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Rapid communications

Vitamin D, glucose tolerance and insulinaemia in elderly men

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Summary Vitamin D status was assessed in 142 elderly Dutchmen participating in a prospective population-based study of environmental factors in the aetiology of non-insulin-dependent diabetes mellitus. Of the men aged 70–88 years examined between March and May 1990, 39 % were vitamin D depleted. After adjustment for confounding by age, BMI, physical activity, month of sampling, cigarette smoking and alcohol intake the 1-h glucose and area under the glucose curve during a standard 75-g oral glucose tolerance test (OGTT) were inversely associated with the serum concentration of 25-OH vitamin D ($r = -0.23$, $p < 0.01$; $r = -0.26$, $p < 0.01$, respectively). After excluding newly diagnosed diabetic patients total insulin concentrations during OGTT were also inversely associated with the concentration of 25-OH vitamin D ($r = -0.18$ to -0.23, $p < 0.05$). Hypovitaminosis D may be a significant risk factor for glucose intolerance. [Diabetologia (1997) 40: 344–347]

Keywords Insulin, glucose tolerance, vitamin D, elderly.

We have been interested in environmental factors which may contribute to the development of non-insulin-dependent diabetes mellitus (NIDDM). Previous analysis of dietary factors in a cohort of elderly Dutch people has shown that regular fish eating reduced the 4-year risk of glucose intolerance in normoglycaemic individuals [1]. Experimentally vitamin D is required for normal insulin secretion and glucose tolerance [2]. Since fish can be an important dietary source of vitamin D this sub-study was designed to determine whether variation in vitamin D status relates to glucose intolerance.

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Abbreviations: 25(OH)D, 25-Hydroxy-vitamin D; NIDDM, non-insulin-dependent diabetes mellitus; OGTT, oral glucose tolerance test.

Subjects, materials and methods

Study population: In 1960 a population-based random sample of 878 men (born 1900–1920) were enrolled in the Zutphen Study, the Dutch contribution to the Seven Countries Study. Clinical examinations and dietary surveys were conducted initially and at regular intervals thereafter. In 1990 all living participants (314) were invited for the 30-year follow-up and 238 (77 %) agreed. Men known by then to have diabetes were excluded ($n = 29$). Complete data on diet and BMI from both the 10- and 30-year surveys and on glucose tolerance at the 30-year survey were available on 158 subjects. Serum for 25-hydroxyvitamin D (25(OH)D) analysis was available on 142 of those who formed the study group.

Protocol: All men were examined as in previously reported surveys [3]. At the 30-year follow-up an OGTT conforming to World Health Organization (WHO) guidelines [4] was performed with samples at 0, 1 and 2 h after the glucose load. Plasma glucose was measured in fluoridated samples using hexokinase methodology. WHO criteria were used to classify the men into those with newly diagnosed NIDDM, impaired glucose tolerance or as normoglycaemic [4]. Serum total insulin was assayed by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden; cross-reaction with proinsulin 100 %).

Clinical examinations and anthropomorphic measurements were made by physicians specifically trained for the study as

previously reported [5]. Height and weight were measured in light clothing without shoes. BMI was calculated by dividing weight by height squared (kg/m^2). Triceps and subscapular skinfold thickness were measured in duplicate. Serum total cholesterol was measured enzymatically in non-fasting samples and triglycerides in fasting serum samples using techniques specified by the Centers for Disease Control, Atlanta, Ga., USA. HDL-cholesterol was measured after magnesium sulphate precipitation of the apo-B containing lipoproteins.

Habitual tobacco usage was assessed using a locally developed questionnaire. Physical activity was assessed with a validated questionnaire designed for retired men (Prof. J. N. Morris, London School of Hygiene and Tropical Medicine, personal communication) modified to become a 15-item sequence. Estimates for time spent on activities (walking, cycling, gardening, odd jobs, sports, hobbies and work) were summed to provide the total weekly minutes of activity. Socioeconomic class was derived from lifelong occupation.

Food and alcoholic beverage consumption data were collected by the cross-check dietary history method [6]. Interviews took place in the months March to June by experienced dieticians and nutritionists in all cohorts, participants being unaware of their glucose tolerance status. The food consumption pattern during the preceding month was determined by questions about the foods eaten during and between meals both at weekends and weekdays and by using an extensive checklist of foods to record the frequency and amounts consumed. Portion sizes were estimated by a portable scale. Food pattern data was compared with the checklist data. Nutrient and alcohol intake was assessed using computerized food table data.

25-OH vitamin D assay. Serum samples stored at –20 °C since 1990 were assayed in 1995 by radioimmunoassay (Incstar; Stillwater, Minnesota, USA; sensitivity 5.0 nmol/l; within and between assay coefficient of variation < 6.6 %; cross reactivity with 24,25-$(OH)_2$ vitamin D and 25,26-$(OH)_2$ vitamin D 100 % and with 1,25-$(OH)_2$ vitamin D 2.5 %).

Statistical analyses. Statistical analyses were carried out using the SAS-program version 6.08. Analysis of (co)variance was used to compare values of risk factors between tertiles of vitamin D concentration and to adjust for age and month of survey. Logarithmic transformation of 25(OH)D data was used as its distribution was skewed. Pearson correlation coefficients between 25(OH)D concentration and risk factors were calculated after adjustment for confounders added into the models as continuous variables, except for the dichotomous variable of cigarette smoking (yes/no). All p values were based on two-sided tests of significance.

Results

The study population was aged 70–88 years (mean ± SD 75.7 ± 4.5) at the 30-year survey, 13 % (19) had impaired glucose tolerance, 6 % newly diagnosed diabetes and 81 % were normoglycaemic. Serum concentration of 25(OH)D ranged from 3.3 to 187.3 nmol/l, 42.0 ± 29.1 [normal reference range 26–180 nmol/l]. Of 25(OH)D values 39 % were below 26 nmol/l. Concentrations fell with increasing age ($r = –0.27, p < 0.05$), by 1.5 nmol/l for each year of age.

Concentrations were higher in samples taken in April and May compared to March (mean difference +19.1 nmol/l). Three men taking vitamin A and D supplements had values in the middle tertile. Vitamin D status did not vary according to socioeconomic class (data not shown).

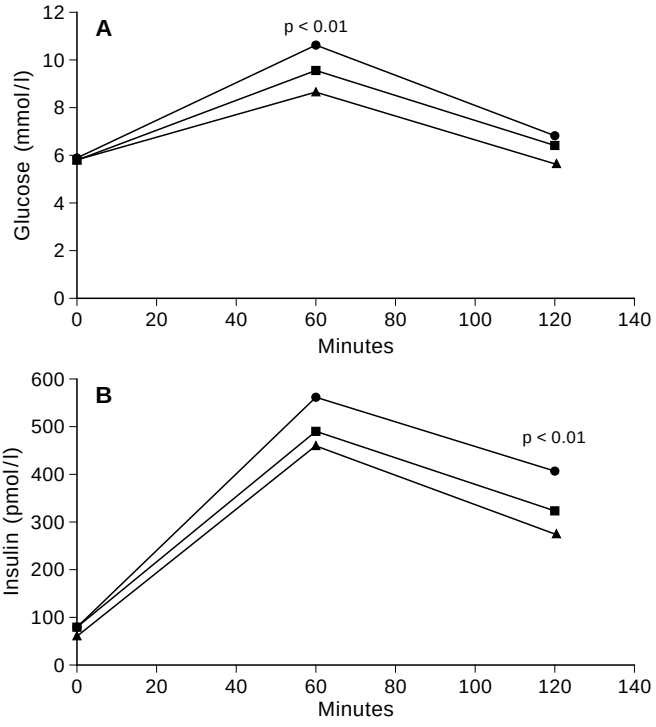

Fig. 1. Glucose (A) and insulin (B) concentrations versus time during oral glucose tolerance test with differing tertile of 25-OH vitamin D concentration; ●, lowest; ■, middle; ▲, highest

25(OH)D concentrations were not significantly predicted by current fish intake, although men in the lowest vitamin D tertile had the lowest intake of fish, fats and oils. The association between 25(OH)D concentration and fish consumption and consumption of fats and oils was borderline significant ($p = 0.13$). Levels of physical activity tended to increase with increasing tertile of 25(OH)D distribution with borderline statistical significance (data not shown). 25(OH)D concentrations were not related to BMI, cigarette smoking or to intakes of energy, alcohol, calcium, protein or carbohydrate.

Neither fasting, 60 min, nor 120 min concentration or the area under the curve for glucose varied with season nor was insulinaemia (fasting, 60 min, 120 min or area under the curve) associated with season. Glucose concentration during OGTT increased with decreasing 25(OH)D concentration independent of age and this relationship remained significant after additional correction for season of the year (i. e. from March to May, the months of sampling in the present study) (Fig. 1 a). After adjusting for physical activity, BMI, skinfold subscapular to triceps ratio, cigarette smoking and consumption of alcohol the association between tertiles of 25(OH)D and 1-h glucose and area under the glucose curve remained statistically significant (Table 1). Additional adjustment

Table 1. Correlation coefficients between serum 25-OH vitamin D concentration and metabolic variables

	Adjusted age and month survey *r*	Adjusted for other variables[d] *r*
Glucose		
Fasting	– 0.06	– 0.02
1 h	– 0.28[c]	– 0.26[b]
2 h	– 0.16	– 0.11
area under curve	– 0.26[b]	– 0.23[b]
Total insulin (n = 134)		
Fasting	– 0.22[a]	– 0.20[a]
1 h	– 0.21[a]	– 0.21[a]
2 h	– 0.23[b]	– 0.18[a]
area under curve	– 0.24[b]	– 0.23[a]
Triglycerides		
Fasting	– 0.15	– 0.13
HDL-cholesterol	0.11	0.07

[a] $p < 0.05$; [b] $p < 0.01$; [c] $p < 0.001$;
Other variables = BMI, skinfold thickness, alcohol, smoking, physical activity
[d] adjusted using multiple linear regression analysis

for the consumption of fish, fats and oils did not alter this relationship.

Insulin concentrations during OGTT increased with decreasing 25(OH)D concentration adjusting for age and month of survey (Fig. 1 b). Additional analysis of insulin variables excluded NIDDM subjects ($n = 8$). Fasting insulin concentration decreased significantly with increasing 25(OH)D. This relationship remained statistically significant after adjusting for possible confounding by BMI, skinfold thickness, physical activity, alcohol consumption and cigarette smoking (Table 1).

HDL-cholesterol was not related to 25(OH)D concentration. Fasting triglyceride levels were inversely related to 25(OH)D though this borderline relationship ($p = 0.08$) was abolished after adjustment for confounders (Table 1).

Discussion

Serum 25(OH)D concentrations reflect body vitamin D stores. In these elderly Dutchmen we have demonstrated that low concentrations of 25(OH)D were common, as in other studies of elderly Europeans [7]. Despite our hypothesis that the benefit of fish consumption was due to increased vitamin D intake, vitamin D status was only marginally affected by dietary factors – the main determinants being non-dietary factors including physical activity. The measure of physical activity probably reflects outdoor sun exposure.

Positive relationships between 25(OH)D and insulin sensitivity have been reported previously in small groups of middle-aged men [8] and in uraemia. This is the largest study we know of in which indices of insulin resistance and glycaemia during OGTT have been investigated in relation to vitamin D status and extensive anthropomorphic and questionnaire data. As this was a cross-sectional study it was unable to determine if hypovitaminosis D increases the risk of developing NIDDM. A prospective randomised controlled trial of vitamin D supplementation is in progress in British Asians (Boucher BJ, personal communication). Most clinical studies in established NIDDM have shown no benefit in giving vitamin D supplementation [9].

A statistical correlation between 25(OH)D and metabolic indices does not demonstrate causality. The association might be due to unidentified factors affecting both 25(OH)D and glucose metabolism. Hyperparathyroidism, found in vitamin D depletion, may be such a link. There is evidence that insulin resistance occurs in primary hyperparathyroidism, which improves after parathyroidectomy [10]. Impaired insulin release, insulin resistance and impaired glucose tolerance developing in subjects with renal failure improves with the administration of $1,25(OH)_2D_3$. Whether this is due to reduction in circulating parathyroid hormone or a direct effect of active vitamin D remains in doubt.

Hypovitaminosis D was common in this group of elderly Caucasian Europeans and this problem is not confined to minority immigrant groups in Europe. Prevention of hypovitaminosis D in groups at high-risk of NIDDM warrants further study as it may contribute to maintaining glucose tolerance and prevent the decline into NIDDM.

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