A role of vitamin D in low-intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus?

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Evidence from different directions, including observational and experimental studies, points to a role of vitamin D status in low-intensity chronic inflammation and insulin resistance in type 2 diabetes mellitus (T2DM). It has been recognised that insulin resistance and low-intensity chronic inflammation are risk factors for T2DM. Thus, vitamin D status can be implicated in the aetiology of TD2M. It is suggested that the relationship between vitamin D and low-intensity chronic inflammation and insulin resistance in T2DM can be mediated in part by the immune-modulating properties of the active form of vitamin D (1-α,25-dihydroxyvitamin D_3; 1,25(OH)$_2$D$_3$), which is able to down regulate the production of pro-inflammatory cytokines – particularly TNF-α, and IL-6. However, an association between vitamin D status and these features, which is independent of BMI, has been also reported. Non-calcaemic effects of vitamin D can be associated with health outcomes other than those traditionally attributed to the vitamin.

Vitamin D: Type 2 diabetes: Insulin resistance: C-reactive protein

Introduction

Various studies have shown a role of vitamin D in glucose homeostasis. In rats, 1-α,25-dihydroxyvitamin D_3 (1,25(OH)$_2$D$_3$) plays an essential role in normal insulin secretion, irrespective of dietary energy intake and serum Ca levels (Kadowaki & Norman, 1985). Vitamin D deficiency is also associated with poor β cell function, and the restoration of vitamin D normalises glucose metabolism (Kadowaki & Norman, 1985). Animal studies have demonstrated lower levels of vitamin D in a type 2 diabetes mellitus (T2DM) model, compared with controls (Ishimura *et al.* 1995).

In human subjects, low vitamin D concentrations have been associated with glucose intolerance and hyperinsulinaemic responses in oral glucose tolerance tests (Chiu *et al.* 2001*a*). Clinical studies have demonstrated a positive correlation between circulating vitamin D (25-hydroxyvitamin D; 25(OH)D) levels and insulin sensitivity (Chiu *et al.* 2001*a*, 2004). There is also experimental evidence of a reduction in insulin resistance, and improvement in glucose levels in oral glucose tolerance tests after vitamin D supplementation in diabetic subjects (Borissova *et al.* 2003; Zittermann, 2003). A recent study in a multi-ethnic sample of >6000 adults who participated in the Third National Health and Examination Survey (NHANES III) in the USA reported an inverse association between vitamin D status, diabetes and insulin resistance (Scragg *et al.* 2004).

One cannot evaluate the role of vitamin D in low-intensity chronic inflammation and insulin resistance in T2DM without taking into consideration that vitamin D status is related to adiposity (Arunabh *et al.* 2003; Parikh *et al.* 2004), and that adiposity is in turn related to both chronic inflammation and insulin resistance (Bays *et al.* 2004). These relationships, which are described below, are shown in Fig. 1.

Adiposity, low-intensity chronic inflammation and insulin resistance

There is increasing evidence from clinical and observational studies that a systemic, sub-clinical, low-intensity inflammatory reaction not only co-exists, but also precedes the development of T2DM (Schmidt *et al.* 1999; Barzilay *et al.* 2001; Pradhan *et al.* 2001; Ford, 2002; Freeman *et al.* 2002; Han *et al.* 2002; Vozarova *et al.* 2002; Engström *et al.* 2003). A characteristic of this inflammatory reaction is the presence of elevated serum acute-phase proteins and pro-inflammatory cytokines (Schmidt *et al.* 1999; Barzilay *et al.* 2001; Pradhan *et al.* 2001; Ford, 2002; Freeman *et al.* 2002;

Abbreviations: CRP, C-reactive protein; IRS, insulin receptor substrate; NHANES, National Health and Examination Survey; 1,25(OH)$_2$D$_3$, 1-α,25-dihydroxyvitamin D$_3$; 25(OH)D, 25-hydroxyvitamin D; T2DM, type 2 diabetes mellitus; VDR, vitamin D receptor.
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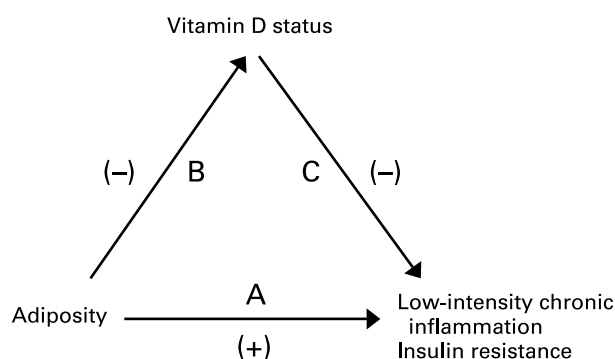
Fig. 1. Relationships among adiposity, vitamin D status, low-intensity chronic inflammation, and insulin resistance. Increased adiposity, as usually seen in type 2 diabetes mellitus (i.e. increased BMI and body fat mass), is positively associated with low-intensity chronic inflammation (i.e. elevated C-reactive protein (CRP) concentrations) and insulin resistance. This is likely to be related to the release of pro-inflammatory cytokines by the adipose tissue, particularly TNF-α and IL-6, which are known to cause insulin resistance and promote the synthesis of CRP by the liver (A). In turn, adiposity is negatively associated with vitamin D status, due to storage of vitamin D in the adipose tissue (B). Vitamin D status is negatively associated with both low-intensity chronic inflammation and insulin resistance. It is proposed that this can be related to down regulation of the production of pro-inflammatory cytokines by the active form of vitamin D $(1,25(OH)_2D_3)$ in immune cells infiltrating the adipose tissue (C).

Han *et al.* 2002; Vozarova *et al.* 2002; Engström *et al.* 2003). These observations have led to the hypothesis of a pathological role of an ongoing cytokine-induced acute-phase response in the pathogenesis of T2DM (Yudkin *et al.* 1999; Pickup, 2004). The inflammatory markers that have shown a strongest predictive capacity in the development of T2DM are C-reactive protein (CRP) and IL-6 (Schmidt *et al.* 1999; Yudkin *et al.* 1999; Barzilay *et al.* 2001; Pradhan *et al.* 2001; Ford, 2002; Freeman *et al.* 2002; Han *et al.* 2002; Vozarova *et al.* 2002; Engström *et al.* 2003; Pickup, 2004). It is important to note that CRP can be considered to be a surrogate marker for the activity of the pro-inflammatory cytokines IL-6 and TNF-α, since these cytokines act synergistically to promote the synthesis of CRP and other acute-phase proteins by the liver (Koj *et al.* 1988; Gewurz *et al.* 1995; Pepys & Hirschfield, 2003).

Epidemiological studies have shown that TNF-α, CRP and IL-6 are positively correlated with BMI and percentage body fat (Das, 2001; Bermudez *et al.* 2002; Pittas *et al.* 2004). CRP levels have been found to be elevated ($>3{\cdot}0$ mg/l) both in overweight and in obese individuals, and in subjects with T2DM (Das, 2001; Bermudez *et al.* 2002; Pittas *et al.* 2004). The strong positive correlation between adiposity and CRP levels may reflect in part the fact that adipocytes are the source of a large proportion of baseline IL-6 as well as TNF-α (Kershaw & Flier, 2004; Pittas *et al.* 2004). It has also been observed that macrophages infiltrate adipose tissue in obese individuals, contributing to the release of pro-inflammatory cytokines (Weisberg *et al.* 2003). These cytokines – particularly TNF-α, IL-6 and IL-1 – are able to cause insulin resistance in adipocytes (Xu *et al.* 2003). It is known that insulin is a potent inhibitor of lipolysis and restrains the release of NEFA from the adipocyte by

inhibiting the enzyme lipoprotein lipase (Bays *et al.* 2004). Thus, insulin resistance in adipocytes can lead to the release of large amounts of NEFA (Xu *et al.* 2003; Bays *et al.* 2004). Chronically elevated plasma NEFA, concentrations can lead to insulin resistance in muscle and liver by impairing tyrosine phosphorylation of insulin receptor substrate (IRS)-1 (Xu *et al.* 2003; Bays *et al.* 2004).

According to recent studies, both lipid-lowering agents (statins) and angiotensin-converting-enzyme inhibitors seem to have anti-inflammatory properties, and findings suggest that they may reduce the risk of diabetes (Yusuf *et al.* 2000; Freeman *et al.* 2001). Additionally, insulin-sensitising agents, such as thiazolidinediones (Jiang *et al.* 1998), as well as physical exercise (Smith *et al.* 1999) also appear to have anti-inflammatory properties. Recent studies on animal models suggest that salicylates can prevent obesity and diet-induced insulin resistance by down regulation of the inhibitor of κB kinase-β (IKK-β) activity (Yuan *et al.* 2001). IKK-β can induce insulin resistance by promoting serine phosphorylation of IRS-1 and subsequent impairment of insulin signalling (Stevenson *et al.* 1999). In agreement with this hypothesis, an experimental study in T2DM patients, who received high-dose aspirin (about 7 g/d for 2 weeks), showed an improvement of fasting and postprandial hyperglycaemia and glucose disposal by 19 to 25 % (Hundal *et al.* 2002).

A recent study in fifty obese and fifty age-matched, normal-weight premenopausal women (Esposito *et al.* 2003) demonstrated a significant association between lower serum concentrations of the anti-inflammatory cytokine IL-10 and the metabolic syndrome (Beutler *et al.* 1985), independent of age and body weight. It is known that individuals with the metabolic syndrome are at increased risk of developing T2DM (Han *et al.* 2002). The same study showed that after 12 months of a lifestyle programme, there was a decrease in body weight, which was associated with a significant decrease in IL-6, CRP, and IL-10 levels in obese women without the metabolic syndrome. In this study, increasing circulating levels of IL-10 were observed in obese women without the metabolic syndrome. This presumably tends to limit the chronic inflammatory state associated with obesity, as IL-10 down regulates the production of pro-inflammatory cytokines. However, the contrary – i.e. low IL-10 levels – was observed in women with the metabolic syndrome (Esposito *et al.* 2003). In summary, these studies suggest that reducing inflammation may have beneficial effects on insulin resistance.

Metabolic effects of pro-inflammatory cytokines

The metabolic effects of IL-1 and TNF-α – two of the main pro-inflammatory, Th1 cytokines – are remarkable. These cytokines are able to inhibit the enzyme lipoprotein lipase, and thus can be conducive to elevated levels of VLDL and hypertriacylglycerolaemia (Beutler *et al.* 1985; Beutler & Cerami, 1985; Tracey & Cerami 1990). In addition, they exert important effects on glucose and energy metabolism. It has been observed that the action of IL-1 in the liver produces dislypidaemia, with elevation of VLDL and reduction of HDL, similar to that observed in the metabolic

syndrome (Beutler & Cerami, 1985). Other effects of stimulation with TNF-α can lead to metabolic changes of the kind observed in the metabolic syndrome, such as hypertriacylglycerolaemia, redistribution of adipose tissue and insulin resistance (Beutler *et al.* 1985; Beutler & Cerami, 1985; Tracey & Cerami, 1990).

It has been demonstrated experimentally both in animals and human subjects that the administration of IL-6 induces gluconeogenesis, which leads to hyperglycaemia and compensatory hyperinsulinaemia (Stevenson *et al.* 1999; Yuan *et al.* 2001). Circulating IL-6 levels correlated positively with circulating triacylglycerol levels in subjects who were otherwise normal and in no acute distress. Moreover, polymorphisms in the promoter region of the IL-6 gene results in constitutively higher circulating IL-6 levels, as well as higher circulating triacylglycerol but not cholesterol levels (Fernandez-Real *et al.* 2000). It has been suggested that in the basal, healthy state IL-6 may affect triacylglycerol levels by decreasing lipoprotein lipase activity (Papanicolaou, 2000).

Recently, it has been demonstrated that IL-6 inhibits insulin receptor signal transduction by inducing a family of negative signal transducers (SOCS), which interact directly with insulin receptors (Senn *et al.* 2002). Moreover, IL-6 inhibits the effect of insulin in rat hepatocytes and in human liver cancer cells HepG2 (Senn *et al.* 2002). There is evidence suggesting that the metabolic effects of TNF-α could be mediated by its capacity of inducing IL-6 production and promoting the expression of IL-6 receptor in the liver and muscle (Cheung *et al.* 1998). These cytokines, which are part of the immune system, can be acting as a link between metabolic and immune processes (Pittas *et al.* 2004). The metabolic effects of these pro-inflammatory cytokines have motivated researchers to consider them as potential therapeutic targets.

In a study to test for a causative role of IL-6 in insulin resistance *in vivo* it was shown that treatment by IL-6 reduced insulin-dependent autophosphorylation of IRS-1 and IRS-2 (Klover *et al.* 2003). Both acute (90 min) and 5 d IL-6 exposure resulted in significant impairment of early insulin signalling events in the liver of treated mice, compared with controls. Importantly, circulating exogenous IL-6 levels only increased basal levels by 30–50 %, similar to increases observed in human obesity (Klover *et al.* 2003). In an *in vitro* study, the acute (10–60 min) and long-term (1–24 h) effects of IL-6 and TNF-α were assessed in differentiated 3T3-L1 cells (Rotter *et al.* 2003). Both IL-6 and TNF-α significantly reduced the mRNA and protein levels of IRS-1 as well as GLUT-4 and PPAR-γ. It was concluded that IL-6 and TNF-α exert long-term inhibitory effects on the expression levels of key signalling molecules for insulin (Rotter *et al.* 2003). In another study, the effects of 4 h treatment of IL-6 on insulin action were examined in awake mice during a 2 h hyperinsulinaemic–euglycaemic clamp (Higashimori *et al.* 2003). Insulin-stimulated whole-body glycolysis and glycogen and lipid synthesis were decreased by 30 and 10 %, respectively, with IL-6. Similarly, IL-6 treatment reduced insulin-stimulated skeletal muscle glycolysis and glycogen synthesis by 50 and 20 %, respectively. These defects in insulin action were secondary to decreases in IRS-1 PI 3-kinase and protein C

kinase activity in skeletal muscle, following IL-6 treatment (Higashimori *et al.* 2003).

Contrary to the metabolic effects of IL-6 and other pro-inflammatory cytokines, there is experimental evidence of anti-diabetogenic effects of IL-10, which provides insight into a novel mechanism by which anti-inflammatory cytokines may prevent insulin resistance (Kim *et al.* 2003). In an *in vivo* study, the effects of 2 h pre-treatment of IL-10 on lipid-induced and IL-6-induced insulin resistance were examined in awake mice during a 2 h hyperinsulinaemic–euglycaemic clamp (Kim *et al.* 2003). Insulin-stimulated IRS-1 associated PI 3-kinase activities in skeletal muscle were reduced by 60 and 40 % following lipid and IL-6 treatment, respectively. IL-10 pre-treatment prevented both lipid-induced and IL-6-induced reduction in skeletal muscle PI 3-kinase, thus preventing insulin resistance in skeletal muscle (Kim *et al.* 2003).

Adiposity and vitamin D status

It has been shown that vitamin D status has a negative correlation with BMI and body fat mass, and obesity is associated with alterations in the vitamin D endocrine system (Arunabh *et al.* 2003; Parikh *et al.* 2004). In a study in 410 healthy women (mean age 47·6 (SD 14·8) years; mean BMI 23·9 (SD 2·9) kg/m^2) the correlation between serum 25(OH)D and percentage of body fat was −0·13 ($P = 0·013$), after adjusting for race, age, season, and dietary vitamin D intake (Arunabh *et al.* 2003). In another study in 302 healthy adults (154 obese; mean BMI 37·3 (SD 5·8) kg/m^2, and 148 non-obese; mean BMI 25·6 (SD 2·9) kg/m^2), serum 25(OH)D was negatively correlated with BMI (r −0·40; $P < 0·0001$) and body fat mass (r −0·41; $P < 0·0001$) (Parikh *et al.* 2004). It is thought that obesity-associated vitamin D insufficiency is related to decreased availability of vitamin D from cutaneous and dietary sources because of its deposition in the adipose tissue (Worstman *et al.* 2000; Arunabh *et al.* 2003; Parikh *et al.* 2004).

A high prevalence of hypovitaminosis D has been reported in women with T2DM, who also had a higher BMI compared with controls (Isaia *et al.* 2001). In a sample of 6228 individuals aged ≥20 years from NHANES III, an inverse relationship between BMI and serum 25(OH)D was observed, after adjusting for confounders (Scragg *et al.* 2004).

Vitamin D status, low-intensity chronic inflammation and insulin resistance

Vitamin D deficiency has been correlated with circulating markers of inflammation in adult subjects (Timms *et al.* 2002). In a study carried out in fifty-four British adults of Bangladeshi origin, aged 35–65 years, free of known diabetes, hypertension or other ongoing illness, who were vitamin D deficient (baseline 25(OH)D < 11 ng/ml) (Timms *et al.* 2002), CRP concentrations were inversely correlated to vitamin D status, and serum 25(OH)D was an independent determinant of CRP at baseline. In the aforementioned study on British adults of Bangladeshi origin, fifty-four vitamin D-deficient subjects were

randomised and given either a 'high' dose (1250 µg; 50 000 IU) or a 'low' dose (12.5 µg; 500 IU) of a depot (oily) injection of cholecalciferol, every 3 months, during 1 year. After follow-up, there was an increase in vitamin D repletion by 100 %, which was similar in both treatment groups (baseline 25(OH)D mean 8.55 (SD 2.47) ng/ml; after supplementation 14.05 (SD 4.9) ng/ml). Baseline CRP was on average 6.12 (SD 5.87) mg/l, and was not statistically different between treatment groups. However, the reductions in CRP were greater in the 'high' than in the 'low' treatment group (39.65 v. 4.8 %, respectively; $P < 0.05$). The authors concluded that vitamin D deficiency is associated with elevated CRP concentrations, which are correctable by supplementation (Timms et al. 2002).

A study carried out in California, in 126 glucose-tolerant, young adults (mean age 26 (SD 6) years), with no evidence or history of chronic or acute illness, showed a positive correlation between 25(OH)D serum levels and insulin sensitivity (r 0.2469; $P = 0.0007$), after controlling for sex, BMI, age, ethnicity, and systolic blood pressure (Chiu et al. 2004). In the same study, the correlation between 25(OH)D and first-phase insulin secretion was -0.2513 ($P = 0.0045$), and -0.3487 ($P = 0.0001$) with second-phase insulin secretion. Notably, these relationships were observed within the whole spectrum of 25(OH)D levels, even among individuals with 25(OH)D concentrations between 50 and 100 ng/ml, and thus were not strictly related to vitamin D deficiency. In this study, serum 25(OH)D had a negative correlation with plasma glucose concentration during a standard 75 g oral glucose tolerance test at 60 min (r -0.2878; $P = 0.0011$), at 90 min (r -0.2872; $P = 0.0011$), and at 120 min (r -0.2988; $P = 0.0007$) (Chiu et al. 2004). Individuals with vitamin D deficiency – 25(OH)D below 20 ng/ml – had a higher risk of insulin resistance and a higher risk for the metabolic syndrome, compared with individuals without vitamin D insufficiency (relative risk 2.72; $P = 0.0097$). Surprisingly, 37 % of the study subjects had serum 25(OH)D below 20 ng/ml (Chiu et al. 2004).

In a study in 187 overweight adults (mean BMI 33.4 (SD 6.8) kg/m^2) the relationships between cacitriopic hormones and insulin sensitivity were examined (Parikh et al. 2003). In a multiple regression model controlling for body fat mass, race, age and sex, serum 25(OH)D was negatively correlated (r -0.19; $P < 0.014$) with Homeostasis Model Assessment for Insulin Resistance, and positively correlated (r 0.264; $P = 0.002$) with Quantitative Insulin Sensitivity Check Index. Intact parato-hormone was positively correlated (r 0.276; $P = 0.0002$) with Homeostasis Model Assessment for Insulin Resistance, and negatively correlated (r -0.23; $P = 0.0026$) with Quantitative Insulin Sensitivity Check Index (Parikh et al. 2003).

In a recent report from NHANES III, in which serum 25(OH)D levels were assessed in a multi-ethnic sample of 6228 adults, an inverse association between quartiles of serum 25(OH)D and diabetes was found (Scragg et al. 2004). In that study, a fourfold reduction in diabetes risk was observed in non-Hispanic whites who were at the highest quartile of serum 25(OH)D (≥ 81.0 nmol/l), compared with those in the lowest quartile (serum 25(OH)D < 44.0 nmol/l),

after adjusting for age, sex, BMI, leisure time physical activity and seasonality (Scragg et al. 2004). A similar trend was observed in Mexican-Americans. These analyses excluded cases with known, diagnosed diabetes (Scragg et al. 2004).

Experimental studies of vitamin D treatment and glucose homeostasis are scarce, usually involve a small number of subjects, use a wide range of treatment regimens, and include different morbid conditions. Despite these dificulties, a recent review (Zittermann, 2003) presented evidence from seven studies in which insulin and glucose response to treatment with vitamin D was assessed. A reduction in blood glucose and insulin levels was observed in five of these studies (Zittermann, 2003). The age of the patients ranged from 17 to 66 years, and duration of treatment ranged from 2 h (intravenous infusion) to 5 months. Doses of vitamin D administered ranged from 0.75 µg calcitriol/d to 2500 µg vitamin D. Reductions in blood glucose ranged between 0.8 and 1.6 mmol/l after a glucose tolerance test (Zittermann, 2003). However, in one study in which subjects received a single injection of 2500 µg vitamin D, an increase of 1.8 mmol/l in blood glucose was observed (Boucher et al. 1995).

A study of vitamin D supplementation in ten women with T2DM and seventeen non-diabetic age- and BMI-matched non-supplemented controls (Borissova et al. 2003) showed a non-significant decrease of 21 % in insulin resistance, after 1 month of receiving 33.3 µg (1332 IU) of cholecalciferol. A significant increase of 34.3 % on first-phase insulin secretion was observed in the treatment group ($P < 0.018$; Borissova et al. 2003).

Immune-regulating effects of vitamin D: plausible mechanism for its actions on low-intensity chronic inflammation and insulin resistance

Most of the actions of the active form of vitamin D ($1,25(OH)_2D_3$) are mediated by the intracellular vitamin D receptor (VDR), a ligand-activated transcription factor, which is a member of the steroid–thyroid hormone receptor family (DeLuca & Zierold, 1998). VDR forms a heterodimer with the retinoid X receptor, and this complex regulates the expression of target genes by binding to vitamin D-responsive elements in their promoter regions (DeLuca & Zierold, 1998). Recent studies have shown a linkage between VDR and the immune system, with the presence of VDR in several immune cells, such as monocytes, macrophages, and activated T and B lymphocytes (O'Kelly et al. 2002; Pichler et al. 2002; Matheu et al. 2003). Interference with cytokine production of monocytes and lymphocytes seems to be a key mechanism by which $1,25(OH)_2D_3$ interacts with the immune system (Pichler et al. 2002; Mahon et al. 2003). Importantly, VDR is present in the adipose tissue (Kershaw & Flier, 2004).

In addition to its well-known bone and Ca- and P-related metabolic functions, the active form of vitamin D ($1,25(OH)_2D_3$) is an important immune-system regulator (Cantorna et al. 2004). Vitamin D has been shown to inhibit cell proliferation and cytotoxic T-cell function in mixed lymphocyte cultures. When added to mitogen-stimulated PMBC, $1,25(OH)_2D_3$ decreases proliferation, immunoglo-

bulin synthesis and cytokines, including IL-1, IL-2, IL-6, TNF-α, and interferon-γ (Lemire, 1995; Becker *et al.* 2002; Lagraine & Lebranchu, 2003). The observation that $1,25(OH)_2D_3$ inhibits the Th1-specific cytokines IL-12 and interferon-γ led to the hypothesis that its immunosuppressive properties were achieved mainly by regulation of Th1 cells (Lemire *et al.* 1995). However, it has been found that $1,25(OH)_2D_3$ not only inhibits the synthesis of interferon-γ, IL-2 and IL-12 production by monocytes, but it also increases synthesis of IL-4, IL-5 and IL-10 (Long & Santos, 1999). The receptor for IL-10 has also been found to be inducible up to 10-fold in HaCaT cells on exposure to $1,25(OH)_2D_3$. Overall, these findings indicate that this hormone inhibits the Th1 response and may promote a Th2 response (Long & Santos, 1999). It has been reported that administration of $1,25(OH)_2D_3$ suppressed the incidence of diabetes in NOD mice, probably by modulating the Th1–Th2 cytokine balance in the pancreatic lesion towards a Th2 profile (Mathieu *et al.* 1992; Adorini, 2003).

There is experimental evidence showing that $1,25(OH)_2D_3$ can down regulate IL-6 production. In human skin fibroblasts and epidermal keratinocytes stimulated with sulfur mustard, $1,25(OH)_2D_3$ suppressed IL-6 secretion by 82 % (Arroyo *et al.* 2003). In this study, stimulation of human skin fibroblasts with sulfur mustard (10^{-4} M for 24 h at 37 °C) resulted in approximately a five-times increase in the secretion of IL-6, and over a ten-times increase for IL-8, which was inhibited by $1,25(OH)_2D_3$, at $\leq 10^{-9}$ M. $1,25(OH)_2D_3$ also suppressed IL-8 secretion by five times and IL-6 secretion by four times on sulfur mustard-stimulated human epidermal keratinocytes at concentrations $\leq 10^{-9}$ M. The effect of $1,25(OH)_2D_3$ on both IL-6 and IL-8 was dose-dependent (Arroyo *et al.* 2003).

Recently, it has been reported that $1,25(OH)_2D_3$ decreases DNA binding of NF-κB in human fibroblasts (Harant *et al.* 1998). The partial inhibition of NF-κB DNA binding by $1,25(OH)_2D_3$ is dependent on *de novo* protein synthesis, suggesting that $1,25(OH)_2D_3$ may regulate expression of cellular factors that contribute to reduced DNA binding of NF-κB (Harant *et al.* 1998, 2000). The ability of $1,25(OH)_2D_3$ to inhibit NF-κB activity in cell types involved in inflammation has contributed to the notion of using $1,25(OH)_2D_3$ as an anti-inflammatory agent (Harant *et al.* 1998, 2000; Arroyo *et al.* 2003).

The effect of vitamin D on the immune system – particularly on animal models of autoimmune and allergic disease – has been reviewed recently (Cantorna *et al.* 2004). Evidence indicates that the effect of treatment with $1,25(OH)_2D_3$ is consistent with inhibition of Th1 cells and induction of Th2 cells (Cantorna *et al.* 2004). It is worth noting that, while $1,25(OH)_2D_3$ has been known as an immunosuppresive agent, mostly for its role in allograft survival, it has shown no effect on infectious disease susceptibility in mice at doses used to improve allograft survival (Cantorna *et al.* 2004).

Of the greatest importance in the context of an adipocytokine-induced process of inflammation and insulin resistance in T2DM is that macrophages – as well as dendritic cells – not only express VDR, but also express the enzyme 1α-hydroxylase (Hewison *et al.* 2004). This gives them the ability to synthesise $1,25(OH)_2D_3$ in the presence of

substrate (25(OH)D) (Hewison *et al.* 2004). Thus – at least theoretically – macrophages infiltrating the adipose tissue in obese individuals could be able to modulate the release of pro-inflammatory cytokines in a paracrine fashion, if circulating 25(OH)D is available in adequate concentrations.

Conclusion

Evidence from different directions points to a protective role of vitamin D on low-intensity chronic inflammation and insulin resistance in T2DM. It is suggested that this role can be related – at least in part – to the immune-modulating actions of $1,25(OH)_2D_3$, which can down regulate the production of pro-inflammatory cytokines by immune cells. However, it is not known yet if this process can take place within the adipose tissue, which seems to be implicated in the development of systemic chronic inflammation and insulin resistance. It is to be noted that an inverse association between vitamin D status and insulin resistance has been observed in a sample of adult participants in the NHANES, which persisted after controlling for BMI (Scragg *et al.* 2004). More studies are needed to clarify these issues.

It is widely recognised that, on one hand, insulin resistance is a major feature of T2DM (Reaven, 1988), while, on the other hand, low-intensity chronic inflammation is related to the development of T2DM and to its cardiovascular complications (Bermudez *et al.* 2002; Pickup, 2004). Thus, vitamin D status can be implicated in the aetiology of TD2M. If this holds true, what should happen from the epidemiological and public health point of view? A brief description of a few consequences of this hypothesis follows:

(a) Since the main source of vitamin D is skin production after UVB light exposure (Holick, 2004*a*), a seasonality effect on T2DM incidence should have to be observed. This has been reported in a registry study on T2DM incidence in Sweden during a 5-year period, in which a higher incidence was observed during the winter months (Berger *et al.* 1999);

(b) Risk of T2DM will have to increase by age, since there is an age-related decrease in vitamin D skin production (Holick, 2004*b*). It is a well-known fact that T2DM risk increases with age (Centers for Disease Control and Prevention, 2003);

(c) Risk of T2DM should be higher in individuals with highly-pigmented skin, compared with individuals with little skin pigmentation, since dark skin absorbs less UVB light and then is less able to synthesise vitamin D (Holick, 2004*a*). This also has been observed (Zittermann, 2003). However, an association between vitamin D status and insulin resistance was not observed in African-Americans in the NHANES study (Scragg *et al.* 2004);

(d) Impairment of vitamin D action – for example, by the presence of VDR gene polymorphisms – should have to be related to a higher risk of T2DM. VDR gene polymorphisms – particularly *TaqI*, *BsmI*, *ApaI* and *FokI* – have been associated with insulin resistance and T2DM in different populations (Chiu *et al.* 2001*b*; Ogunkolade *et al.* 2002; Oh & Barrett-Connor, 2002). However, findings on this respect are still inconclusive.

Finally, vitamin D deficiency has been called an 'unrecognised epidemic' in the USA (Holick, 2004*a*),

while at the same time there is growing evidence implicating vitamin D status in a number of chronic ailments, as autoimmune disease, cancers and CVD (Holick, 2004*a*; Raiten & Picciano, 2004). Moreover, current vitamin D recommendations – which were originally set to prevent rickets (Vieth, 1999) – are likely to be reviewed soon (Weaver & Fleet, 2004). All this guarantees an increasing interest in the role of vitamin D on health in the near future. In this respect, the need for carefully designed studies, with clearly defined outcomes and accurate measures of vitamin D status, as well as the study of possible mechanisms, cannot be overemphasised.

Acknowledgements

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