging roles of vitamin D in reducing insulin resistance

The pathogenesis of diabetes is complex, and one factor proposed to mediate an increase in insulin resistance is inflammation, such as occurs in obesity. Several studies support a role for vitamin D and 1,25(OH)₂D, as an anti-inflammatory agent. For example, 1,25(OH)₂D inhibits the release of the pro-inflammatory cytokine TNFα and regulates the activity of NFκB (102–106), which functions as a mediator of TNFα pro-inflammatory actions, at multiple levels. In addition, 1,25(OH)₂D down-regulates the increased levels of inflammatory markers (TNFα, IL-6, IL-1, IL-8, cyclooxygenase-2, intercellular adhesion molecule-1 and B7-1) in monocytes from type 2 diabetic patients compared with monocytes from healthy controls (107). In other models, 1,25(OH)₂D inhibits the synthesis and actions of pro-inflammatory PG by inhibiting cyclooxygenase-2 expression, increasing the expression of the enzyme which inactivated PG (15-PG dehydrogenase) and decreasing PG receptors. 1,25(OH)₂D influences several pathways known to regulate inflammatory responses, including increasing mitogen-activated protein kinase phosphatase 5 which down-regulates p38 mitogen-activated protein kinase activity (108). Therefore, vitamin D may also function to reduce the risk of diabetes by acting to reduce inflammatory responses.

New roles of vitamin D to regulate insulin resistance are emerging. One such area is the role of vitamin D in the non-enzymic glycation of proteins. The process occurs more rapidly in diabetic patients than in normal individuals and measures of HbA1c in blood provide a clinical indicator of integrated blood glucose homeostasis during the previous 2–3 months. Glycated albumin similarly indicates glucose metabolism during the previous 21 d period. In addition to the role of glycation products in vascular complications, there is evidence that links impaired insulin signalling in skeletal muscle cells to glycated albumin (109). Recent studies support a role for vitamin D as a vascular protective agent against the effects of advanced glycation endproducts, which are proposed to mediate the devastating consequences of diabetes on cardiac complications (110).

Summary and future directions

There is substantial evidence to support an association between optimal vitamin D status, insulin sensitivity and health even for individuals with normal glucose homeostasis. Multiple mechanisms have been proposed with supportive results in the literature for the action of vitamin D to improve glucose homeostasis. Vitamin D may reduce risk factors for diabetes by reductions in fat mass and gains in lean mass, through the direct action of its active metabolite, through suppression of PTH, or a combination of these responses. Vitamin D may also mediate insulin sensitivity by improving Ca status, increasing local production of 1,25(OH)₂D, thus leading to transcriptional regulation of specific genes, or by suppressing serum levels of PTH. Likewise, 1,25(OH)₂D may act to enhance insulin synthesis and release, increase insulin receptor expression, and suppress inflammation, all three mechanisms acting to reduce the potential risks for insulin resistance. More directly, vitamin D may influence levels of PTH, which have recently been linked to insulin signalling at the level of the adipocyte. These effects of vitamin D, either acting in concert or alone, all serve to improve insulin sensitivity. The challenge that lies ahead is in determining the mechanisms and relative strengths of these pleiotrophic actions of vitamin D in order to make informed recommendations for vitamin D intakes that promote health and reduce the risks of onset of insulin resistance and progression to type 2 diabetes.

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References


