Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells

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Abstract

Aims/hypothesis. We investigated the effects of constant and intermittently increased glucose concentrations on human proximal tubule cells and cortical fibroblasts in primary culture.

Methods. Cells were grown to confluence and then exposed for 4 days to 6.1 mmol/l D-glucose (normal), 25 mmol/l D-glucose (high), or 6.1 mmol/l alternating with 25 mmol/l D-glucose on a daily basis.

Results. In proximal tubular cells, exposure to high glucose caused an 11 % increase in thymidine uptake ($p < 0.05$), a 230 % increase in secretion of transforming growth factor beta 1 (TGF- β 1; $p < 0.05$) and a 393 % increase in platelet derived growth factor. Intermittent exposure to high glucose caused thymidine uptake to further increase by 42 % ($p < 0.01$) and TGF- β 1 secretion by 352 % ($p < 0.01$) but no additional increase in platelet-derived growth factor secretion was observed. Cellular protein content in-

creased by 27 % ($p < 0.05$) and collagen synthesis by 29 % ($p < 0.05$), changes that were not observed in cells constantly exposed to high glucose. In cortical fibroblasts constant exposure to high glucose caused a 35 % increase in thymidine uptake ($p < 0.01$). Intermittently high glucose increased thymidine incorporation a further 58 % ($p < 0.001$), collagen synthesis by 65 % ($p < 0.01$) and insulin-like growth factor binding protein 3 secretion by 216 % ($p < 0.01$).

Conclusion/interpretation. In cultured human tubulointerstitial cells, increased glucose concentrations change cell growth, collagen synthesis and cytokine secretion. These effects are enhanced following intermittent exposure to high glucose, indicating that short lived excursions in glycaemic control have important pathological effects on the human tubulointerstitium. [Diabetologia (1999) 42: 1113–1119]

Keywords Diabetic nephropathy, tubulointerstitium, cell growth, extracellular matrix, cytokine.

Most studies of diabetic nephropathy have concentrated on functional and structural changes in the glomerulus. It is increasingly recognised, however, that chan-

ges within the tubulointerstitium are important in determining the outcome of a variety of progressive kidney diseases including diabetic nephropathy [1, 2]. These changes have included proximal tubule cell (PTC) atrophy and compensatory growth, interstitial fibrosis and the accumulation of extracellular matrix [3, 4, 5]. Indeed such tubulointerstitial changes were the dominant lesion in around one third of patients in a biopsy study of patients with Type II (non-insulin-dependent) diabetes mellitus [6]. It has been suggested that such changes are critical in the development of progressive dysfunction that does not appear to occur when only glomerular changes are observed [7, 8].

The Diabetes Control and Complications Trial (DCCT) definitively showed that the risk of develop-

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Abbreviations: PTC, Proximal tubule cell, CF, cortical fibroblast, TGF- β 1, transforming growth factor beta-1, PDGF, platelet derived growth factor, IGF-1, insulin-like growth factor-1, IGFBP-3, insulin-like growth factor binding protein 3.

ing diabetic kidney disease was increased in patients with poor diabetes control [9]. Even among the patients with "good" glycaemic control in this study the cumulative incidence of diabetic nephropathy was, however, around 15%. This suggests that relatively short lived excursions of glycaemic control, that may have little impact on long-term markers of glycaemia such as HbA_{1c} , can have an impact on tissue complications of diabetes.

There are a number of models available that enable the effects of high glucose on the tubulointerstitium to be studied. The streptozotocin-induced diabetic rat model allows the effects of hyperglycaemia on the kidney to be determined but any changes observed may occur, or be modulated by, haemodynamic and circulating factors as well as by other renal structures such as glomerular cells and vascular endothelium. There also seem to be important species differences with all streptozotocin-induced diabetic rats developing histological changes in the kidneys but few going on to develop renal failure. This is in contrast to patients with diabetes in up to 30% of whom renal failure may develop. Tissue culture has been used to study the effects of high glucose concentrations on the tubulointerstitium to avoid the problems of circulating and haemodynamic factors as well as the modulating influences of other cell types. Such studies have tended to use immortalised cell lines such as the porcine LLC-PK1 and although these enable the direct effects of high glucose concentrations to be studied, they appear to have abnormal growth characteristics and lose some of their phenotypic features. In addition, these experiments have exposed cultured cells to constant high glucose concentrations, which does not reflect the situation in patients with diabetes and possibly allow cells to adapt to such conditions.

Only one study has compared in vitro the effects of intermittent with constant high glucose concentrations on cell growth and extracellular matrix accumulation [10]. In this study of cultured rat mesangial cells intermittent high glucose concentrations further enhanced the increased DNA and collagen synthesis compared with cells exposed to constant high glucose concentrations.

In our in vitro model of the human tubulointerstitium, cultured human proximal tubule cells (PTC) and cortical fibroblasts (CF) have been shown to reproduce the normal cellular morphology, immunohistochemistry and function observed in vitro [11, 12, 13]. Such cultured cells enable us to investigate the direct effects of high glucose concentrations on the tubulointerstitium without confounding haemodynamic and circulating factors. This model also allows us to compare the effects of exposure to both constant and intermittent high glucose concentrations on tubulointerstitial cells.

It has been well established that such tubulointerstitial cells secrete a variety of cytokines which have both autocrine and paracrine actions [13–19]. Proximal tubule cells secrete the cytokines transforming growth factor beta 1 (TGF- β 1) and platelet-derived growth factor (PDGF) both of which promote cell growth and collagen synthesis. The cortical fibroblasts produce insulin-like growth factor 1 (IGF-1) and its regulatory protein insulin-like growth factor binding protein 3 (IGFBP-3) which appear to have independent effects in regulating cell growth and extracellular matrix production. Our previous studies have shown that the autocrine and paracrine effects of these cytokines modulate tubulointerstitial growth and that these effects are dysregulated in the tubulointerstitial disease that characterises cyclosporin-induced nephrotoxicity [20]. It has been suggested that the activity of these cytokines change in diabetic nephropathy, the direct role of increased or intermittently increased glucose concentrations, in mediating these responses has not, however, been explained.

The aims of our study therefore were to compare the effects of exposure to constant and intermittent high glucose concentrations on human PTCs and CFs in culture. Cell growth was assessed by measuring thymidine incorporation as an index of DNA synthesis as well as protein content and collagen synthesis as a marker of extracellular matrix production. We also measured the production of the PTC-derived cytokines PDGF and TGF- β 1, and CF-derived IGF-1 and its main regulatory protein IGFBP-3.

Methods

Patients. Under aseptic conditions segments of macroscopically and histologically normal renal cortex were obtained from ten patients (five men, five women) undergoing nephrectomy for small (< 6 cm) tumours (seven renal cell carcinomas and three transitional cell carcinoma of the renal pelvis). The average age of the patients was 68 years. The patients were otherwise healthy and receiving no medication. Written informed consent was obtained from each patient before surgery and ethical approval for the study was obtained from the Royal North Shore Human Research Ethics Committee.

Cell culture. The methods for primary culture of both human proximal tubule cells and cortical fibroblasts are described in detail elsewhere [11, 13]. Briefly, renal cortical tissue was dissected from the capsule and medulla, minced and digested with collagenase (class 2, 383 U/mg; Worthington, Freehold, N.J., USA) and passed through a 100 μ m mesh. The filtrate was resuspended in 45% percoll (Pharmacia, Uppsala, Sweden) and separated into four distinct bands by isopycnic ultracentrifugation. The upper band was removed for renal cortical fibroblast culture and the lowermost band for PTC culture.

Proximal tubule cells were resuspended in serum-free hormonally defined media consisting of 1:1 (vol:vol) Dulbecco's modified Eagle's media and Hams F-12 (DMEM/F-12) ICN Pharmaceuticals Inc., Costa Mesa, Calif., USA), supplemented with 5 μ g/ml human transferrin (Sigma Chemical, St. Louis,

Mo., USA), 5 µg/ml (0.87 µmol/l) bovine insulin (Sigma), 0.05 µmol/l hydrocortisone (Sigma), 10 ng/ml (1.64 nmol/l) epidermal growth factor (Collaborative Research, Bedford, Mass., USA), 50 µmol/l prostaglandin E₁ (Sigma), 50 nmol/l selenium (Sigma) and 5 pmol/l tri-iodothyronine (Sigma). The tubular fragments were plated at a density of 1.5 mg pellet per cm² in 75 cm² flasks (Corning, New York, N.Y., USA). Media were changed every 48 h. The cells were incubated in humidified 95% air:5% CO₂ at 37°C and were subcultured at near confluence using seeding densities of 4000 cells per cm². Such cells were designated passage 1. Renal cortical fibroblasts (CF) were resuspended in DMEM/Hams F-12 containing 10% fetal calf serum (Trace Biochemicals, Sydney, Australia) and were seeded at an initial density of 2.25 mg pellet per cm². Subculture at confluence was subsequently carried out using a seeding density of 7000 cells per cm². As the doubling times for CF were greater than for PTC, the higher seeding densities of the CF allowed both to be studied at confluence approximately 6–7 days after plating.

The ultrastructure, growth and immunohistochemistry of both PTC and CF have been well characterised in our laboratory and shown to have normal function and hormonal responsiveness in culture [11, 12, 13, 14]. We did not see any cellular atypia by cytological examination.

Experimental protocol. All experiments were carried out on quiescent, confluent, passage 2 PTC and CF. Cells were made quiescent by two washes followed by incubation for 24 h in basic media (DMEM/Hams F-12 containing 5 µg/ml human transferrin). After this time flow cytometry showed that the growth of the cells was arrested in the G-0 phase of the cell cycle. Cells were then incubated for a further 4 days in glucose specific basic media. Media was changed on a daily basis with cells exposed to constant normal (6.1 mmol/l) D-glucose, constant high (25 mmol/l) D-glucose, normal and high D-glucose alternating on a daily basis and an osmotic control of 6.1 mmol/l D-glucose with 18.9 mmol/l L-glucose. Media was collected from all experiments 24 h after the final media change to assay for the cytokines TGF-β₁, PDGF, IGF-1 and the regulatory binding protein of IGF-1, IGFBP-3.

Assessment of cell growth. The details of these methods have been described elsewhere [13, 14, 21]. Cell counts were determined using a standard haemocytometer. Tritiated thymidine uptake into cells over the final 24 h of the study, as an index of DNA synthesis and hence hyperplasia was determined. To assess hypertrophy the protein content of cells was determined using a spectrophotometric method, following sonic disruption.

Collagen synthesis assay. Proximal tubule cell and CF collagen synthesis was assessed by measuring 3 h tritiated proline incorporation [22, 23]. Under glucose specific conditions, confluent cells were incubated with 7.4 × 10⁵ bq (20 µCi/ml) 2,3-³H-proline (Amersham, Buckinghamshire, UK), 0.284 mmol/l L-ascorbic acid (Sigma) and 0.234 mmol/l β-aminopropionitrile (Sigma). This media was then aspirated and precipitated overnight with 1 ml 10% trichloroacetic acid and 0.02% unlabelled proline. The cells were solubilised in 200 µl 0.2 mol/l NaOH and neutralised with 200 µl 0.2 mol/l HCl before precipitation as above. Pellets were obtained by centrifugation at 176 g for 5 min and then washed three times in 1 ml of 5% trichloroacetic acid containing 0.01% unlabelled proline. The pellets were then dissolved in 200 µl NaOH and titrated back to pH 7.6 with 0.2 mol/l HCl. A 100-µl aliquot was then incubated at 37°C for 1 h with 400-µl TRIS-CaCl₂-N-ethylmaleimide buffer (50 mmol/l TRIS-HCl, 5 mmol/l CaCl₂, 2.5 mmol/l N-ethyl-

maleimide, 0.02% NaN₃) in both the presence and absence of 2 mg/ml collagenase (Worthington class II, Worthington Biochemical, Lakewood, N.J., USA). The reaction was terminated by precipitation with 500 µl ice-cold 20% TCA and 1% tannic acid. After centrifugation at 1250 g supernatants and pellets were rendered soluble in 200 µl 0.2 mol/l NaOH and then counted in a beta-counter (1215 Rackbeta II, LKB Wallac, Turku, Finland).

Assay for TGF-β₁. Active TGF-β₁ concentration in 200 µl of media collected from PTCs was measured using an enzyme-linked immunosorbent assay (ELISA), (Amersham Life Science, Buckinghamshire, UK). The total protein content of lysed cells was measured and TGF-β₁ was expressed in ng per milligram of protein. The intra-assay CV was 1.6–3.3% and the inter-assay CV was 7.6–19.1%.

Assay for PDGF. The concentration of PDGF-AB 200 µl of media collected from PTCs was measured using an enzyme-linked immunosorbent assay (ELISA), (Amersham Life Science). The total protein content of lysed cells was measured and PDGF was expressed in ng per milligram of protein. The assay had a within assay CV of 1.3–6.3% and an inter-assay CV of 5.5–10%.

Assay for IGF-1. Insulin-like growth factor binding proteins were dissociated from IGF-1 and extracted using the Bio-Spin P-10 separation method described previously [13]. Concentrations of IGF-1 were then measured by assaying 100 µl aliquots in duplicate in a previously described radioimmunoassay (RIA) [24]. The IGF-1 specific antibody was a gift from Professor R.C. Baxter (Sydney, Australia). Maximum specific binding of IGF-1 tracer to antibody was in excess of 30%. The cross reactivity of this assay with IGF-2 was negligible. The intra-assay coefficient of variance was less than 5% and the inter-assay coefficient of variance was less than 10%.

Measurement of IGFBP-3. The IGFBP-3 RIA carried out on collected media using a specific rabbit polyclonal antibody (a gift from Professor R.C. Baxter), the details of which are described elsewhere [13]. The assay had an intra-assay CV of 2.4–4% and an inter-assay CV of 12–16.5%.

Measurement of lactate dehydrogenase. To assess cell viability following the exposure of cells to different concentrations of glucose, lactate dehydrogenase activity was measured using an enzymatic assay (Boehringer Mannheim, Mannheim, Germany) in fresh media collected from PTCs and CFs on the final day of the experiment. The intra-assay CV was 1.5–3% and results are expressed in units per litre.

Statistical analysis. All experimental conditions were replicated sixfold using PTC and CF cultures from at least three separate human donors. Thymidine uptake, protein content and total collagen synthesis were expressed as a change from the control value (6.1 mmol/l D-glucose) which was regarded as 100%. Results are expressed as means ± SEM. Statistical comparisons between groups were made by analysis of variance (ANOVA), with pairwise multiple comparisons made by Fisher's protected least-significant differences test. Analyses were by the software package, Statview version 4.5 (Abacus Concepts, Berkeley, Calif., USA). A *p* value less than 0.05 was considered significant.

Fig. 1a, b. Thymidine uptake in **a** PTC, and **b** CF. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). *, $p < 0.05$ vs 6.1 mmol/l glucose, ** $p < 0.01$ vs 6.1 mmol/l glucose, *** $p < 0.0001$ vs 6.1 mmol/l glucose. #, $p < 0.05$, 6.1/25 mmol/l glucose vs 25 mmol/l glucose, ##, $p < 0.01$ 6.1/25 mmol/l glucose vs 25 mmol/l glucose. ■ PTC, □ CF

Fig. 2a, b. Protein content in **a** PTC and **b** CF. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). *, $p < 0.05$ vs 6.1 mmol/l glucose. ■ PTC, □ CF

Results

Cell counts. The cell count for PTCs exposed to 6.1 mmol/l glucose was $14\,278 \pm 1944.2$ cells cm^{-2} and for CFs was $18\,658 \pm 2636.6$ cells cm^{-2} . There were no statistically significant differences between cells of either type exposed to constant or intermittently raised glucose concentrations compared with control cells exposed to 6.1 mmol/l glucose.

Thymidine uptake. After PTCs were exposed to high (25 mmol/l) glucose, there was an increase in thymidine uptake of 11% ($\pm 4\%$, $p < 0.05$), compared with cells exposed to normal glucose (6.1 mmol). This effect was further enhanced to 42% ($\pm 9\%$, $p < 0.001$) when cells were exposed to alternating normal and high glucose concentrations (Fig. 1a). When CFs were exposed to 25 mmol/l glucose there was also an increase in thymidine incorporation compared with cells exposed to normal glucose concentrations of 35% ($\pm 10\%$, $p < 0.01$). Similarly thymi-

Fig. 3a, b. Collagen synthesis in **a** PTC and **b** CF. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). *, $p < 0.05$ vs 6.1 mmol/l glucose, ** $p < 0.01$ vs 6.1 mmol/l glucose, ##, $p < 0.01$ 6.1/25 mmol/l glucose vs 25 mmol/l glucose. ■ PTC, □ CF

dine uptake was enhanced when CFs were exposed to alternating normal and high glucose concentrations with a 58% ($\pm 5\%$, $p < 0.001$) increase compared with normal glucose (Fig. 1b).

Protein content. There was no statistically significant increase in the cell protein content of PTCs exposed to constant high glucose concentrations. Cells exposed to alternating normal and high glucose concentrations showed, however, a significant increase of 27% ($\pm 11\%$, $p < 0.05$), (Fig. 2a). In CFs there were no statistically significant changes in cell protein content after exposure to either constant or intermittent high glucose concentrations (Fig. 2b).

Collagen synthesis. Collagen synthesis was unchanged in PTCs exposed to constant high concentrations of glucose. After such cells were exposed to intermittent high glucose a 29% ($\pm 10\%$, $p < 0.05$) increase was, however, observed (Fig. 3a). In CFs exposed to constant high glucose a similar pattern was observed with collagen synthesis unchanged after this exposure but a 65% ($\pm 28\%$, $p < 0.01$) increase noted in cells exposed to intermittent high glucose (Fig. 3b).

Secretion of TGF- β 1 by proximal tubule cells. After exposure to 6.1 mmol/l glucose, the concentration of active TGF- β 1 in collected media was $24.6 (\pm 8.2)$ ng/mg protein. After exposure to constant high glucose this was increased by 230% ($\pm 39\%$, $p < 0.05$). This effect was further enhanced following exposure of PTCs to alternating normal and high concentrations of glucose with active TGF- β 1 secretion increased by 352% ($\pm 69\%$, $p < 0.0001$) compared with cells exposed to constant normal glucose (Fig. 4). No active TGF- β 1 was detected in media collected from CFs.

Fig. 4. Active TGF- β 1 secretion by PTCs. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). *, $p < 0.05$ vs 6.1 mmol/l glucose, *** $p < 0.0001$ vs 6.1 mmol/l glucose. #, $p < 0.05$, 6.1/25 mmol/l glucose vs 25 mmol/l glucose. ■ PTC

Fig. 5. PDGF secretion by PTCs. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). *, $p < 0.05$ vs 6.1 mmol/l glucose. ■ PTC

Secretion of PDGF by proximal tubule cells. After exposure to 6.1 mmol/l glucose, the concentration of PDGF in collected media was $4.5 (\pm 1.7)$ ng/mg protein. This was increased in PTCs exposed to constant high and alternating high glucose concentrations by 393 % (± 108 %, $p < 0.05$ and 409 % ± 140 %, $p < 0.05$) respectively (Fig. 5). The difference between cells exposed to constant and alternating high glucose concentrations was not statistically significant. No PDGF was detected in media collected from CFs.

Secretion of IGF-1 by cortical fibroblasts. The mean concentration of IGF-1 in media collected from CFs exposed to 6.1 mmol/l glucose was 18.7 ng/mg protein. There were no statistically significant changes in the secretion of this cytokine following the exposure of CFs to either constant or intermittently raised glucose concentrations (Fig. 6). No IGF-1 was detected in the media collected from PTCs.

Secretion of IGFBP-3 by cortical fibroblasts. The mean concentration of IGFBP-3 in media collected from CFs exposed to 6.1 mmol/l glucose was 459 ± 180 ng/mg protein. Unlike IGF-1 this was, however, enhanced in the presence of intermittent high

Fig. 6. IGF-1, **a** and IGFBP-3, **b** secretion by CFs. OC, osmotic control (6.1 mmol/l D-glucose and 18.9 mmol/l L-glucose). ** $p < 0.01$ vs 6.1 mmol/l glucose, #, $p < 0.05$, 6.1/25 mmol/l glucose vs 25 mmol/l glucose. □ CF

glucose concentrations by 216 % (± 40 %, $p < 0.01$). In CFs exposed to constant high glucose IGFBP-3 was also increased by 128 % (± 31 %) but this increase did not reach statistical significance and remained lower than in cells exposed to alternating concentrations of glucose (Fig. 6). No IGFBP-3 was detected in the media collected from PTCs.

Lactate dehydrogenase activity. In CFs there were no statistically significant differences in LDH activity in the media from cells exposed to 6.1 mmol/l glucose 13 ± 3 units/l⁻¹, 25 mmol/l glucose 17.5 ± 4.5 units/l⁻¹, and 6.1/25 mmol/l glucose, 19.5 ± 1.5 units/l⁻¹.

Similarly in the PTCs there were no statistically significant differences in LDH activity in media collected from cells exposed to 6.1 mmol/l glucose, 20.5 ± 3.5 units/l⁻¹, 25 mmol/l glucose, 27.5 ± 2.5 units/l⁻¹ or 6.1/25 mmol/l glucose, 22.5 ± 1.5 units/l⁻¹.

Discussion

This study has shown that cultured human tubulointerstitial cells exposed to high glucose concentrations have increased cell growth and collagen synthesis, replicating the observations that have been made in histological studies of patients with diabetic kidney disease. Moreover these effects were further enhanced in cells that were exposed to intermittent rather than constant high glucose concentrations. These findings suggest that variability in glycaemic control could be more deleterious to the tubulointerstitium than constant high concentrations of glucose. To ensure that these effects were not the result of glucose induced cytotoxicity, cell viability was assessed by measuring lactate dehydrogenase release. No differences were detected between cells exposed to normal or high glucose concentrations for either of these variables and the PTC and CF appeared to adapt to exposure to a constant high glucose concentration.

In contrast such an adaptive response is not observed in cells intermittently exposed to high glucose concentrations. This adaptation has the effect of down regulating, to an extent, the adverse cellular growth response and production of extracellular matrix.

It is possible that the lack of increase in cell number was because cells were grown to confluence before they were exposed to the different experimental conditions for the final 4 days of the study. Under such circumstances, which resemble those *in vivo*, thymidine uptake precedes increases in cell numbers by several days.

Some of these effects probably occur as a result of changes in the secretion of cytokines which we have previously shown to have both autocrine and paracrine actions in the tubulointerstitial cells [13–19]. Both PDGF and TGF- β 1 secretion were increased in media collected from PTCs. The increased incorporation of thymidine in these cells is likely therefore to occur as the result of the autocrine action of PDGF, whereas increased TGF- β 1 is likely to result in increased accumulation of extracellular matrix, detected in these studies as an increase in collagen synthesis. We have previously shown that in PTCs the effect of TGF- β 1 is to convert a proliferative stimulus to a hypertrophic stimulus, particularly in combination with increased IGF-1 activity [25]. It is likely therefore that the increased TGF- β 1 secretion by PTCs exposed intermittently to raised glucose concentrations contributes to the increased total cellular protein observed in these cells.

In cortical fibroblasts increased production of cell-associated IGFBP-3 may result in increased biological activity of IGF-1. Paracrine and autocrine action of IGF-1 has been strongly implicated in the development of diabetic nephropathy [26, 27, 28]. Although intrarenal production of IGFBP-3 has been recognised for some time its precise role in the regulation of IGF-1 activity in diabetes is not clear. The few previous studies investigating the effects of diabetes on IGFBP in the kidney reported changed expression of mRNA in animal models of diabetes [26, 27] but IGFBP concentrations were not reported and these results cannot necessarily be extrapolated to patients with diabetes. In a cyclosporin A-induced model of tubulointerstitial disease, cell growth and collagen synthesis changed as a result of dysregulation of the IGF-1/IGFBP-3 axis [20]. It has also recently emerged that IGFBP-3 has biological activity in its own right, possibly through an interaction with the TGF- β 1 V receptor [30, 31]. Thus the twofold increase in IGFBP-3 in cortical fibroblasts intermittently exposed to high concentrations of glucose, may directly result in the increased collagen synthesis and contribute to the cell growth we observed.

The mechanisms by which the response of tubulointerstitial cells to constant increases in glucose con-

centrations are down regulated are not well defined but may occur as a result of down-regulated glucose transport and therefore intracellular glucose or accompanying intracellular sodium concentrations [32]. The effects of high glucose concentrations have been best described in the proximal tubule cells of rat model of diabetes induced by streptozotocin. The activity of the luminal surface sodium-dependent glucose transporters SGLT1 and SGLT2, responsible for glucose uptake, can be either up or down regulated depending on the glucose concentration [33, 34, 35, 36]. The expression of the apical transporters responsible for the efflux of glucose from the PTC, glucose transporter (GLUT)1, GLUT2 and GLUT4 in response to raised glucose are also variable, with GLUT 1 apparently unaffected and GLUT2 expression increased in the PTC [37]. The effect on GLUT4 in the PTC is not known. In skeletal muscle this transporter is, however, down-regulated following exposure to high concentrations of glucose [38]. Interestingly, alterations in the expression of glucose transporters described above have also been shown to result in the release of cytokines such as PDGF and TGF- β 1 [39]. Little is known about the regulation of glucose transport in the cortical fibroblast. It appears likely therefore that tubulointerstitial cells exposed to constant high concentrations of glucose adapt to these conditions, possibly by changes in glucose transport. Cells exposed only intermittently, however, may not adapt and therefore show biological effects downstream, such as increased cell growth and collagen synthesis.

There are no previous reports of the effects of intermittent high glucose on human tubulointerstitial cells. The results of this study are, however, consistent with those in which cultured rat mesangial cells were exposed to constant and intermittent high glucose concentrations and increases in DNA synthesis and collagen synthesis were observed in the cells alternately exposed to normal and high concentrations of glucose [10].

In conclusion we have shown that the exposure of human PTCs and CFs to intermittent high glucose enhances the effects of the exposure of such cells to constant high glucose concentrations. The changes in cell growth and collagen synthesis were similar to those in patients with diabetic nephropathy and are probably due to the autocrine and paracrine actions of cytokines such as TGF- β 1, PDGF and changes in the IGF-1/IGFBP axis. Experiments in which cultured cells are exposed *in vitro* to constant high concentrations of glucose do not replicate the conditions experienced by the cells of patients with diabetes, in whom glucose concentrations vary considerably. Under such conditions the effects of diabetes on the tubulointerstitium are possibly underestimated. These results can be extrapolated to suggest that even in patients with apparently good diabetes control, short-

lived excursions of glycaemic control may give rise to clinically meaningful tubulointerstitial pathology. In addition, pharmacological agents with the potential to modulate the actions of the cytokines PDGF, TGF- β 1 and IGF-1 and its binding proteins would appear to have the potential to treat or prevent the tubulointerstitial component of diabetic nephropathy.

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