Review article

Inadequate vitamin D status: does it contribute to the disorders comprising syndrome 'X'?

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Environmental factors are important in the aetiology of glucose intolerance, type II diabetes and IHD. The lack of vitamin D, which is necessary for adequate insulin secretion, relates demographically to increased risk of myocardial infarction. These disorders are connected, degenerative vascular disease increasing with glucose intolerance and diabetes and, with its risk factors, comprising syndrome 'X'. Evidence is presented suggesting that vitamin D deficiency may be an avoidable risk factor for syndrome 'X', adding another preventative measure to current recommendations which are aimed at reducing the worldwide epidemic of these disorders. Experimentally, vitamin D deficiency progressively reduces insulin secretion; glucose intolerance follows and becomes irreversible. Relationships between vitamin D status, glucose tolerance and 30 min insulin secretion during oral glucose tolerance tests are reported in British Asians; insulin secretion, but not glycaemia, improving with short-term supplementation. Studies showing reduction in blood pressure and in risk of heart attack and diabetes with exercise (usually outdoor), rarely consider the role of vitamin D status. Glycaemia and insulin secretion in elderly European men, however, relate to vitamin D status, independent of season or physical activity. Prolonged supplementation can improve glycaemia. Hypertension improves with vitamin D treatment with or without initial deficiency. Vitamin D status and climate are reviewed as risk factors for myocardial infarction; the risk reducing with altitude despite increasing cold. Glycaemia and fibrinogenaemia improve and insulin secretion increases in summer. Variation in vitamin D requirements could arise from genetic differences in vitamin D processing since bone density can vary with vitamin D-receptor genotype. Vitamin D receptors are present in islet β cells and we report insulin secretion in healthy Asians differing profoundly with the Apa I genotype, being independent of vitamin D status. Those at risk of vitamin D deficiency include the elderly, those living indoors or having a covered-up style of dress, especially dark-skinned immigrants, and pregnant women, and these are groups recognized as being at increased risk of diabetes.

Whilst it is well known that adequate supplies of vitamin D are vital for the development and integrity of bones, it is not generally appreciated that insulin production by the pancreatic β cell is also dependent on vitamin D, although there is a considerable literature in this field. It has been known for 30 years that Ca plays a part in insulin secretory responses to glucose, and the role of deficiency of vitamin D in the development of glucose intolerance and of diabetes in man and in animals has been extensively investigated. Both increased insulin resistance and reduced insulin secretion have been found with reduction in activity of, or in stores of, vitamin D (Milner & Hales, 1967; Cade & Norman, 1986; Labriji-Mestaghanmi *et al.* 1988). For a diabetologist in the East London Borough of Tower Hamlets, where one-quarter of Britain's Bangladeshi population lives, the 4–5-fold increase in prevalence of

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non-insulin-dependent diabetes (NIDDM) in this ethnic group, compared with that in Caucasians, remains an ever present challenge (McKeigue et al. 1992). In addition, we still see clinical osteomalacia locally, so that we have had to consider whether lack of vitamin D might be contributing to the increased prevalence of NIDDM in local Asian residents, in addition to the possible diabetogenicity of dietary factors, such as betel-nut (Areca catechu; pan), in this population (Boucher et al. 1994). The high prevalence of both diabetes and osteomalacia in British Asians noted in 1986 led to the comment that 'whether their vitamin D deficiency contributes to the pathogenesis of their diabetes is an area ripe for investigation' (Dandona et al. 1986).

Syndrome 'X'

Syndrome 'X', as described by Reaven (1995), is the term used to describe a cluster of disorders linked to insulin resistance, with the potential risk of glucose intolerance and eventual diabetes, and is especially common in immigrant Asians. Reavens' syndrome covers both the changes in the blood which increase the risk of clotting and those, such as the hyperlipidaemias, accelerating degenerative changes in the vasculature; factors which together cause increases in the incidence of strokes and heart attacks. Since Asians resident in the UK have an increased death rate from heart attack and stroke compared with those of indigenous Caucasians (Balarajan, 1996), this group of disorders presents a major health problem.

The scale of the health problems associated with syndrome 'X' can be appreciated when one considers that this term describes a clustering of disorders leading to degenerative diseases of the vasculature which account for one-third of deaths in the Western world. Impaired glucose tolerance (IGT) and NIDDM are common and carry equal increases in risk of heart disease. IGT and NIDDM are increasingly common, NIDDM affecting 3 % of Caucasians, 7 % of Americans over 40 years of age and at least 10 % of urban Asians in the Western world. The prevalence of NIDDM can be as high as 40–60 % in some populations which may carry a similar prevalence of IGT in addition, and the prevalence of both disorders increases in all groups with age. Atheromatous disease in diabetics increases standardized mortality rates 2–3-fold, 50 % of diabetics develop hypertension and 15–20 % of hypertensives have IGT, carrying the same risks as diabetes even though progression to diabetes is not invariable (Ferranini et al. 1987; Hjermann, 1992).

Specific disorders found in syndrome 'X', with or without the overt development of glucose intolerance, include hypertriacylglycerolaemia, raised LDL- and reduced HDL-cholesterol, hypertension, central (intra-abdominal) obesity and reduced insulin secretion, increased insulin resistance being an early and fundamental feature (see Table 1). Fasting hyperinsulinaemia, reflecting insulin resistance, impairment of glucose tolerance and eventual diabetes are common features, whilst increases in circulating free fatty acids, fibrinogen, plasminogen-activator inhibitor and reductions in fibrinolysis are also found (Kannel et al. 1991; Dhawan et al. 1994).

Table 1. Features of syndrome 'X' and risk factors for non-insulin-dependent diabetes mellitus

Features of syndrome 'X'
Increased insulin resistance
Hypertriacylglycerolaemia
Central obesity
Hyperlipidaemia
Hypertension
Increased fibrinogen, PAI1 (thrombogenic factors)
Glucose intolerance
Increased risk of diabetes
Risk factors for non-insulin dependent diabetes
Obesity (especially central)
Reduced physical activity
Inappropriate diet, e.g. high fat intake
Increased insulin resistance
Toxins (nitroso-compounds, medications)
Small stature
Low full-term birth weight

PAI1, plasminogen-activator factor 1.

Environment v. genetic origin of syndrome 'X'?

The recent increase in prevalence of this group of disorders in the last 30 years has led to the idea that they must have resulted from some evolutionary advantage. The 'Thrifty Genotype' hypothesis proposes this to be an enhanced ability to survive famine by increasing body fat stores in times of plenty (Neel, 1962). Alternatively the 'Thrifty Phenotype' hypothesis suggests that dietary deficiency in pregnancy acts as a trigger for the disorders comprising syndrome 'X', since the prevalence of adult diabetes, hypertension and hypertriacylglycerolaemia has been demonstrated to increase progressively with reduction in full-term birth weight in many different population groups, studied both in childhood and over the age of 50 years (Hales & Barker, 1992; Barker et al. 1993; Phillips et al. 1994). Insulin resistance, an integral part of this syndrome, has recently been shown to be increased in otherwise healthy adults who had been small babies, despite the absence of any other detectable feature of syndrome 'X' (Phillips et al. 1994). Reducing dietary protein intake from 200 to 80 g/kg in isoenergetic diets during gestation can induce permanent alterations in metabolic processes in the liver in experimental animals (Petrie et al. 1997), together with increases in cortisol secretion (Phillips et al. 1997). The mechanisms by which such fetal programming is induced are being worked out and include permanent alterations in liver enzyme activities (Desai et al. 1997a). However, it is not necessarily the case that protein will prove to be the only maternal nutrient of importance in this respect.

Recent dramatic falls in the prevalence of NIDDM in less than 20 years, from the 40 % found in well-nourished Nauruans who were the offspring of a malnourished population, to the 20 % found in their descendants in the current generation of Nauruans (Dowse et al. 1991), suggest that genetic factors are unlikely to outweigh nutritional or other environmental factors in the causation of type 2 diabetes mellitus in that population.

Whilst genetic differences between populations are thought to play a large part in determining susceptibility

to NIDDM, environmental factors, or 'triggers', are also active in its induction (see Table 1; WHO Study Group, 1985; Khaw, 1994; Boucher, 1995).

The hypothesis

In view of the requirement of the β cell for vitamin D and of the high prevalence of vitamin D deficiency in many population groups, such as the elderly and immigrant Asians, the postulate that vitamin D deficiency is an avoidable risk factor for IGT and NIDDM, and thus also of diseases related to syndrome 'X', appears to be worthy of more general consideration. Syndrome 'X' may comprise unrelated phenomena (Cruickshank, 1995), but an overview of factors that could link disparate but common disorders is recommended when attempting to identify environmental triggers in common degenerative disorders where genetic predisposition may be necessary, but is not a sufficient explanation (LaPorte, 1995). The present review attempts such an approach in examining the postulate that vitamin D insufficiency could contribute to the development of syndrome 'X' in man.

Vitamin D metabolism

The ways in which vitamin D insufficiency, loss of activation, or inhibition of action can arise are clearly relevant to consideration of the available evidence. Sunlight induces cholecalciferol formation in man, and in the absence of adequate exposure to u.v. light, a minimal daily dietary intake of $10 \mu g$ is required. The basic pathways involved in the formation and activation of the effector hormone calcitriol (1,25-dihydroxyvitamin D; $1,25(OH)_2D$) are shown in Fig. 1. An outline of what is known of its receptor and post-receptor mechanisms follows. Hormonal vitamin D acts as a promotor for gene transcription of factors active in bone and in cells of the immune system. It also promotes cell differentiation and inhibits cell proliferation in many tissues and cell lines (Holick, 1995; Darwish et al. 1997). It is likely, therefore, that its action on the β cell is to promote transcription of genes relevant to insulin secretion and release.

Evidence from animal studies

Normal insulin secretion is dependent on $1,25(OH)_2D$ in both intact animals and isolated islets (Frankel et al. 1980; Kadowski et al. 1984; Nyomba et al. 1984; Billaudel et al. 1989; Levy et al. 1994). One mechanism of action is likely to be the Ca dependence of one of the β cell proinsulin-cleavage endopeptidases (Rhodes & Alarcon, 1994). Ca is also necessary for exocytosis of insulin from the β cell, and for β cell glycolysis which plays a part in signalling circulating glucose concentration (Milner & Hales 1967; Rutter et al. 1997). First-phase insulin release in response to glucose is impaired in early vitamin D deficiency, followed in sustained deficiency by a reduction in second-phase insulin secretion. Severe failure of insulin secretion then develops. In the early stages of experimental dietary vitamin D deficiency, both first- and second-phase

responses can be restored by the adequate administration of dietary vitamin D in the intact animal, or by adding $1,25(OH)_2D$ to incubations of isolated islets from vitamin D-depleted animals. In established vitamin D deficiency, recovery of β cell function cannot be achieved in short-term supplementation experiments (Kadowski, 1985; Cade & Norman, 1986).

A decrease in 25-hydroxy-vitamin D (25(OH)D) 1-α-hydroxylase (EC 1.14.13.13) activity in the kidneys, which is thought to be secondary to insulin deficiency, has been reported in experimental diabetes (Ikeda et al. 1987), but not specifically with diabetes, in man so far. This would provide the potential for a vicious circle, reducing insulin secretion, thereby worsening glycaemia.

$1,25(OH)_2D$ has been shown to increase mRNA for pre-proinsulin after binding to the β cell vitamin D nuclear receptors (VDR; Ozono et al. 1990). Reduced VDR activity in the kidney and in the endothelium of the gut in genetically-diabetic mice has been postulated to be secondary to hypoinsulinaemia (Ishida et al. 1988), but, if genetically determined, (see p. 321) could also contribute to the development of diabetes.

Evidence from studies in man

Insulin sensitivity has been shown to relate directly to vitamin D status in healthy people, independent of confounding factors such as weight, central obesity and age. This relationship extends to subjects without significant vitamin D deficiency as currently defined (r 0·77, $P < 0.05$; Lind et al. 1989a). Increases in insulin resistance associated with reduction in vitamin D status have also been described, especially in uraemia where loss of 1α-hydroxylation due to loss of nephrons produces an acquired deficiency state. This has been shown to improve after treatment with $1,25(OH)_2D$ (Gedik et al. 1986; Lind et al. 1989a; Kocian, 1992; Mak, 1992b; Beaulieu et al. 1993; Kumar et al. 1994; Orwoll et al. 1994). Vitamin D status has been shown to relate inversely to the severity of hypertension in Caucasian populations, both with and without vitamin D deficiency, and to have a similar inverse relationship to hypertriacylglycerolaemia (Lind et al. 1989b; Scragg et al. 1992; Barger-Lux & Heaney, 1994). Serum 25(OH)D has also been found to relate directly to circulating apolipoprotein A-1 ($P < 0.001$ in both men and women) and also to HDL-cholesterol ($P < 0.05$ in men and $P < 0.005$ in women) in 358 Belgians (Auwerx et al. 1992). Increased risk of myocardial infarction itself may be a feature of low vitamin D status, and the evidence for this has been comprehensively discussed by Scragg et al. (1992, 1995a) and Scragg (1996). An increased prevalence of myocardial infarction has been found in northern compared with southern European communities (e.g. Scotland $v.$ England or Finland $v.$ Italy) i.e. in those populations where vitamin D deficiency is more common (Balarajan et al. 1987). Both freedom from deficiency of vitamin D and an increased Ca intake have been suggested as protective against IHD (Knox, 1973; McCarron & Morris, 1987). Hypertension, a well-recognized risk factor for myocardial infarction, has been found to be increased with reduction in
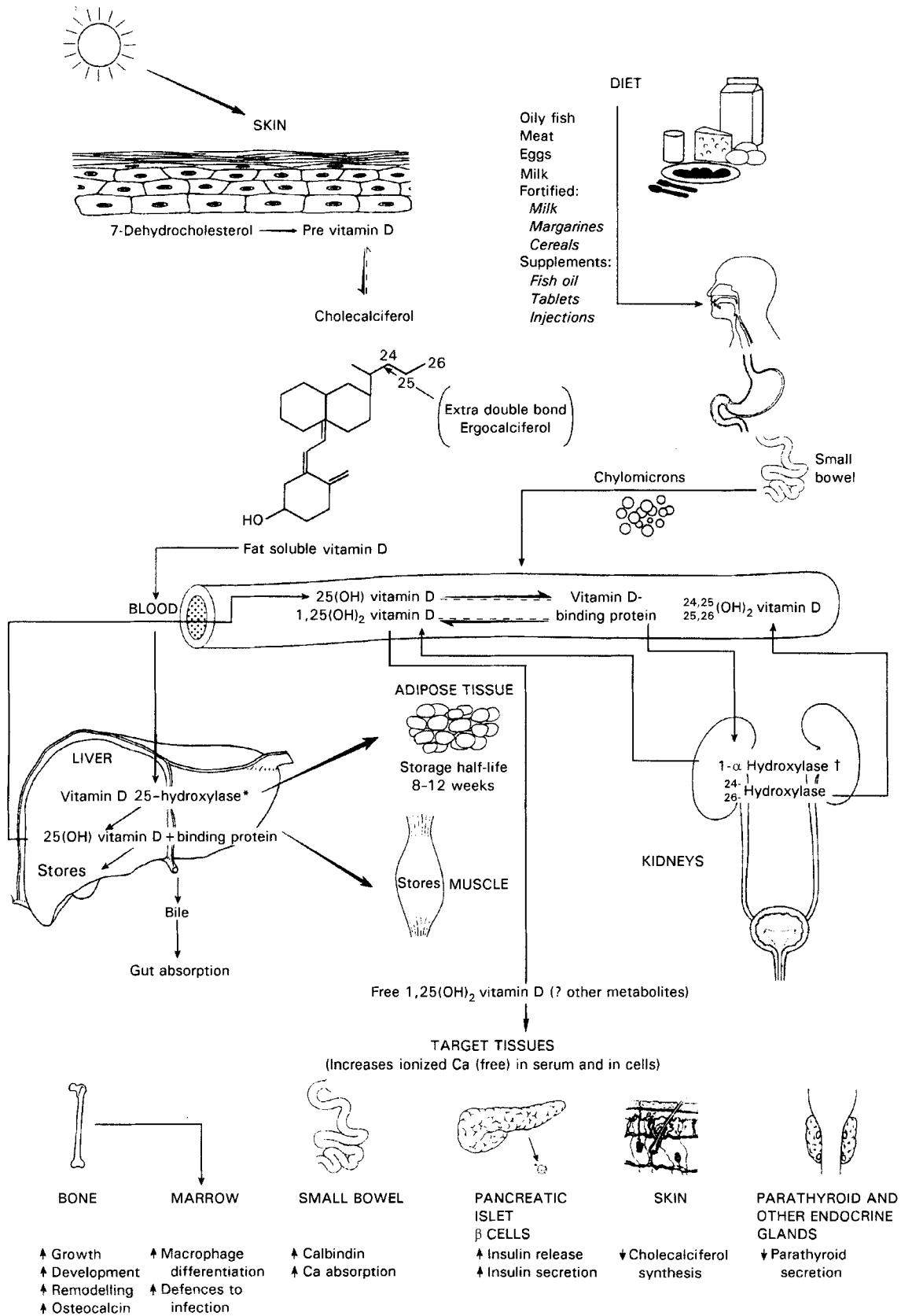
Fig. 1. The major metabolic pathways for vitamin D metabolism. 1-α hydroxylase, 25-hydroxyvitamin D 1-α hydroxylase (EC 1.14.13.13); 24-hydroxylase, 26-hydroxylase, 25-hydroxyvitamin D 24 (or 26)-hydroxylase. *Feedback regulation includes suppression of the activity of this enzyme by 25(OH) vitamin D. †Feedback regulation includes activation of this enzyme by parathyroid hormone and suppression by ionized calcium and 1,25(OH)₂ vitamin D. (From Stanbury & Mawer, 1990; Hewison, 1992; Haddad, 1995.)

available Ca and to improve with Ca intake and with vitamin D supplementation in several studies, even when subjects were not initially deficient (Lind *et al.* 1988; Kishimoto *et al.* 1993). Hypertension is well recognized as a feature of primary hyperparathyroidism. Improvement in the hypertension of spontaneously hypertensive rats with supplementary Ca is described. This is inhibited by continued administration of parathyroid hormone. In addition, correction of abnormal insulin resistance and glucose intolerance are well known after surgical cure of primary hyperparathyroidism (Kishimoto *et al.* 1993), with cure of NIDDM in one instance (Quin & Gumpert, 1997). The secondary hyperparathyroidism of vitamin D deficiency, as reported in other Indo-Asian diabetic patients (Serhan *et al.* 1997), may contribute, therefore, to the production of both glucose intolerance and of hypertension and to their progressive deterioration once diabetes is established.

Seasonal effects in man

Blood pressure, serum triacylglycerol and fibrinogen concentrations are higher in January and February than in July and August in UK Caucasians (Woodhouse *et al.* 1993, 1994; Scarabin *et al.* 1994). These findings have been attributed to ambient temperature, cold being a risk factor for arterial occlusion. (Lloyd, 1991; Wilmshurst, 1994; Khaw, 1995; The Eurowinter Group, 1997), but could also relate to the well-recognized seasonal fluctuations in vitamin D status found in the UK (McLaughlin *et al.* 1974; Maxwell, 1994). Fasting triacylglycerol and blood glucose concentrations, known to be lower in summer than winter in man, move in the opposite direction to seasonal changes in insulin secretion (Fahlen *et al.* 1971), showing improvement with the seasonal increases in vitamin D status seen in many European communities (van der Wielen *et al.* 1995).

The finding that IHD becomes less prevalent at increasing altitude, as temperatures drop but exposure to u.v. radiation is increased, supports the suggestion that vitamin D status may be more important than ambient temperature in determining long-term circulatory risk in these populations (Scragg, 1996). Neither blood pressure nor serum cholesterol, however, were altered in a recent trial using a single dose of oral vitamin D given in the winter to elderly people re-studied at about 5 weeks later in January and February, although the data for deficient subjects was not examined separately and the authors note that longer periods may be required for any benefits to develop (Scragg *et al.* 1995*b*).

It was on this background that we included measurement of vitamin D status in a study of a population of UK Asians which examined glucose tolerance in relation to dietary and anthropomorphic factors in 1990–1 (Boucher, 1995). Vitamin D deficiency was expected to be rare as a result of increasing medical awareness locally of the need for vigilance in the detection of vitamin D deficiency, and for Asians, in particular, to be advised on the maintenance of adequate vitamin D status. What was found, however, was a 95 % prevalence of vitamin D insufficiency (serum 25(OH)D < 20 nmol/l) in forty-four subjects deemed to be 'at risk' of glucose intolerance on spot glucose testing, as compared with 80 % in a small but comparable group of controls from within the same population. We found vitamin D status (as measured by circulating 25(OH)D) in Bangladeshi Asians to relate largely to diet in the winter months, specifically to the consumption of fish, ghee and, to a lesser degree, the consumption of eggs. Sunshine exposure has a major role in men, since vitamin D status varies with season of the year in our group as in Caucasians in the UK. The seasonal effect was much less, however, in women who are therefore especially dependent on diet throughout the year in this ethnic group. Previous findings showing glucose tolerance and insulin secretory capacity to relate directly to vitamin D status in man were confirmed in this group as follows. We found plasma true insulin, reflecting insulin secretory capacity at 30 min on oral glucose tolerance testing, to improve with increasing vitamin D status, (r 0·73, $P = 0·0001$) whilst glucose tolerance was reduced with reduction in vitamin D status ($r - 0·31, P = 0·04$), independent of other risk factors such as age, sex or anthropomorphic features relating to body build, in the group as a whole.

Subjects not known to have diabetes were subdivided into those 'at-risk' of diabetes (spot blood glucose values > 6·4 mmol/l if taken < 2 h after food or > 4·4 mmol/l if taken > 2 h after food) or 'not-at-risk' of diabetes (with spot blood glucose values less than those defining the 'at-risk' group). The same relationships of vitamin D status to insulin secretion and glucose tolerance were found in both subgroups as in the whole group, and were independent of age, weight, sex or body build. (We have found similar relationships in fifty-five vitamin D-deficient subjects but not in 120 non-deficient subjects in a current ongoing study (N Mannan, BJ Boucher, P Mills, K Noonan, D Syndercomb Court and CN Hales, unpublished results).) We also showed that giving a single parenteral dose of 2500 μg vitamin D increased plasma insulin 30 min after a standard 75 g oral glucose load 12 weeks later by a mean of + 160 % in our deficient subjects. Since the improvement in insulin secretory responses to glucose was greatest in those whose insulin responses to oral glucose were nearest to normal to start with, and since we saw no improvement in glycaemia despite these changes, we have a study in progress to determine whether subjects with vitamin D deficiency, 'at risk' of, but without diabetes at oral glucose tolerance testing, can be protected from progression towards NIDDM or from other features of syndrome 'X' by supplementation with vitamin D at 1250 μg three monthly over 1 year.

We have also been able to study a group of 142 elderly Dutchmen from the Zutphen subgroup of subjects within the Seven Nation Study, in collaboration with E Feskens and D Kromhout (Baynes *et al.* 1997). Of these subjects 39 % were vitamin D deficient. We found that vitamin D status related to physical (outdoor) activity rather than to diet. Glucose tolerance in these subjects was inversely related to vitamin D status independent of weight, age, physical activity, diet or of season itself ($r - 0·26, P < 0·01$). This relationship was independent of fish intake and of the intake of specific fats and oils in this subgroup,

despite the finding of an overall long-term protective effect of increasing fish consumption against NIDDM in Seven Nation Study subjects (Feskens *et al.* 1991).

It is of interest, therefore, that whilst our markedly-deficient British Asians (mean serum 25(OH)D 10·05 (SD 7·73, range 5·25–43·75) nmol/l) showed no specific relationship for fasting serum insulin (r 0·03, $P = 0·84$), but a positive relationship between 30 min serum insulin concentration (r 0·59, $P = 0·002$) and vitamin D status during an oral glucose tolerance test (n 44), the relationship between fasting insulin in our study on less-severely-deficient elderly Dutchmen (range of serum 25(OH)D 3·3–187·3 (normal range 26–180) nmol/l) and vitamin D status was an inverse one ($r - 0·22$, $P < 0·05$). These findings suggest that insulin resistance, the main abnormality in elderly Dutchmen, may with further reduction in vitamin D status, as in our Asian subjects, lead to predominant failure of insulin secretion.

Vitamin D in pregnancy

The possibility that maternal vitamin D deficiency could contribute to the syndrome 'X'-related health risks recently identified as being associated with low birth weight should also be considered. Whilst fetal survival in the presence of maternal malnutrition could be genetically determined, it has been shown experimentally that malnutrition, whether general or of protein, at certain specific stages of pregnancy leads to lack of fetal growth from which catch-up growth is not possible. Such effects are most marked if feeding is reduced during phases of rapid cell division, and it has been suggested that man is 'uniquely likely' to be susceptible to intra-uterine insults in terms of loss of subsequent growth of organs and of final adult height, since human progeny are born at such a relatively advanced stage of development (Widdowson & McCance, 1975). Since VDR are present in the islet β cell (Roth *et al.* 1982; Johnson *et al.* 1994), we should also consider whether vitamin D could play a part in programming factors other than birth weight. Vitamin D requirements are increased 4–5-fold in pregnancy, and experimental dietary depletion has been shown to be specifically associated with reduced fetal growth in animals. Vitamin D deficiency is also well known to reduce size and weight in the newborn human infant (Maxwell *et al.* 1981; Brommage & De Luca, 1984; Marya *et al.* 1987; Kuoppala, 1988; Nap & Hazewinkel, 1994). Whilst inadequate protein intake is a prime suspect, this finding suggests that deficiency of vitamin D could be an additional factor in the aetiology of the 'small baby' syndrome defining the 'Thrifty Phenotype' hypothesis. Supplementation is recommended for some groups of pregnant North American women at risk of vitamin D deficiency (Specker, 1994), and it would be of interest to know whether birth weight, subsequent insulin sensitivity or the glucose tolerance of their offspring may thus be altered.

Recent work has confirmed a reduction in weight and size of mice born to mice with dietary deficiency of vitamin D, with an increase in fasting glycaemia but no change in glucose tolerance in 3-month-old female offspring. Follow-up glucose tolerance testing, hopefully with blood pressure monitoring, of these offspring as they age will be of particular interest (Desai *et al.* 1997*b*).

Vitamin D in treatment of syndrome 'X'

The possibility that the prevalence of syndrome 'X', and its devastating sequelae, could be reduced by dietary supplementation in pregnancy requires investigation. If demonstrated, this would be a valuable addition to current policies, since compliance with nutritional advice is often good whilst mothers are concerned for the well-being of the unborn child. It is, in contrast, notoriously difficult to achieve compliance with policies advocating long-term lifestyle changes, such as reduction in dietary fat intake, loss of weight and increased physical activity in adults (Tremoli *et al.* 1986; Henry, 1994). Prolonged treatment of osteomalacia with vitamin D has been reported to improve glucose tolerance, although short-term treatment has generally been ineffective, despite some increase in insulin secretion, as in our own recent study (Clark *et al.* 1981; Ljunghall *et al.* 1987; Kocian, 1992; Kumar *et al.* 1994; Orwoll *et al.* 1994; Boucher *et al.* 1995). The efficacy of vitamin D treatment appears to be reduced by increasing duration of diabetes. No benefits have been found with vitamin D supplementation in terms of glucose tolerance in non-deficient subjects, although, as mentioned previously, blood pressure has been lowered.

1,25(OH)$_2$D supplementation, when activation of vitamin D by 1-α hydroxylation of 25(OH)D is deficient due to loss of nephrons, can normalize insulin secretion and glucose tolerance (Lind *et al.* 1988; Mak, 1992*a*, 1994; Kautsky-Willer *et al.* 1995). If 1-α-hydroxycholecalciferol is given intravenously for at least 3 months, it reduces triacylglycerol and increases HDL-cholesterol concentrations in the plasma. Correction of hypocalcaemia in a small group of patients with either rickets or hypoparathyroidism improved both insulin secretion and glycaemia (Bansal *et al.* 1975). Prophylactic 1,25(OH)$_2$D, or equivalent analogues, are in routine use to prevent renal osteodystrophy. This treatment has the additional beneficial effects of preserving insulin sensitivity and preventing glucose intolerance; indeed, it can also correct these abnormalities if started early (Kautsky-Willer *et al.* 1995).

Variation in requirements for vitamin D

Several factors could contribute to differences in vitamin D requirement between individuals, between populations, and with age. These include variations in absorption, in activation, and in rates of removal of 1,25(OH)$_2$D. We have shown, in studies into the marked increase in prevalence of vitamin D deficiency amongst the elderly, that absorption of vitamin D from the gut, for example, is reduced with age (Corless *et al.* 1975; Barragry *et al.* 1978). Reduced Ca absorption from the gut in response to 1,25(OH)$_2$D has also been reported in the elderly as well as reduction in renal 25(OH)D 1-α-hydroxylase activity, with loss of nephrons with age (as judged by 1,25(OH)$_2$D production in response to parathyroid hormone; Riggs *et al.*

1981; Ebeling *et al.* 1992). The combination of these changes increases susceptibility of the elderly to the development of clinical bone disease, especially when the adequacy of dietary vitamin D is borderline and exposure to u.v. from sunshine is minimal (van der Wielen *et al.* 1995; Scragg, 1996). These phenomena must similarly increase the risk of glucose intolerance with age.

Analysis of the data from the Survey in Europe on Nutrition in the Elderly: A Concerted Action, of about 2000 people from nineteen towns and villages in twelve countries across Europe, has shown an increased prevalence of vitamin D deficiency in the elderly from West to East and also from North to South of the Alps (van der Wielen *et al.* 1995). Glucose intolerance increased with increasing deficiency in about 900 of these subjects in whom 25(OH)D was measured, whilst insulin resistance, as judged by fasting insulin concentration, increased with increasing vitamin D deficiency, independent of the effects of anthropomorphic markers of diabetic risk ($r - 0.31$, $P = 0.05$ and $r - 0.21$, $P = 0.02$ respectively; A Teuscher and AU Teuscher, European Association for the Study of Diabetes subgroup on Dietetics and Nutrition 1996, personal communication). Vitamin C has been shown to be necessary for hydroxylation of cholecalciferol to its active form (Cantatore *et al.* 1991) and, therefore, deficiency may enhance the effects of vitamin D insufficiency.

Vitamin D receptor, post-receptor mechanisms

Earlier work on Asian boys with and without rickets despite comparable diet, vitamin D intake and exposure to sunshine led O'Hara-May & Widdowson (1976) to postulate that individuals might differ in vitamin D 'requirements' in respect of bone disease. Since the VDR is expressed in islet β cells, and alleles of the VDR gene are believed to relate to severity of osteoporosis in some populations (Morrison *et al.* 1994; Ferrari *et al.* 1995), there is also a possibility that genetic variation in islet β cell VDR activity could be associated with differences in insulin secretory responsiveness. Preliminary examination of our initial findings for insulin secretion during oral glucose tolerance tests, with VDR genotype, has shown significant variation with the AA allele, but not with the BB allele in Bangladeshis (findings with the TT allele are borderline), despite comparability of vitamin D status between subgroups (Hitman *et al.* 1998). Vitamin D action is effected after dimerization of its ligand-bound receptors (VDR), often with retinoid X receptors, and modulated by 9-*cis* retinoic acid (Colston, 1993) inducing transcription of target genes. The interaction of vitamin A and related compounds with post-receptor mechanisms provides an explanation for previously-reported interactions of vitamins A and D. Large oral doses of vitamin A have been shown experimentally to inhibit the expected toxicity of large doses of vitamin D and to induce rachitic changes in animals fed on normal diets (Metz *et al.* 1985). Vitamin A induces acute glucose intolerance in man (Chertow *et al.* 1982), and we have found it to induce hyperglycaemia in CD1 mice (Motahar & Boucher, 1997). Inhibition of islet

insulin release is found *in vitro* (Chertow & Baker, 1978). These findings suggest that consumption of unduly large amounts of vitamin A or related compounds could enhance the diabetogenic risks of vitamin D deficiency. This topic is likely, therefore, to warrant full review from an appropriate expert in the future, especially now that the retinoid X (α and β) receptors have now been found in insulin-secreting cells from the rat (Chertow *et al.* 1993, 1997).

Serum vitamin D-binding proteins

Vitamin D stores are estimated by measurement of 25(OH)D bound to vitamin D-binding protein. It is not known whether there is any variation in availability of reserves of vitamin D in relation to the known genotypes of the vitamin D-binding (group-specific component) protein, although their distribution varies with latitude and with skin pigmentation. An increase in prevalence of type 2 diabetes (NIDDM) has been found with the F1-F1 phenotype, which is associated with reduction in insulin secretion. Other combinations of alleles are found in relation to diabetes in the Bantu (Constans *et al.* 1985; Kamboh & Ferrell, 1986; Szathmary, 1987; Iyengar *et al.* 1989; Chen *et al.* 1990; Braun *et al.* 1992) and this aspect of vitamin D availability warrants further consideration.

Immune defence mechanisms

Vitamin D is known to have a role in promoting immune defence mechanisms and to be protective against infections such as tuberculosis (Hewison, 1992; Maxwell, 1994; Strachan *et al.* 1995). It can also be protective against some autoimmune processes. The insulitis leading to spontaneous insulin-dependent diabetes in the non-obese diabetic mouse can, for example, be prevented by the administration of the active hormone $1,25(OH)_2D$ and, indeed, by its non-hyperglycaemic analogues (Mathieu *et al.* 1995), which may provide interesting therapeutic options for consideration. Subjects with NIDDM in populations with a high risk of progression to insulin requirement may therefore be put at increased risk if they are also vitamin D deficient. The finding of a particularly high incidence of progression to insulin requirement in patients with NIDDM in association with certain human leucocyte antigen markers for insulin-dependent diabetes is especially intriguing, particularly since islet cell antibodies are a feature in such patients (Groop, 1988). Our group has recently found certain VDR alleles to be significantly associated with insulin-dependent diabetes, in association with both islet cell antibodies and anti-glutamic acid decarboxylase (*EC* 4.1.1.15) antibodies in family studies in southern Indians (McDermott *et al.* 1997).

Sunshine *v.* exercise or both?

Maxwell (1994) has already suggested to The Nutrition Society that many diseases with increased prevalence in northern latitudes (e.g. seasonal affective disorder, osteoporotic fractures and IHD) could relate to lack of vitamin D due to reduced exposure to sunshine. If disorders clustering

with IHD and IGT were similarly linked, one could extend these hypotheses to include not only NIDDM but syndrome 'X'. The implication would be that the prevalence of such disorders might be reduced by maintenance of adequate vitamin D status, adding to the currently recognized benefits of increased physical exercise and weight control.

Outdoor exercise is well known to improve vitamin D status. The cardiovascular benefits of increased vitamin D status and of outdoor exercise have already been emphasized, but most workers in this field have not examined vitamin D status when assessing the effects of physical activity. In studies that have specifically examined the possible role of vitamin D in atherogenic risk, vitamin D status has been found to correlate with blood pressure and LDL-cholesterol but not other risk markers of IHD in subjects without vitamin D deficiency. Single large oral doses of cholecalciferol did not improve risk markers, such as cholesterol, in these subjects (Scragg et al. 1992, 1995c). Any benefits of treatment with vitamin D are perhaps more likely to be found in deficient subjects, although significant reduction in blood pressure is found in treating non-deficient people (Lind et al. 1988).

Fish oil

There is a large amount of literature suggesting a protective effect of dietary fish and of supplemental fish oils against IHD through the n-3 fatty acids (Kromhout et al. 1985; Feskens et al. 1993; McKeigue, 1994). The prevalence of diabetes and IHD has been shown to be reduced with increasing fish consumption in Scandinavians, and with consumption of salmon or of seal oil in Alaska. Unfortunately, vitamin D status was not reported in these studies. The amounts of vitamin D found in fish can vary greatly, Pacific salmon (*Salmo onchrhynchus*) being said to be a good source of vitamin D whilst Atlantic salmon (*Salmo salar*), for example, is not. Therapeutic fish-oil preparations can contain 0–0·25 µg vitamin D/g so that it is to be hoped that future studies will include consideration of both vitamin D status and of the content of such foods or treatment (Fraser, 1995; Pritchard et al. 1995).

Who may be at risk?

Whilst vitamin D is produced in the skin following exposure to u.v. light, there are large areas of the Northern hemisphere where outdoor exposure to sunlight is insufficient for the normal self-regulatory production of active vitamin D to maintain adequate levels throughout the year. Progressive reduction in available sunlight has been shown to be mirrored by increases in population serum cholesterol concentrations in a global meta-analysis (Grimes et al. 1996).

Industrialization and resultant urbanization reduce exposure to sunlight throughout the Western world, and increase dependence on dietary sources of vitamin D. It is not surprising, therefore, that rickets was the first disease recognized as being caused by industrial smoke pollution (Hutchinson & Shah, 1921). Inadequate exposure to sunlight is common in people with black skins living in northern countries, but is also seen in many places with sunny climates. Rickets was, for example, common in affluent urban communities in Bombay in studies contributing to the discovery that sunlight prevents rickets. Affluent communities were found to live an indoor lifestyle, with children routinely kept indoors from birth to the age of 18 months (Park, 1923; Ihde, 1975). Vitamin D deficiency is currently widespread in the elderly in California, in Saudi men, the Bedouin and in the elderly across Europe from Britain to Italy, as well as in New Zealand and parts of Finland (Sedrani, 1984; Parviainen et al. 1992; McGrath et al. 1993; Brown & Brenton, 1994; van der Wielen et al. 1995). If vitamin D deficiency contributes significantly to the risks of syndrome 'X' and glucose intolerance, then clearly large numbers of people are currently at risk, and correction of such deficiency would be desirable.

British diets provide barely enough vitamin D to meet the minimal requirements for those not adequately exposed to u.v. light (10 µg daily; Department of Health and Social Security, 1979), let alone the increased requirements of pregnancy. The introduction of vitamin D supplementation of milk and some breakfast cereals in various countries should be reducing the prevalence of urban type 2 diabetes, if not of syndrome 'X'. Since Western communities are, however, being advised both officially and in the lay press to avoid eating fat and to beware of the carcinogenic risks of sunlight, specific efforts may have to be made to maintain adequate vitamin D status. The last time that this was done in the UK was during and after the Second World War, when the prevalence of type 2 diabetes fell strikingly. This fall was attributed to diet, especially to the high-fibre Ca-enriched National Loaf (Trowell, 1974). Wartime Britons were also supplied with cod-liver oil, one of 'five welfare foods for children' (Ministry of Food, 1945) which could have contributed to this phenomenon. Icelanders currently take cod-liver oil regularly and have less childhood insulin-dependent diabetes than is seen in other European countries; it would be interesting to know whether their prevalence of type 2 diabetes or of IHD is rising or falling (Iyer, 1991; Helgasen et al. 1992).

Standard vitamin D supplementation for babies fell out of favour after outbreaks of infantile hypercalcaemia due to over-zealous use of supplemented baby foods (Misselwitz et al. 1990), as it did for adults when excessive intake of fish oils was found to carry atherogenic risks and to increase circulating cholesterol (Davies, 1989; Moon et al. 1992). Supplementation in the UK is now limited to margarine, some yoghurt, breakfast cereals and, uncommonly, milk, although increased intake of fish is widely recommended as beneficial for the heart by doctors, nutritionists and the media.

If avoidance of vitamin D deficiency were to be the subject of future health promotions, a risk–benefit analysis of dietary supplementation, medication or u.v. light would be required in order to develop a safe and practicable policy for use in health promotion. Modest exposure to sunlight might well prove to be the safest for those with black skin, and is also especially effective in the elderly (Haddad, 1992), and carries no risk of vitamin D intoxication (see Fig. 1). Safe regimens for oral supplementation in the elderly are also available (Bryne et al. 1995).

Whilst antioxidant vitamins are under active consideration in relation to cardiovascular risk factors by many groups, vitamin D was not covered by presentations reported from a recent meeting of The Nutrition Society on the nutritional, environmental, dietary or lifestyle factors relevant to diabetes and insulin resistance in the symposium '*Diabetes towards the year 2000*' (The Nutrition Society, 1997). The following conclusions have been drawn, therefore, from the data presented to bring the 'poor relation', vitamin D, to the attention of workers in the field as a risk factor for diabetes, and for syndrome 'X', which is worthy of more general attention.

Conclusions and recommendations

1. The evidence available suggests that assessment of vitamin D status should be considered in the planning of future studies on environmental factors in the aetiology of NIDDM.

2. Assessment of vitamin D status should be included in studies of measures for the prevention of diabetes, unless deficiency is unknown in study subjects.

3. The proposition that vitamin D deficiency may contribute to the abnormalities leading to degenerative disease of the vasculature directly, rather than simply by increasing the risk of diabetes, warrants further investigation.

4. It would be helpful if vitamin D status were assessed, by measurement of circulating 25(OH)D concentration, in any future studies which concern measures of physical activity or diet in relation to insulin sensitivity, secretion, glycaemia or other aspects of syndrome 'X', since many of the features characterizing syndrome 'X' are recognized as being associated with reduced physical (outdoor) activity which itself improves vitamin D status, an effect which could confound studies on the benefits of exercise.

5. 'Normal ranges' for circulating 25(OH)D concentration have been used to define vitamin D status in healthy people adequately exposed to sunlight. Minimal recommended daily intakes of dietary vitamin D are defined by the capacity to prevent rickets and osteomalacia and are expected to maintain serum 25(OH)D concentration within the normal range. It is possible that there is an optimum for vitamin D status. Adverse effects may result from higher as opposed to lower 'normal' concentrations of serum 25(OH)D, in terms of hyperlipidaemia or other metabolic changes; optimal vitamin D status, therefore, may need to be re-defined. It would be especially desirable to examine this hypothesis in pre-morbid populations with a high risk of degenerative heart disease.

6. The effects of treating deficient subjects with vitamin D should be clearly distinguished from the effects of supplementation in non-deficient subjects, both in the examination of findings relevant to glycaemia, and of findings relevant to lipidaemia, thrombogenesis or other markers of syndrome 'X', since such effects may vary with status.

7. Whilst the avoidance of vitamin D deficiency remains a desirable measure for the promotion of good health, attempts to maintain serum concentrations of 25(OH)D at or above the upper limit of current normal ranges should be avoided until the questions posed by the current state of knowledge have been answered.

8. Studies of the effects of variation in dietary intake of vitamin A and vitamin A metabolites on insulin secretion and glucose tolerance in man are required with concomitant assessment of vitamin D status.

9. The possibility that the adverse effects of large intakes of dietary fish oils on glycaemia may relate to their very considerable vitamin A content requires investigation, since supplementation with vitamin D, free of vitamin A, is also widely available.

References

Auwerx J, Bouillon R & Kesteloot H (1992) Relation between 25-hydroxyvitamin D3, apolipoprotein A-1, and high density lipoprotein cholesterol. *Arteriosclerosis and Thrombosis* **12**, 671–674.

Balarajan R (1996) Ethnicity and variations in mortality from coronary heart disease. *Health Trends* **28**, 45–51.

Balarajan R, Yuen P & Machin D (1987) Inequalities in health: Changes in RHA's in the past decade. *British Medical Journal* **294**, 1561–1564.

Bansal S, Rizui SNA, Rao MB & Vaishana H (1975) Effect of hypocalcaemia on glucose tolerance, insulin release and free fatty acid levels in human subjects. *Postgraduate Medical Journal* **51**, 471–475.

Barger-Lux MJ & Heaney RP (1994) The role of calcium intake in preventing bone fragility, hypertension, and certain cancers. *Journal of Nutrition* **124**, 1406S–1411S.

Barker DJP, Hales CN, Fall CHD, Osmond C, Phipps K & Clark PMS (1993) Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. *Diabetologia* **36**, 62–67.

Barragry JM, France MW, Corless D, Gupta SP, Switala S, Boucher BJ & Cohen RD (1978) Intestinal cholecalciferol absorption in the elderly and in younger subjects. *Clinical Science and Molecular Medicine* **55**, 213–220.

Baynes K, Boucher BJ, Feskens EJM & Kromhout D (1997) Vitamin D, glucose tolerance and insulinaemia in elderly men. *Diabetologia* **40**, 344–347.

Beaulieu C, Kestekian R, Havrankova J & Gascon-Barre M (1993) Calcium is essential in normalizing intolerance to glucose that accompanies vitamin D depletion in vivo. *Diabetes* **42**, 35–43.

Billaudel B, Faure A, Labriji-Mestaghanmi H & Sutter BCJ (1989) Direct in vitro effect of 1,25-dihydroxyvitamin D3 on islet insulin secretion in vitamin deficient rats: Influence of D3 pretreatment. *Diabete et Metabolisme* **15**, 85–87.

Boucher BJ, Ewen SW & Stowers JM (1994) Betel nut (*Areca catechu*) consumption and the induction of glucose intolerance in adult CD1 mice and in their F1 and F2 offspring. *Diabetologia* **37**, 49–55.

Boucher BJ (1995) Strategies for reduction in the prevalence of NIDDM; the case for a population-based approach to the development of policies to deal with environmental factors in its development. *Diabetologia* **38**, 1125–1129.

Boucher BJ, Mannan N, Noonan K, Hales CN & Evans SJW (1995) Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in East London Asians. *Diabetologia* **38**, 1239–1245.

Braun A, Bichmaier R & Cleve H (1992) Molecular analysis of the gene for the human vitamin-D-binding protein (group-

specific component): allelic difference of the common genetic GC types. *Human Genetics* **89**, 401–406.

Brommage R & De Luca HF (1984) A maternal defect is responsible for growth failure in vitamin D-deficient rat pups. *American Journal of Physiology* **246**, E216–E220.

Brown AC & Brenton B (1994) Dietary survey of Hopi native American elementary students. *Journal of the American Dietetic Association* **94**, 517–522.

Byrne PM, Freaney R & McKenna MJ (1995) Vitamin D supplementation in the elderly: review of safety and effectiveness of different regimes. *Calcified Tissues International* **56**, 518–520.

Cade C & Norman AW (1986) Vitamin D3 improves impaired glucose tolerance and insulin secretion in the vitamin D-deficient rat in vivo. *Endocrinology* **119**, 84–90.

Cantatore FP, Loperfido MC, Magli DM, Mancini L & Carrozzo M (1991) The importance of vitamin C for hydroxylation of vitamin D3 to 1,25(OH)$_2$D3 in man. *Clinical Rheumatology* **10**, 162–167.

Chen LZ, Easteal S, Board PG, Summers KM, Bhatia KK & Kirk RL (1990) Albumin-vitamin-D-binding protein haplotypes in Asian-Pacific populations. *Human Genetics* **85**, 89–97.

Chertow BS & Baker GR (1978) The effects of vitamin A on insulin release and glucose oxidation in isolated rat islets. *Endocrinology* **103**, 1562–1572.

Chertow BS, Blaner WS, Rajan N, Primerano DA, Meda P, Cirulli V, Krozowksi Z, Smith R & Cordle MB (1993) Retinoic acid receptor, cytosolic retinol-binding and retinoic acid binding protein mRNA transcripts and proteins in rat insulin-secreting cells. *Diabetes* **42**, 1109–1114.

Chertow BS, Driscoll HK, Goking NQ, Primerano D, Cordle MB & Mathews KA (1997) Retinoid-X receptors and the effects of 9-*cis*-retinoic acid on insulin secretion from RINm5F cells. *Metabolism* **46**, 656–660.

Chertow BS, Sivitz WI, Baranetsky NG, Styer TB, Sorensen BJ, Schickman CH, Norris RM & Ozuk BA (1982) Vitamin A palmitate decreases intravenous glucose tolerance in man. *Acta Vitaminologica et Enzymologica* **4**, 291–298.

Clark SA, Stumpfe WE & Sar M (1981) Effect of 1,25 dihydroxyvitamin D3 on insulin secretion. *Diabetes* **30**, 382–386.

Colston KW (1996) New concepts in hormone receptor action. *Lancet* **342**, 67–68.

Constans J, Hazout S, Garruto RM, Gajdusek DC & Spees EK (1985) Population distribution of the human vitamin D binding protein: anthropological considerations. *American Journal of Physical Anthropology* **68**, 107–122.

Corless D, Boucher BJ, Cohen RD, Beer M & Gupta SP (1975) Vitamin-D status in long-stay geriatric patients. *Lancet* **i**, 1404–1406.

Cruickshank JK (1995) Challenging the orthodoxy of insulin resistance. *Lancet* **346**, 772–773.

Dandona P, Fonseca V & Menon RK (1986) Vitamin D deficiency and the endocrine pancreas. *Diabetologia* **29**, 468–469.

Darwish HM & DeLuca HF (1996) Recent advances in the molecular biology of vitamin D action. *Progress in Nucleic Acid Research and Molecular Biology* **53**, 321–344.

Davies H (1989) Coronary heart disease: The significance of coronary pathology in infancy and the role of mitogens such as vitamin D. *Medical Hypotheses* **30**, 179–185.

Department of Health and Social Security (1979) *Ultraviolet Light and Vitamin D Metabolism. Report on Health and Social Subjects* no. 15. London: H.M. Stationery Office.

Desai M, Bryne J, Zhang SJ, Petry A, Lucas A & Hales CN (1997a) Programming of hepatic insulin-sensitive enzymes in offspring of rat dams fed an isocaloric protein restricted diet. *American Journal of Physiology* **272**, Suppl., G1083–G1090.

Desai M, Petrie CJ, Wang CL, O'Connell DA & Hales CN (1997b) Effects of maternal and early vitamin D deficiency on glucose and lipid metabolism in rat offspring. *Diabetic Medicine* **14**, Suppl., S38 Abstr.

Dhawan J, Bray CL, Warburton R, Ghambhir DS & Morris J (1994) Insulin resistance, high prevalence of diabetes, and cardiovascular risk in immigrant Asians. Genetic or environmental effect? *British Heart Journal* **72**, 413–421.

Dowse GK, Zimmet PZ, Finch CF & Collins VR (1991) Decline in incidence of epidemic glucose intolerance in Nauruans: implications for the 'thrifty genotype' hypothesis. *American Journal of Epidemiology* **133**, 1093–1104.

Ebeling PR, Sandgren ME, DiMagno EP, Lane AW, DeLuca HF & Riggs BL (1992) Evidence of an age-related decrease in intestinal responsiveness to vitamin D: Relationship between serum 1,25-dihydroxyvitamin D3 and intestinal vitamin D receptor concentrations in normal women. *Journal of Clinical Endocrinology and Metabolism* **75**, 176–182.

Fahlen M, Oden A, Bjorntorp P & Tibblin G (1971) Seasonal influence on insulin secretion in man. *Clinical Science* **41**, 453–458.

Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Graziadei L, Pedrinelli R, Brandi L & Bevilacqua S (1987) Insulin resistance in essential hypertension. *New England Journal of Medicine* **317**, 350–357.

Ferrari S, Rizzoli R, Chevally T, Slosman D, Eisman JA & Bonjour J (1995) Vitamin-D-receptor-gene polymorphisms and change in lumbar-spine bone mineral density. *Lancet* **345**, 423–424.

Feskens EJM, Bowles CH & Kromhout D (1991) Inverse association between fish intake and risk of glucose intolerance in normoglycaemic elderly men and women. *Diabetes Care* **14**, 935–942.

Feskens EJM, Bowles CH & Kromhout D (1993) Association between fish intake and coronary heart disease mortality. *Diabetes Care* **16**, 1021–1034.

Frankel BJ, Heldt AM & Grodsksy GM (1980) Vitamin D deficiency inhibits pancreatic secretion of insulin. *Science* **209**, 823–825.

Fraser DR (1995) Fat-soluble vitamins. Vitamin D. *Lancet* **345**, 104–107.

Gedik O & Akalin S (1986) Effects of vitamin D deficiency and repletion on insulin and glucagon secretion in man. *Diabetologia* **29**, 142–145.

Grimes DS, Hindle E & Dyer T (1996) Sunlight, cholesterol and coronary heart disease. *Quarterly Journal of Medicine* **89**, 579–589.

Groop L, Miettenen A, Groop PH, Meri S, Koskimies S & Bottazzo GF (1988) Organ-specific autoimmunity and HLA-DR antigens as markers for beta-cell destruction in patients with type II diabetes. *Diabetes* **37**, 99–103.

Haddad JG (1992) Vitamin D – solar rays, the milky way, or both? *New England Journal of Medicine* **326**, 1213–1215.

Hales CN & Barker DJP (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia* **35**, 595–601.

Helgasen T, Danielsen R & Thorsson AV (1992) Incidence and prevalence of Type 1 (insulin-dependent) diabetes mellitus in Icelandic children 1970–1989. *Diabetologia* **35**, 880–883.

Henry RR (1994) Prospects for primary prevention of type 2 diabetes mellitus. *Diabetes Reviews International* **3**, 2–5.

Hewison M (1992) Vitamin D and the immune system. *Journal of Endocrinology* **132**, 173–175.

Hitman GA, Mannan N, McDermott MF, Aganna E, Hales CN & Boucher BJ (1998) Vitamin D polymorphisms influence insulin secretion in Bangladeshi Asians. *Diabetes* (In the Press).

Hjermann I (1992) The metabolic cardiovascular syndrome, syndrome X, Reavens syndrome, insulin resistance syndrome, atherothrombogenic syndrome. *Journal of Cardiovascular Pharmacology* **55**, S5–S10.

Holick MF (1995) Noncalcaemic actions of 1,25-dihydroxyvitamin D3 and clinical applications. *Bone* **17**, 107S–111S.

Hutchinson HS & Shah SJ (1921) The aetiology of rickets, early and late. *Quarterly Journal of Medicine* **15**, 167–194.

Ihde AJ (1975) Studies on the history of rickets. *Pharmacy in History* **17**, 15–58.

Ikeda K, Matsumoto T, Morita K, Kawanobe Y, Ezawa I & Ogata E (1987) Role of insulin in the stimulation of renal 25-hydroxyvitamin D3-1 alpha-hydroxylase by phosphorous deprivation in rats. *Metabolism: Clinical and Experimental* **36**, 555–557.

Ishida H, Cunningham NS, Henry HL & Normal AW (1988) The number of 1,25-dihydroxyvitamin D3 receptors is decreased in both intestine and kidney of genetically diabetic db/db mice. *Endocrinology* **122**, 2436–2443.

Iyengar S, Hamman RF, Marshall JA, Majumder PP & Ferrell RE (1989) On the role of vitamin D binding globulin in glucose homeostasis: Results from the San Luis Valley Diabetes Study. *Genetic Epidemiology* **6**, 691–698.

Iyer P (1991) Rock and roll ghost town (Iceland). In *Falling off the Map: Some Lonely Places in the World*, pp. 77–94. Reading: Black Swan.

Johnson JA, Grande JP, Roche PC & Kumar R (1994) Immunohistochemical localization of the 1,25(OH)₂D3 receptor and calbindin D28k in human and rat pancreas. *American Journal of Physiology* **267**, E356–E360.

Kadowski S & Norman AW (1984) Dietary vitamin D is essential for normal insulin secretion from the perfused rat pancreas. *Journal of Clinical Investigation* **73**, 759–766.

Kamboh MI & Ferrell RE (1986) Ethnic variation in vitamin D-binding protein (Gc): a review of isoelectric focusing studies in human populations. *Human Genetics* **72**, 281–293.

Kannel WB, Wilson PW & Zhang TJ (1991) The epidemiology of impaired glucose intolerance and hypertension. *American Heart Journal* **121**, 1268–1273.

Kautsky-Willer A, Pacini G, Barnas U, Ludvik B, Streli C, Graf H & Prager R (1995) Intravenous calcitriol normalizes insulin sensitivity in uremic patients. *Kidney International* **47**, 200–206.

Khaw KT (1994) Genetics and environment: Geoffrey Rose revisited. *Lancet* **343**, 838–839.

Khaw KT (1995) Temperature and cardiovascular mortality. *Lancet* **345**, 353–355.

Kishimoto H, Tsumura K, Fujioka S, Uchimoto S & Morji H (1993) Chronic parathyroid hormone administration reverses the antihypertensive effect of calcium loading in young spontaneously hypertensive rats. *American Journal of Hypertension* **6**, 234–240.

Knox E (1973) Ischaemic-heart-disease mortality and dietary intake of calcium. *Lancet* **i**, 1465–1467.

Kocian J (1992) Diabetic osteopathy. Favourable effect of treatment of osteomalacia with vitamin D and calcium on high blood glucose levels (In Czech). *Vnitrni Lekarstvi* **38**, 353–356.

Kromhout D, Bosschieter EB & De Lezenne Coulander C (1985) The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *New England Journal of Medicine* **312**, 1205–1209.

Kumar S, Davies M, Zakaria Y, Mawer EB, Gordon C, Olukoga AO & Boulton AJ (1994) Improvement in glucose tolerance and beta-cell function in a patient with vitamin D deficiency during treatment with vitamin D. *Postgraduate Medical Journal* **70**, 440–443.

Kuoppala T (1988) Alterations in vitamin D metabolites and minerals in diabetic pregnancy. *Gynaecology and Obstetric Investigation* **25**, 99–105.

Labriji-Mestaghanmi H, Billaudel B, Garnier PE & Sutter BCJ (1988) Vitamin D and pancreatic islet function. 1. Time course for changes in insulin secretion and content during vitamin D deprivation and repletion. *Journal of Endocrine Investigation* **11**, 577–587.

LaPorte RE (1995) Patterns of disease: Diabetes mellitus and the rest. *British Medical Journal* **310**, 545–546.

Levy J, Gavin JR & Sowers JR (1994) Diabetes mellitus: a disease of abnormal cellular calcium metabolism? *American Journal of Medicine* **96**, 260–263.

Lind L, Lithell H, Skarfors E, Wide L & Ljunghall S (1988) Reduction of blood pressure by treatment with alphacalcidol. A double-blind, placebo-controlled study in subjects with impaired glucose tolerance. *Acta Medica Scandinavica* **223**, 211–217.

Lind L, Pollare T, Hvarfner A, Lithell H, Sorensen OH & Ljunghall S (1989a) Long-term treatment with active vitamin D (alphacalcidol) in middle-aged men with impaired glucose tolerance. Effects on insulin secretion and sensitivity, glucose tolerance and blood pressure. *Diabetes Research* **11**, 141–147.

Lind L, Wengle B, Wide L & Ljunghall S (1989b) Reduction of blood pressure during long-term treatment with active vitamin D (alphacalcidol) is dependent on plasma renin activity and calcium status. A double-blind, placebo-controlled study. *American Journal of Hypertension* **2**, 20–25.

Ljunghall S, Lind L, Lithell H, Skarfors E, Selinus I, Sorensen OH & Wide L (1987) Treatment with one-alpha-hydroxycholecalciferol in middle-aged men with impaired glucose tolerance – a prospective randomized double-blind study. *Acta Medica Scandinavica* **222**, 361–367.

Lloyd EL (1991) The role of cold in ischaemic heart disease: a review. *Public Health* **105**, 205–215.

McCarron DA & Morris CD (1987) The calcium deficiency theory of hypertension. *Annals of Internal Medicine* **107**, 919–1022.

McDermott MF, Ramachandran A, Aganna E, Ogunkolade W, Curtis D, Boucher BJ, Snehalatha C & Hitman GA (1997) Allelic variation in the vitamin D receptor influences susceptibility to IDDM in Indian Asians. *Diabetologia* **40**, 971–975.

McGrath N, Singh V & Cundy T (1993) Severe vitamin D deficiency in Auckland. *New Zealand Medical Journal* **106**, 524–526.

McKeigue P (1994) Diets for secondary prevention of coronary heart disease: can linoleic acid substitute for fish oil? *Lancet* **343**, 1445.

McKeigue PM, Pierpoint T, Ferrie JE & Marmot MG (1992) Relationship of glucose intolerance and hyperinsulinaemia to body fat pattern in South Asians and Europeans. *Diabetologia* **35**, 785–791.

McLaughlin M, Fairney A, Lester E, Raggatt PR, Brown DJ & Wills MR (1974) Seasonal variation in serum 25-hydroxy cholecalciferol in healthy people. *Lancet* **i**, 536–537.

Mak R (1994) Renal disease, insulin resistance, and glucose intolerance. *Diabetes Reviews* **2**, 19–28.

Mak RH (1992a) Intravenous 1,25 dehydroxycholecalciferol corrects glucose intolerance in haemodialysis patients. *Kidney International* **41**, 1049–1054.

Mak RHK (1992b) Amelioration of hypertension and insulin resistance by 1,25-dihydroxycholecalciferol in hemodialysis patients. *Pediatric Nephrology* **6**, 345–348.

Marya RK, Rathee V, Lata V & Mudgil S (1987) Effects of vitamin D supplementation in pregnancy. *Gynaecological and Obstetric Investigation* **12**, 155–161.

Mathieu C, Waer M, Casteels K, Laureys J & Bouillon R (1995) Prevention of Type 1 diabetes in NOD mice by non hypercalcaemic doses of a new structural analogue of 1,25-dihydroxyvitamin D, KH1060. *Endocrinology* **136**, 866–872.

Maxwell JD (1994) Seasonal variation in vitamin D. *Proceedings of the Nutrition Society* **53**, 533–543.

Maxwell JD, Ang L & Brooke IR (1981) Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. *British Journal of Obstetrics and Gynaecology* **88**, 987–991.

Metz AL, Walser MM & Olson WG (1985) The interaction of dietary vitamin A and vitamin D related to skeletal development in the turkey poult. *Journal of Nutrition* **115**, 929–935.

Milner RDG & Hales CN (1967) The role of calcium and magnesium in insulin secretion from rabbit pancreas studied in vitro. *Diabetologia* **3**, 47–49.

Ministry of Food (1945) *Food Facts: Welfare Foods for Children.* London: H.M. Stationery Office.

Misselwitz J, Hesse V & Markestad T (1990) Nephrocalcinosis, hypercalcuria and elevated levels of 1,25-dihydroxyvitamin D in children. Possible link to vitamin D toxicity. *Acta Paediatrica Scandinavica* **79**, 637–643.

Moon J, Bandy B & Davison AJ (1992) Hypothesis; etiology of atherosclerosis and osteoporosis: are imbalances in the calciferol endocrine system implicated? *Journal of the American College of Nutrition* **11**, 567–583.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L, Nguyen TV, Sambrook PNS & Eisman JAE (1994) Prediction of bone density from vitamin D receptor alleles. *Nature* **367**, 284–287.

Motahar V & Boucher BJ (1997) Induction of hyperglycaemia in CD1 mice by the betel-nut (*Areca catechu*) nitrosamine MNPN, inhibition by beta-carotene and enhancement by vitamins A and E. *Clinical Science* **99**, 14p Abstr.

Nap RC & Hazewinkel HA (1994) Growth and skeletal development in the dog in relation to nutrition; a review. *Veterinary Quarterly* **16**, 50–59.

Neel JV (1962) Diabetes mellitus; a thrifty genotype rendered detrimental by 'progress'? *American Journal of Human Genetics* **14**, 353–362.

Nyomba BL, Bouillon R & De Moor P (1984) Influence of vitamin D status on insulin secretion and glucose tolerance in the rabbit. *Endocrinology* **115**, 191–197.

O'Hara-May J & Widdowson EM (1976) Diets and living conditions of Asian boys in Coventry with and without signs of rickets. *British Journal of Nutrition* **36**, 23–26.

Orwoll E, Riddle M & Prince M (1994) Effects of vitamin D on insulin and glucagon secretion in non-insulin-dependent diabetes mellitus. *American Journal of Clinical Nutrition* **59**, 1083–1087.

Ozono K, Seino Y, Yano H & Yamoaka K (1990) 1,25-Dihydroxyvitamin D3 enhances the effect of refeeding on steady state preproinsulin messenger ribonucleic acid levels in rats. *Endocrinology* **126**, 2041–2045.

Park EA (1923) The aetiology of rickets. *Physiological Reviews* **3**, 106–163.

Parviainen MT, Kumpusalo E, Halonen P, Neittaanmali L & Pekkarinen H (1992) Epidemiology of vitamins A, E, D and C in rural villages in Finland: biochemical, nutritional and socioeconomic aspects. *International Journal for Vitamin and Nutrition Research* **62**, 238–243.

Petri CJ, Desai M, Ozanne SE & Hales CN (1997) Early and late nutritional windows for diabetes susceptibility. *Proceedings of the Nutrition Society* **56**, 233–242.

Phillips DI, Barker DJP, Hales CN, Hirst S & Osmond C (1994) Thinness at birth and insulin resistance in adult life. *Diabetologia* **37**, 150–154.

Phillips DIW, Barker DJP, Fall CHD, Whorwood CB, Walker BR & Wood PJ (1997) Low birthweight and raised plasma cortisol concentrations in adult life. *Diabetic Medicine* **14**, S11 Abstr.

Pritchard BNC, Smith CCT, Ling KL & Betteridge DJ (1995) Fish oils and cardiovascular disease. *British Medical Journal* **310**, 819–820.

Quin ID & Gumpert JRW (1997) Remission of non-insulin-dependent diabetes mellitus following resection of a parathyroid adenoma. *Diabetic Medicine* **14**, 80–81.

Reaven GM (1995) The fourth Musketeer – from Alexandre Dumas to Claude Bernard. *Diabetologia* **38**, 3–13.

Rhodes CJ & Alarcon C (1994) What beta cell defect could lead to hyperproinsulinaemia in NIDDM?; some clues from recent advances made in understanding the proinsulin-processing mechanism. *Diabetes* **43**, 511–517.

Roth J, Bonner-Weir S, Norman AW & Orci L (1982) Immunohistochemistry of vitamin D dependent calcium binding protein in chick pancreas; exclusive localization in beta cells. *Endocrinology* **116**, 2216–2218.

Riggs BL, Hamstra A & DeLuca HF (1981) Assessment of 25-hydroxyvitamin D 1 alpha-hydroxylase reserve in postmenopausal osteoporosis by administration of parathyroid extract. *Endocrinology and Metabolism* **53**, 833–835.

Rutter GA, Pouli AE & Hutton JC (1997) A phogrin-aequorin chimera to measure free Ca^{2+} concentration in the vicinity of secretory granules. *Diabetic Medicine* **14**, S34 Abstr.

Scarabin PY, Bara L, Nicaud JP, Cambou DA, Luc G, Evans AE & Cambien F (1994) Seasonal variation of plasma fibrinogen in elderly people. *Lancet* **343**, 975–976.

Scragg R (1996) Sunlight, vitamin D, and cardiovascular disease. In *Calcium Regulating Hormones and Cardiovascular Function*, pp. 213–237 [MF Crass and LV Aviol, editors]. Boca Raton, FL: CRC Press.

Scragg R, Holdaway I, Jackson R & Lim T (1992) Plasma 25-hydroxyvitamin D3 and its relation to physical activity and other heart disease risk factors in the general population. *Annals of Epidemiology* **2**, 697–703.

Scragg R, Jackson R, Holdaway IM, Lim T & Beaglehole R (1995a) Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. *International Journal of Epidemiology* **19**, 559–563.

Scragg R, Khaw KT & Murphy S (1995b) Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. *European Journal of Clinical Nutrition* **49**, 640–646.

Scragg R, Khaw KT & Murphy S (1995c) Life-style factors associated with winter serum 25-hydroxyvitamin D levels in elderly adults. *Age and Ageing* **24**, 271–275.

Sedrani SH (1984) Vitamin D status of Saudi men. *Tropical and Geographical Medicine* **36**, 181–187.

Serhan E, Khokov MA & Singh BM (1997) Hypovitaminosis D in Indo-Asian patients with diabetes. *Diabetic Medicine* **14**, S42 Abstr.

Specker BL (1994) Do North American women need supplemental vitamin D during pregnancy or lactation? *American Journal of Clinical Nutrition* **59**, 484S–490S.

Stanbury SW & Mawer EB (1990) Metabolic disturbances in acquired osteomalacia. In *The Metabolic and Molecular Basis of Acquired Disease*, pp. 1717–1739 [RD Cohen, B Lewis, KGMM Alberti and AM Denman, editors]. London: Balliere Tindall.

Strachan DP, Powell ICJ, Thaker A, Millard F & Maxwell JD (1995) Vegetarian diet as a risk factor for tuberculosis in immigrant South London Asians. *Thorax* **50**, 175–180.

Szathmary EJ (1987) The effect of Gc genotype on fasting insulin level in Dogrib Indians. *Human Genetics* **75**, 368–372.

The Eurowinter Group (1997) Cold exposure and winter mortality from ischaemic heart disease, cerebrovascular disease, respiratory disease, and all causes in warm and cold regions of Europe. *Lancet* **349**, 1341–1346.

The Nutrition Society (1997) Symposium on *'Diabetes towards the year 2000' (Nutrition Society Summer Meeting 24–26 June 1996). Proceedings of the Nutrition Society* **56**, 225–290.

Tremoli E, Petroni A, Socini A, Maderna P, Colli S, Paoletti R, Galli C, Ferro-Luzzi A, Strazullo P, Mancini M, Puska P, Iacono J & Daugherty R (1986) Dietary interventions in north Karelia, Finland and south Italy. Modification of thromboxane Br formation in platelets of male subjects only. *Arteriosclerosis* **59**, 101–111.

Trowell H (1974) Diabetes mellitus death-rates in England and Wales 1920–70 and food supplies. *Lancet* **ii**, 998–1002.

van der Wielen RP, Lowik MR, van den Berg H, de Groot LC, Haller J, Moreiras O & van Staveren WA (1995) Serum vitamin D concentrations amongst elderly people in Europe. *Lancet* **346**, 207–210.

WHO Study Group on Diabetes (1985) Diabetes Mellitus: report of a WHO study group on diabetes mellitus. *World Health Organization Technical Report Series* no 727. Geneva: WHO.

Widdowson EM & McCance RA (1975) A review: New thoughts on growth. *Pediatric Research* **9**, 154–156.

Wilmshurst P (1994) Temperature and cardiovascular mortality. *British Medical Journal* **309**, 1029–1030.

Woodhouse PR, Khaw KT & Plummer M (1993) Seasonal variation of blood pressure and its relationship to ambient temperature in an elderly population. *Journal of Hypertension* **11**, 1267–1274.

Woodhouse PR, Khaw KT, Plummer M, Foley A & Meade TW (1994) Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. *Lancet* **343**, 435–439.