Glucose intolerance and impairment of insulin secretion in relation to vitamin D deficiency in East London Asians

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Summary Vitamin D deficiency reduces insulin secretion and still occurs in East London Asians in whom the prevalence of diabetes mellitus is at least four times that of Caucasians. Vitamin D status was assessed in 44 of 65 non-diabetic subjects 'at risk' of diabetes (spot blood glucose level > 6.0 mmol/l < 2 h post cibum, or > 4.6 mmol/l > 2 h post cibum on two separate occasions) and in 15 of 60 age and sex-matched 'low-risk' control subjects who attended for oral glucose tolerance test (OGTT) after screening of 877 omnivorous subjects not known to have diabetes. It was found that 95 % of at-risk and 80 % of low-risk subjects were vitamin D deficient (serum 25-hydroxy-vitamin D < 11 ng/ml). Diabetes was present in 16, impaired glucose tolerance in 12 and normoglycaemia in 19 at-risk subjects, impaired glucose tolerance in 2, and normoglycaemia in 13 low-risk subjects. Correlations of 30-min OGTT blood glucose, specific insulin and C-peptide levels with 25-hydroxy-vitamin D concentrations in 44 at-risk subjects were -0.31 ($p = 0.04$), 0.59 ($p = 0.0001$) and 0.44 ($p = 0.006$). In 15 'not-at-risk' subjects 30-min OGTT specific insulin and C-peptide levels correlated with 25-hydroxy-vitamin D, $r = 0.39$ ($p = 0.04$) and 0.16 ($p = 0.43$), respectively. Serum alkaline phosphatase concentration was higher in at-risk than not-at-risk

subjects (59.6 vs 46.5 IU/l, $p = 0.012$); corrected calcium concentrations were comparable (2.38 vs 2.39 mmol/l, $p = 0.7$). Following treatment with 100,000 IU vitamin D by i.m. injection, specific insulin, C-peptide [30 min on OGTT] and 25-hydroxy-vitamin D concentrations had risen 8–12 weeks later [means $\pm$ SD] from 57 ± 62 to 96.2 ± 82.4 mU/l [$p = 0.0017$], 1.0 ± 0.4 to 1.7 ± 0.8 pmol/ml [$p = 0.0001$] and 3.6 ± 1.8 to 13.5 ± 7.4 ng/ml [$p = 0.0001$], (but not to low-risk group values of 179 ± 89 mU/l, 2.7 ± 1.14 pmol/ml and 8.16 ± 6.4 ng/ml), respectively. Both total serum alkaline phosphatase and corrected calcium concentrations rose following vitamin D treatment in the at-risk subjects by 11.1 ± 8.22 (from 44 to 55 IU/l) and 0.15 ± 0.18 , (2.43 to 2.57 mmol/l), respectively ($p = 0.004$). Abnormal glucose tolerance was unchanged by vitamin D treatment. The value of early and sustained repletion with vitamin D in diabetes prophylaxis should be examined in communities where vitamin D depletion is common. [Diabetologia (1995) 38: 1239–1245]

Key words Non-insulin-dependent diabetes mellitus, vitamin D, vitamin D deficiency, total insulin, specific insulin, proinsulin, 32,33 split proinsulin, C-peptide, glucose intolerance.

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Abbreviations: OGTT, Oral glucose tolerance test; IGT, impaired glucose tolerance; p.c., post cibum; CV, coefficient of variation; NEFA, non-esterified fatty acids.

The prevalence of non-insulin-dependent diabetes mellitus (NIDDM) is greater in Asian immigrants to the United Kingdom than in the Caucasian population; furthermore, Asians develop NIDDM earlier in life than their Caucasian contemporaries [1, 2]. Vitamin D depletion still presents as rickets and osteomalacia in Asians in the UK [3]. In experimental animals vitamin D is necessary for normal release of insulin and for the maintenance of normal glucose toler-

ance. Following prolonged deficiency, impairment of insulin secretion develops and diabetes becomes irreversible [4, 5]. Administration of 1,25 calcitriol improves insulin secretion and corrects early glucose intolerance in patients with renal failure [6]. Calcium appears to be necessary for normal insulin secretion [7, 8] and correction of hypocalcaemia in nutritional rickets and hypoparathyroidism has been shown to correct both insulin secretion and impaired glucose tolerance in a group of eight patients [9]. We therefore examined the vitamin D status by oral glucose tolerance test (OGTT) of subjects screened as "at-risk" of diabetes, during a study of dietary factors in diabetes aetiology in East London Asians. Once vitamin D deficiency was found to be common in those screened as at-risk of diabetes we proceeded to examine glucose tolerance, secretion of insulin and of proinsulin-like molecules (as well as fasting serum calcium, parathormone and alkaline phosphatase concentrations) in relation to vitamin D status.

Subjects and methods

One thousand British Asians of Bangladeshi origin living in the London Borough of Tower Hamlets were contacted at the surgeries of their general practitioners as consecutive "well" attenders, or through practice records. The study was approved by the district ethics committee. After giving informed consent volunteers answered a dietary questionnaire and provided a personal history of diabetes. Spot blood glucose level was measured on a finger-prick sample using BM 1-44 stix (Boehringer Mannheim Ltd UK, Lewes, Sussex, UK) with a reflolux meter by a single observer (N.M.). The results were validated using a glucose oxidase specific micro-technique (Beckman Instruments, Palo Alto, Calif., USA), with a coefficient of variation for glucose levels below 25 mmol/l of less than 3%. Only subjects "at-risk" of diabetes and their "not-at-risk" control subjects are considered further in this report. Sixty-five subjects not previously known to have diabetes, all of whom were meat and fish eaters, but with spot blood glucose levels greater than 6.0 mmol/l less than 2 h post cibum (p.c.), or greater than 4.6 mmol/l at times greater than 2 h p.c. on two separate occasions, were regarded as at-risk of diabetes [10] and were invited for a standard 75-g OGTT [11]; 57 attended. In 44 of these subjects 0-, 30- and 120-min venous sampling was achieved. Multiple venous sampling being unacceptable to many subjects from the ethnic group under study, finger-prick sampling was used for one or more sampling times in the remaining 13 subjects. Plasma glucose concentrations were measured by standard hexokinase methodology using a Discrete Analyser (Du Pont Dimension SIMS Clinical Chemistry System; Wilmington, Del., USA). Aliquots of serum separated within 2 h of sampling were stored at -20°C before assay. Serum total insulin was measured by an in-house radioimmunoassay using Guildhay antibody and National Institute of Biological Standards (UK) human insulin standard 66/304, (CV between or within assay $<7.5\%$; cross-reaction with pro-insulin 100%). Specific human insulin was measured by enzyme immunoassay (Medgenix, Florius, Belgium) (CV $<10\%$, cross-reaction with pro- and 32,33 split proinsulin, 0%). C-peptide concentrations were measured by radioimmunoassay (Medgenix) (CV $<7.2\%$). Proinsulin and 32,33 split proinsulin

concentrations were assayed by immunoradiometric assay (CV $<7.5\%$) and serum 25-hydroxy-vitamin D concentrations, reflecting vitamin D status [12], by radioimmunoassay (Incstar, Minn., USA; lower limit of detection 2.1 ng/l, within and between assay CV $<6.6\%$). Total fasting serum calcium, alkaline phosphatase and albumin concentrations were measured with the Bayer DAX 48 Analyser (Basingstoke, UK). Calcium concentrations were corrected to a serum albumin level of 46 g/l. Serum intact parathormone was measured by immunoradiometric assay in at-risk subjects (Nichols Institute Diagnostics Ltd, Saffron Walden, Essex, UK; within and between assay CV $<5\%$).

Of the 42 at-risk subjects identified as vitamin D-deficient (serum 25 hydroxy vitamin D <11.0 ng/ml [12]) at initial OGTT, 22 accepted the offer of treatment from their general practitioner with a single bolus injection of 100,000 IU of vitamin D and returned for repeat OGTT 8–12 weeks later, with finger-prick sampling at 0 and 120 min for blood glucose estimation, and limited venous sampling at 30 min. The first OGTTs were carried out between December 1991 and May 1992. Sixty-five subjects matched for age and sex to those of the at-risk group but with spot blood glucose levels below the at-risk values given above were invited for OGTT, and 15 attended, all of whom were meat and fish eaters. These subjects acted as not-at-risk control subjects for assessment of glycaemic and vitamin D status. Post-repletion OGTTs following vitamin D treatment of the at-risk group of OGTTs in the low-risk group were carried out concurrently between January and March 1993.

Statistical analysis

Statistical analyses used paired and independent sample *t*-tests and non-parametric equivalents and multiple regression with the computer program STATA (Stata Corp, College Station, Texas, USA), version 3.1, on an IBM PC compatible computer. Parametric tests including correlation used log transformed data where they were positively skewed; Spearman rank correlation was also used. The significant correlations given were each confirmed to remain significant when recalculated after exclusion of any outliers. Correlations of differences between post- and pre-treatment results were made with the mean of the pre- and post-treatment findings, to minimize the effect of regression towards the mean [13]. Data are given as mean $\pm$ SD.

Results

Of the 993 subjects who completed the questionnaire and were screened, 116 were found to have known diabetes (12%). Spot blood glucose tests identified 65 subjects as at-risk for diabetes as defined above. Forty-seven of these subjects completed OGTTs with venous sampling for glucose at 0, 30 and 120 min and of these 19 were normal, 12 showed impaired glucose tolerance (IGT) and 16 had diabetes as defined by World Health Organization criteria [11]. The prevalence of diabetes in the group as a whole, NIDDM in all cases, was therefore at least 14%. There were 22 women and 22 men in the at-risk and 10 women and 5 men in the not-at-risk group in whom 25-hydroxy-vitamin D was measured (chi-squared = 0.09 for the

Fig. 1. Serum parathormone and 25-hydroxy-vitamin D concentrations in 44 Asian subjects, not known to have diabetes, at-risk of diabetes on screening blood glucose test (spot values > 6.0 mmol/l less than 2 h p.c. or > 4.6 mmol/l more than 2 h p.c. on two occasions)

difference in sex ratio ($p = 0.77$)). The mean ages of subjects in these groups were 42, 48, 48, and 55 years, respectively and the mean $\pm$ SD ages of at-risk compared to not-at-risk subjects were 44.9 ± 13.0 and 49.9 ± 8.7 respectively ($p = 0.17$). There were no differences between body mass index (BMI) in the at-risk group as a whole at 25.9 ± 3.95 kg/m² [or in the subgroup treated with vitamin D, at 26.2 ± 4.5 kg/m² ($p = 0.46$)] and the BMI of the not-at-risk group at 25.2 ± 5.2 kg/m² ($p = 0.55$). Of the 44 at-risk subjects providing adequate serum sampling for immunoassays at OGTT, 42 were vitamin D-depleted (serum-25-hydroxy-vitamin D levels < 11 ng/ml). Vitamin D concentrations were 5.2 ± 4.6 , 3.7 ± 1.4 , and 3.2 ± 1.5 ng/ml in those who had normal, IGT, or diabetic

results, respectively on OGTT, and significantly higher at 8.16 ± 6.4 ng/ml in the not-at-risk control than in the at-risk subjects, $p = 0.01$. The 25-hydroxy-vitamin D was not affected by sex, or related to age or to BMI in any group of subjects. Mean serum parathormone concentration in the at-risk group was 31.0 ± 3.0 , (range 18.3–39.3) as compared to 23.8 ± 2.4 ng/l, (range 19.6–28.0) in 21 healthy Caucasians of comparable age examined at the same time of year (and assayed together with the at-risk samples, $p = 0.0001$ [14]). There was a negative, though not striking, correlation of parathormone with serum-25-hydroxy-vitamin D in the 33 subjects in the at-risk group in whom adequacy of venous sampling allowed fasting parathormone to be measured, $r = -0.27$, $p = 0.09$ (Fig. 1). Specific serum insulin concentrations at 30 min on OGTT were lower in the 44 at-risk subjects compared to those of the 15 not-at-risk subjects, 37 ± 26 vs 178 ± 89 mU/l ($p = 0.0001$, Mann-Whitney test) as were C-peptide concentrations at 30 min; 1.1 ± 0.45 pmol/ml in the at-risk group vs 2.7 ± 1.1 pmol/ml in the not-at-risk subjects ($p = 0.001$, Mann-Whitney test) (Table 1). There was no relationship of 30-min OGTT specific insulin concentration with age or with BMI, and no difference in the findings by sex. During OGTT 30-min plasma glucose concentrations were negatively related to vitamin D status in the at-risk group as well as in the combined at-risk and not-at-risk subjects ($r = -0.31$ and -0.32 , respectively, $p = 0.04$), as were 2-h values in the at-risk group ($r = -0.31$, $p = 0.04$). Both total and specific human insulin and C-peptide concentrations at 30 min on OGTT in the untreated at-risk group were correlated positively with serum-25-hydroxy-vitamin D concentration ($r = 0.73$, $p = 0.0001$; $r = 0.59$, $p = 0.002$ and $r = 0.44$, $p = 0.006$, respectively [Spearman ranking]) (Fig. 2). Multiple regression analysis confirmed that total and specific insulin

Table 1. OGTT data in 44 at-risk, 15 not-at-risk subjects, and in 22 of the 44 at-risk subjects treated with vitamin D

Variable		Group		
		At risk		Not-at-risk
		Pre-vitamin D	Post-vitamin D	
	(n = 44)	(n = 22) ^g		(n = 15)
30 min				
Plasma glucose (mmol/l)	10.8 ± 3.6	9.95 ± 3.4	11.75 ± 4.5^g	9.5 ± 1.8^c
Specific insulin (mIU/l)	36.9 ± 26.1	36.8 ± 24.4	96.2 ± 82.4^a	178 ± 88.5^b
C-peptide (pmol/ml)	1.08 ± 0.45	0.76 ± 0.62	1.7 ± 0.2^a	2.7 ± 1.14^c
Proinsulin (pmol/l)	15.4 ± 12.9	15.33 ± 11.9	13.4 ± 9.9^e	13.1 ± 10.5^f
32,33 Proinsulin (pmol/l)	40 ± 30.2	41.8 ± 30.2	37.8 ± 27.6^d	48.13 ± 35.2^g
120 min				
Plasma glucose (mmol/l)	11.04 ± 5.36	11.13 ± 5.73	12.5 ± 6.6^a	7.2 ± 1.57^b

^a $p < 0.01$ for differences in means in 22 "at-risk" group; pre- vs post-vitamin D treatment;

^b $p < 0.01$ and ^c $p < 0.0001$ for differences in means in 44 "at-risk" vs "low risk" control subjects;

^d $p > 0.5$ and ^e $p > 0.3 < 0.5$ for differences in means as for ^a;

^f $p > 0.5$ and ^g $p > 0.3 < 0.5$ as for ^b and ^c

^g See methods for details

Fig. 2. Serum 30-min specific insulin with fasting 25-hydroxy-vitamin D concentrations in 44 subjects not known to have diabetes, and at-risk of diabetes on screening blood glucose (spot values > 6.0 mmol/l less than 2 h p.c. or > 4.6 mmol/l more than 2 h p.c. on two occasions). 25-hydroxy-vitamin D values of 2.1 ng/l, the lower limit of detection of the assay, are shown spread out over 1.5–2.1 ng/l for clarity

Fig. 3. (a) Serum C-peptide and (b), specific insulin concentrations at 30 min on OGTT in 44 subjects not known to have diabetes and not-at-risk or at-risk of diabetes on screening, (where at-risk is defined by spot blood glucose values > 6.0 mmol/l less than 2 h p.c. or > 4.6 mmol/l more than 2 h p.c. on two occasions), and in the subgroup of 22 of the 44 at-risk subjects who had been treated with 100,000 IU of vitamin D by injection 8–12 weeks before repeat OGTT. Mean $\pm$ SD

and C-peptide concentrations were each correlated with 25-hydroxy-vitamin D concentrations independently of age, sex or BMI. Corrected serum calcium concentrations were comparable in at-risk and not-at-risk subjects, mean 2.37 compared to 2.39 mmol/l ($p = 0.69$), whilst serum alkaline phosphatase concentrations were higher in the at-risk subjects, mean 59.6 U/l, compared to 46.5 U/l in not-at-risk subjects ($p = 0.012$).

Measured 8–12 weeks after vitamin D injection, serum alkaline phosphatase had risen from 44 to 55 U/l, a rise of 11.1 ± 8.22 U/l, $p = 0.00005$, and corrected serum calcium from 2.43 to 2.57 mmol/l, a rise of 0.15 ± 0.18 mmol/l, $p = 0.004$, no value rising above the normal range. Specific insulin concentration at 30 min on OGTT had risen in the 22 treated subjects by 43.0 ± 65 mU/l, ($p = 0.0001$) and C-peptide concentration had risen by 0.74 ± 0.61 pmol/l, ($p = 0.0001$), (Table 1, Figs. 3, 4, 5 for changes in individuals). Serum 25-hydroxy-vitamin D concentrations were 13.5 ± 7.4 ng/ml in the 22 treated subjects compared to their pre-treatment values of $3.6 \pm$

1.8 ng/ml, $p = 0.0001$. The correlations of 30-min OGTT specific insulin and C-peptide with 25-hydroxy-vitamin D concentration in the 22 at-risk subjects studied after vitamin D treatment were $r = 0.47$ ($p = 0.059$) and $r = 0.12$ ($p = 0.4$). 30-min OGTT proinsulin concentration was somewhat higher in the at-risk group before (15.33 ± 11.95 pmol/l) than after treatment (13.8 ± 8.6 pmol/l), or than in the not-at-risk group, (13.1 ± 10.5 pmol/l). 32,33 split proinsulin concentration was similar in the at-risk (40 ± 30.15 pmol/l) and the not-at-risk (48.13 ± 35.2 pmol/l) groups. Following vitamin D treatment 32,33 split proinsulin concentration fell marginally from 41.8 ± 30.2 to 37.8 ± 27.6 pmol/l in contrast to the significant rise found in specific insulin concentration.

None of the 15 not-at-risk control attenders had diabetes, though two (12.5%) had IGT; their 30-min OGTT true insulin and C-peptide concentrations were within the normal ranges at 178 ± 88.53 mU/l and 2.7 ± 1.14 pmol/ml, respectively and 12 were vitamin D depleted. The 25-hydroxy-vitamin D concentration was significantly higher in these subjects at

Fig. 4. Serum 25-hydroxy-vitamin D concentrations in 44 subjects not known to have diabetes, not-at-risk and at-risk of diabetes on blood glucose screening, (where at-risk is defined by spot glucose values of >6.0 mmol/l less than 2 h or >4.6 mmol/l, more than 2 h p.c. upon two occasions) and in 22 of the at-risk subjects 8–12 weeks after treatment with vitamin D, 100,000 IU in oil, by intramuscular injection. Mean $\pm$ SD

Fig. 5. Individual changes in C-peptide and specific insulin (30-min OGTT concentrations) before and after treatment of 22 subjects not known to have diabetes and found to be at-risk of diabetes (where at-risk is defined by spot glucose values >6.0 mmol/l less than 2 h p.c. or >4.6 mmol/l more than 2 h p.c. on two occasions), with vitamin D

8.18 ± 7.14 ng/ml ($p=0.001$), than in the 22 at-risk subjects before vitamin D treatment. OGTT (30-min) specific insulin concentration in the not-at-risk control group, as in the at-risk group, correlated positively with 25-hydroxy-vitamin D concentration ($r=0.389$, $p=0.05$) but the correlation of C-peptide with vitamin D status was not significant in this group ($r=0.16$, $p=0.12$). No at-risk subject re-at-

tending for OGTT after vitamin D treatment was on oral hypoglycaemic agents though dietary advice had been given to subjects initially found to have diabetes. Glucose tolerance at the second OGTT, approximately 1 year after the first attendance and at a comparable time of year, was unaltered in 15 subjects, worsened in six (normal to IGT in two, IGT to diabetes in four) and improved in two (IGT to normal). The changes in 30-min OGTT 32,33 split proinsulin concentrations were negatively correlated to the means of the concentrations of 25-hydroxy-vitamin D, measured before and after treatment with vitamin D ($r=-0.32$, $p=0.12$, $n=19$, three samples being inadequate for assay), but there was no relationship between changes in split proinsulin and vitamin D status. Neither pro- nor 32,33 split proinsulin concentrations at 30 min on OGTT related to vitamin D status in either the untreated at risk subjects ($r=0.12$, $p=0.45$ and $r=0.067$, $p=0.69$ respectively, $n=40$), or in the not-at-risk control subjects ($r=-0.20$, $p=0.48$ and $r=-0.06$, $p=0.84$, respectively $n=14$, one sample being inadequate for proinsulin assay).

Discussion

There is no single accepted method of screening for diabetes. The method chosen for the present survey has been used previously in screening British Asians for diabetes [10]. While not all the at-risk subjects identified in the present survey agreed to OGTT, and not all attenders for OGTT agreed to full venous sampling, a problem also found in communities in India [15], the numbers studied were sufficient to demonstrate a relationship between vitamin D status and both insulin and C-peptide concentrations at 30 min on OGTT, reflecting secretion/release into the bloodstream, in subjects at-risk and not-at-risk of diabetes at screening. Vitamin D has been shown to be required for maintenance of normal glucose tolerance and insulin release in animals [4, 5], and is believed to act in the same way in man, possibly by ensuring the availability of free calcium ions [9, 16]. Prolonged depletion leads to failure of insulin secretion which over time becomes irreversible [17]. While rickets and osteomalacia are still seen due to vitamin D depletion in our local Asian community [3], such problems are less common than was the case 10 or more years ago. The finding of inadequate vitamin D repletion, as evidenced by reduced serum 25-hydroxy-vitamin D concentrations, in 42 of 44 at-risk and 11 of 15 not-at-risk subjects was therefore unexpected. The reduction in absorption of vitamin D found with age [18] was unlikely to account for the reduction in concentrations of vitamin D in at-risk subjects since they were younger than the not-at-risk subjects. It was also unlikely that the increased risk

of vitamin D depletion in female Asians [19] accounted for the greater depletion found in the at-risk group since the proportion of females to males was greater in the not-at-risk group (2:1), than in the at-risk group (1:1). True insulin secretion and abnormalities of insulin secretory profiles have not to our knowledge been examined previously in relation to vitamin D status in man, though proinsulin cleavage endopeptidases have been found to be calcium dependent [20], and increases in immunoassayable insulin during OGTT reported in patients with NIDDM given one- α -hydroxycalciferol for 3 weeks [21].

The reduction in parathormone concentration with increased vitamin D status seen in our subjects, and the rise in corrected serum calcium and in serum alkaline phosphatase following treatment with vitamin D suggest that reduction in serum 25-hydroxy-vitamin D concentration was associated with deficiency of vitamin D. The finding of significant correlations for both 30-min OGTT C-peptide and specific insulin with vitamin D status in both the at-risk and not-at-risk groups suggests that vitamin D repletion may be of value for the maintenance of normal insulin secretion in man. 25-hydroxy-vitamin D concentrations were not as high in the not-at-risk group of British Asians as is normal for British Caucasians at comparable times of year [22], though they were higher than in at-risk subjects. It is possible, therefore, that long-term vitamin D depletion could contribute to the increased prevalence of diabetes seen in Asians migrating to Northern Europe as compared to that of indigenous Caucasians. Early correction of vitamin D depletion has been shown to restore both insulin secretion and normoglycaemia in dialysis patients developing glucose intolerance with failing 1-hydroxylation of 25-hydroxy-vitamin D [6, 23] and in earlier case reports of treatment of vitamin D deficiency in subjects with glucose intolerance [9]. As in previous reports of treatment with vitamin D or its active di-hydroxy form in four vitamin D-depleted patients with established diabetes [17], and in 35 patients with NIDDM [24], we found no improvement in abnormal glucose tolerance in our 22 vitamin D-treated subjects despite improvement in insulin output. It is unlikely therefore that such therapy would be of value in established human diabetes. Furthermore, there has been no suggestion of increases in insulin secretion with vitamin D supplementation in replete adults [25]. It is unlikely that the increases in insulin secretion we describe following vitamin D treatment were related to dietary treatment of diabetes since the increases were largest in those who remained normoglycaemic. While the failure to correct diabetes in our study was disappointing, and specific insulin secretion was not fully restored despite improvement in insulin secretory profiles, the increases in serum 25-hydroxy-vitamin D that were achieved were modest and did not reach normal values. Maintenance of vitamin D reple-

tion over time might prove more effective. The finding that insulin and C-peptide responsiveness after 1,25 (OH) $_2$ D treatment were negatively correlated with duration of diabetes is of interest in this context [25], as is the report of improved glycaemia in diabetic patients with osteomalacia after treatment with large doses (42,000–85,000 IU) of vitamin D daily [26]. Diabetes in a male Dutch population group over 60 years old has been shown to be reduced in relation to increases in long-term fish consumption: though vitamin D intake was not discussed [27], the vitamin D-rich herring family was noted to contribute to fish intake. This finding strengthens the suggestion that maintenance of vitamin D repletion might contribute to the avoidance of diabetes, a hypothesis which we are about to test with a prospective double blind trial of treatment of vitamin D depletion.

The increased cardiovascular mortality suffered by British Asians, and its onset at an earlier age than in indigenous Caucasians, has been attributed to the increased prevalence of diabetes in this and in similar ethnic groups [28]. The 32,33 split-proinsulin concentrations have been shown to be increased in early glucose intolerance and to correlate with risk factors for cardiovascular disease [29], but it is not known whether correction of abnormal insulin secretory profiles might have wider implications. It has been suggested that disorders which increase in prevalence with increasing latitude, and present more commonly in winter, such as ischaemic heart disease, could prove to relate to vitamin D deficiency [30]. This suggestion is supported by the reductions in NEFA concentrations [9, 21] and in insulin resistance [9] reported with correction of vitamin D depletion in man, to which secondary hyperparathyroidism has been suggested to contribute [31]. Examination of dietary information from the survey of which the present study is a part has shown initial serum 25-hydroxy-vitamin D concentrations in at-risk subjects at OGTT to be related to the consumption of fish; a relationship found in both at-risk and not-at-risk subjects ($p < 0.04$ and < 0.02 , respectively) [14]. The vitamin D content of fish, especially oily fish, could therefore contribute to its known cardioprotective effects [32].

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