Letters to the Editor

Bicentric evaluation of a teaching and treatment programme for Type 1 (insulin-dependent) diabetic patients

Dear Sir,

Mühlhauser et al. [1] reported in this Journal improvement in metabolic control and other measures of diabetic care following a teaching and treatment programme for Type 1 diabetic patients and have assumed that their study substantiates the feasibility of applying this programme to other hospitals.

A similar teaching and treatment programme was carried out for 2 years in our Department [2]. Teaching was organised for 5 days (Monday–Friday) for groups of five to eight patients and included 12 h of theoretical and practical teaching delivered by physicians, nurses and dieticians. Four self-service lunches were provided under the guidance of the dieticians. All insulin-treated patients admitted to the diabetic ward were accepted in the study unless they were blind or deaf; from 1 February 1981 to 1 February 1982, 94 patients were included (65 Type 1 and 29 Type 2 diabetic patients). Patients were asked to monitor every day by testing urine or blood for glucose with test strips. Insulin dosage was adjusted in the light of the results. Glycaemic control was assessed 1 and 2 years later using fasting HbA_{1c} determinations by a microcolumn method using the Bio-Rad kit (normal range 6.5%–7.5% of total haemoglobin; Bio-Rad, Richmond, California, USA).

The mean HbA_{1c} level at entry was $10.6 \pm 2.3\%$; one year later it had dropped to $10.1 \pm 1.8\%$ (92 patients; paired t-test: $p < 0.05$) and 2 years later it was $9.6 \pm 1.7\%$ (75 patients; $p < 0.01$). During the 2-year study no statistically significant change was observed in the number of daily injections or insulin dosage. This suggests that improvement in blood glucose control was due to better self-care by the patients and not to correction of errors in their previous insulin regime.

Results of this study support the assumption of Mühlhauser et al. concerning the general feasibility of such a teaching programme, and the resulting improvement in glycaemic control.

Yours sincerely,

N. Larpent and J. Canivet

References

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High glucose concentrations do not influence replication and prostacyclin release of human endothelial cells

Dear Sirs,

In a recent publication [1], Stout described a direct inhibitory effect of glucose on the replication of cultured human endothelial cells from umbilical cord veins. From clinical reports it is known that arteriosclerotic lesions in diabetic patients develop more rapidly than in normal subjects [2]. Moreover, reduced synthesis of prostacyclin (PGI₂) by the vessel wall has been reported in experimental [3] and human diabetes [4, 5]. According to the 'response to injury' hypothesis of arteriosclerosis, damage to the vascular endothelium is an important prerequisite for the development of arteriosclerosis [6]. If high glucose concentrations in cell culture inhibit endothelial cell replication and function, the accelerated development of arteriosclerosis in diabetes could be explained by hyperglycaemia.

We have therefore investigated both cell replication (number of cells and [³H] thymidine incorporation to DNA) and PGI₂ production (measured as 6-keto-prostaglandin F_{1α}) in primary cultures of human endothelial cells exposed to low (5.6 mmol/l) and high (22.4 mmol/l) glucose concentrations.

Human endothelial cells from five individual cords were isolated according to the method of Jaffe et al. [7]. They were plated in microtitre plates (Microtest 11, 3040F, Falcon, Basle, Switzerland) and grown

Fig. 1. Proliferation and thymidine incorporation of human endothelial cells in primary cultures. Cells from five umbilical cords were cultured and the points represent the mean $\pm$ SEM of four cultures for each cell sample. Half of the cultures were used to determine the number of cells per well, the other half to measure [³H] thymidine incorporation. Number of cell per well observed with 5.6 mmol/l (○) and 22.4 mmol/l glucose (●). [³H] Thymidine incorporation found using 5.6 mmol/l (□) and 22.4 mmol/l glucose (■)

Fig. 2. Prostacyclin (PGI₂) release from cultured human endothelial cells. Cell culture media were exchanged daily and aliquots were taken to determine the amounts of PGI₂ using a specific radioimmunoassay for 6-keto-prostaglandin F_{1α}. ○: 5.6 mmol/l glucose (control), ●: 22.4 mmol/l glucose. Values are given as mean ± SEM (*n* = 5)

in 0.25 ml/well of medium 199 (Gibco, Paisley, Scotland), 20% heat-inactivated, pooled human serum (Seromed, Munich, FRG), 2 mmol/l L-glutamine, 100 units/ml penicillin-streptomycin and either 5.6 mmol/l glucose (control) or 22.4 mmol/l glucose (Merck, Darmstadt, FRG). The medium was exchanged daily. Half of the wells plated with human endothelial cells were used to determine the number of cells, the other half to measure [³H] thymidine incorporation. For cell count, cells were detached with 0.1 ml of 0.05% trypsin (Gibco Europe, Basle, Switzerland), 0.02% EDTA for 15 min at 37 °C and fixed by adding 0.1 ml of 1.0% paraformaldehyde, 0.145 mol/l NaCl and 1.3 mmol/l EDTA. Cell numbers were determined using a Coulter counter (Coulter Electronics, Luton, Beds., UK). For [³H] thymidine incorporation, cells were labelled with 1 µCi of [³H] thymidine/well (sp. act. 5 Ci/mmol, Amersham International, Amersham, Bucks., UK) for 18 h at 37 °C. Cells were detached by 0.05% trypsin, 0.02% EDTA and collected on glass fibre filters (Whatman 934-AH) using a cell harvester (Dynatech, Kloten, Switzerland). Filters were placed in 10 ml of scintillation fluid (Econofluor, New England Nuclear, Boston, Mass., USA) and radioactivity incorporated by cells in individual wells was determined.

No differences were observed in the number of cells or in [³H] thymidine incorporation using either 5.6 or 22.4 mmol/l glucose (Fig. 1). The daily synthesis and release of PGI₂ from these cultures also was not affected by elevated glucose concentrations (Fig. 2). Generally, we observed large amounts of PGI₂ produced by human endothelial cells during the first day in culture and then a decrease, followed by a fairly constant secretion of PGI₂ into the culture medium. The same results were obtained with either normal human serum or fetal calf serum to culture the cells.

Our results do not confirm those of Stout [1]. They show that in cell culture neither replication of endothelium nor the PGI₂ generation (one of the physiological functions of endothelium) is inhibited by high glucose concentrations. These data do not support the hypothesis that glucose might directly impair endothelial cell turnover [1] and therefore augment the cascade of events leading to arteriosclerosis as suggested by Ross and Glomset [6].

Yours sincerely,

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