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Thiamine corrects delayed replication and decreases production of lactate and advanced glycation end-products in bovine retinal and human umbilical vein endothelial cells cultured under high glucose conditions

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Summary This study aimed at verifying whether thiamine, a co-enzyme which decreases intracellular glycolysis metabolites by allowing pyruvate and glyceraldheyde 3-phosphate to enter the Krebs cycle and the pentose-phosphate shunt, respectively, corrects delayed replication caused by high glucose concentrations in cultured human umbilical vein (HUVEC) and bovine retinal endothelial cells (BREC). After incubation in physiological (5.6 mmol/l) or high (28.0 mmol/l) glucose with or without 150 μmol/l thiamine, cells were counted and proliferation assessed by mitochondrial dehydrogenase activity. Lactate was measured in both cell types as an index of glycolytic activity and fluorescent advanced glycosylation end-products (AGE) concentration was determined in the HUVEC lysate. Both cell counts and proliferation assays in either of the cell types confirmed the impairment to cell replication induced by high glucose. When thiamine was added to cells kept under high glucose conditions, the number of surviving cells was significantly increased and the reduced cell proliferation appeared to be corrected. Lactate assays confirmed the increased production of this metabolite by BREC and HUVEC in high glucose, which was reduced by thiamine. Fluorescent AGE determination showed that thiamine may prevent non-enzymatic glycation in HUVEC. Thiamine restores cell replication, decreases the glycolytic flux and prevents fluorescent AGE formation in endothelial cells cultured in high glucose, suggesting that abnormal levels of glycolytic metabolite(s) may damage cells. [Diabetologia (1996) 39: 1263–1268]

Keywords Diabetes mellitus, diabetic microangiopathy, endothelium, glucose, thiamine, glycolysis, lactate, advanced glycosylation end-products.

High glucose concentrations impair the replication of cultured human umbilical vein (HUVEC) [1–3] and bovine retinal endothelial cells (BREC) [4] and this may be secondary to enhanced glycolytic flux. Altered concentrations of intermediate phosphorylated metabolites could play a role in determining cell damage. In particular, increased availability of glyceraldehyde 3-phosphate (G3P), which is much more active than glucose in promoting protein glycation and AGE formation [5], might be one of the mechanism(s) involved.

Thiamine (vitamin B_1) acts as a coenzyme for transketolase [6], pyruvate-dehydrogenase [7] and α-ketoglutarate-dehydrogenase [8]. The former shifts G3P from glycolysis to the pentose phosphate shunt, the second transforms pyruvate in acetyl-coenzyme A, which then enters the Krebs cycle, and the third catalyses the oxidation of ketoglutaric acid to succinyl-CoA within the Krebs cycle.

The aim of this study was to ascertain whether thiamine would modify glycolysis, AGE production and replication in HUVEC and BREC cultured in high glucose concentrations.

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Abbreviations: AGE, Advanced glycation end-products; BREC, bovine retinal endothelial cells; DMEM, Dulbecco's modified Eagle's medium; G3P, glyceraldehyde 3-phosphate; HUVEC, human umbilical vein endothelial cells; PBS, phosphate buffered saline Dulbecco "A"; TPB, tryptose phosphate broth.

Materials and methods

Cell cultures. HUVEC were obtained and cultured with a partial modification of Jaffe's method [9], as previously described [3]. BREC were obtained from bovine retinas with a partial modification of the methods of Wong et al. [10] and McIntosh et al. [11], as previously described [4].

HUVEC were cultured in Dulbecco's modified Eagle's medium (DMEM, 5.6 mmol/l glucose) (Sigma Chemical Co., St. Louis, Mo.,USA), added with 20% fetal calf serum (Sigma), whereas BREC were cultured in DMEM with the addition of 10% human serum (a gift from Turin Blood Bank) and 10% tryptose phosphate broth (TPB) (Sigma), until confluent.

After trypsinization, cells were cultured in the appropriate plates or flasks (see below) with standard media containing 5.6 or 28.0 mmol/l glucose (Sigma), with or without 150 μmol/l thiamine (Sigma).

Cell counts. Cells in primary culture were detached by trypsin/EDTA (Sigma) and seeded in 6-well plates (approximately 20 000 cells well, 2500 cells cm^2), with 2 ml of standard medium. After 24 h, the medium was completely removed and changed with the appropriate media (physiological or high glucose, with or without thiamine), as described above. The media were changed every 48 h. After 7 days, the wells were washed twice with phosphate buffered saline Dulbecco "A" (PBS) (Oxoid, Unipath Ltd., Basingstoke, UK); the cells were then detached by trypsin/EDTA and counted in Bürker chambers.

Proliferation assay. The assay is based on the detection of mitochondrial dehydrogenase activity, through cleavage of yellow tetrazolium salts to purple formazan crystals, in metabolically active cells [12]. Cells in secondary cultures were seeded in 24-well plates (approximately 10 000 cells/well, 5000 cells/cm^2). After 24 h, appropriate media (500 μl/well) were added as described above. After a further 48-hour incubation, 50 μl 5 mg/ml MTT (3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) solution (Boehringer Mannheim Biochemica, Mannheim, Germany) was added to each well. The cells were incubated at 37 °C for 4 h and then 500 μl of solubilization solution (10% sodium dodecylsulphate (SDS) in 0.01 mol/l HCl) (Boehringer) was added. The plates were left overnight at 37 °C and then the absorbance at 580 nm of each well was measured spectrophotometrically.

Lactate assay. Cells were seeded in sterile Petri dishes 3.5 cm in diameter (approximately 20 000 cell/well, 2500 cell/cm^2) and incubated for 24 h with 2 ml standard medium. The latter was then changed with the above experimental media and cells were incubated for 2 and 7 days to confirm the data obtained at the same time points of the cell counts and proliferation assays. Twenty-four hours before the assay, the media were removed and DMEM without serum and phenol red pH indicator (both of which interfere with the spectrophotometer readings) was added instead. Lactate concentrations in the media were calculated as generation of NADH by lactate dehydrogenase, as detected spectrophotometrically at 340 nm. Results are expressed as mmol/l lactate produced in 1 h by 100000 cells.

Fluorescent AGE determination. HUVEC were cultured for 20 days with the above experimental media in 25 cm^2 flasks (seeding density: approximately 5000 cell/cm^2). Cells were then detached by trypsin, washed and lysed by NaOH 0.1 N. Total protein concentrations were determined by the Lowry method (Sigma) and spontaneous fluorescence was detected at 370 nm excitation and 440 nm emission by a fluorimeter (Jenway mod. 6200, Dunmow, Essex, UK), as described by Monnier et al. [13]. Results are given as fluorescence/total protein ratio. Fluorescence was used as a non-specific marker of AGE production.

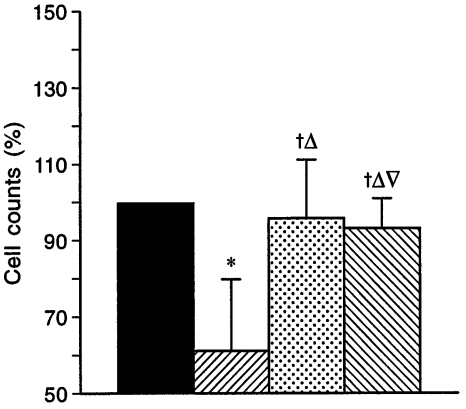

Fig. 1. HUVEC recovered after culture in glucose 5.6 ■ and 28.0 ▨ mmol/l, with □ or without ▧ 150 μmol/l thiamine, expressed as percentages of the cell counts in glucose 5.6 mmol/l. * $p < 0.01$ vs 5.6 glucose; † NS vs 5.6 glucose; △, $p < 0.05$ vs 28.0 glucose; ▽, NS vs 5.6 glucose + thiamine

Statistical analysis

Because of the wide batch-to-batch variations, data are expressed as percentages (mean ± SD) of the results obtained in 5.6 mmol/l glucose within each experiment, unless otherwise stated. Statistical comparison among groups was carried out by analysis of variance and then both Bonferroni's *t*-test and Wilcoxon's rank-sum test were used to confirm the significances of the differences between any two groups. Results were considered significant if the *p*-value was 0.05 or less.

Results

After 1 week of culture in the relevant media, both HUVEC and BREC grown under high glucose conditions (28.0 mmol/l) were significantly less numerous than those grown in physiological glucose (5.6 mmol/l).

HUVEC counts in high glucose ($n = 6$) were 61.6 ± 18.4% of those in physiological glucose ($p < 0.01$), 96.0 ± 15.6% in normal glucose and thiamine (NS vs normal glucose, $p < 0.05$ vs high glucose alone), and 93.5 ± 7.9% in high glucose plus thiamine (NS vs normal glucose, $p < 0.05$ vs high glucose and NS vs normal glucose plus thiamine) (Fig. 1).

BREC counts in high glucose ($n = 10$) were 82.5 ± 9.6% ($p < 0.01$ vs physiological glucose), 106.3 ± 20.8% in physiological glucose plus thiamine (NS vs physiological glucose alone, $p < 0.05$ vs high glucose alone), and 127.5 ± 24.0% in high glucose plus thiamine ($p < 0.05$ vs physiological glucose alone, $p < 0.01$ vs high glucose alone, NS vs physiological glucose plus thiamine) (Fig. 2).

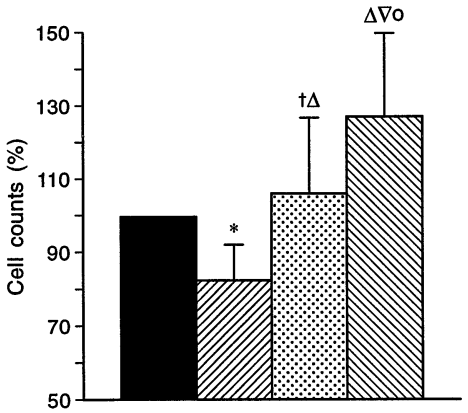

Fig. 2. BREC recovered after culture in glucose 5.6 ■ and 28.0 ▨ mmol/l, with □ or without ▨ 150 μmol/l thiamine, expressed as percentages of the cell counts in glucose 5.6 mmol/l. * $p < 0.01$ vs 5.6 glucose; † NS vs 5.6 glucose; △, $p < 0.05$ vs 28.0 glucose; ▽, NS vs 5.6 glucose + thiamine; ◯, $p < 0.05$ vs 5.6 glucose

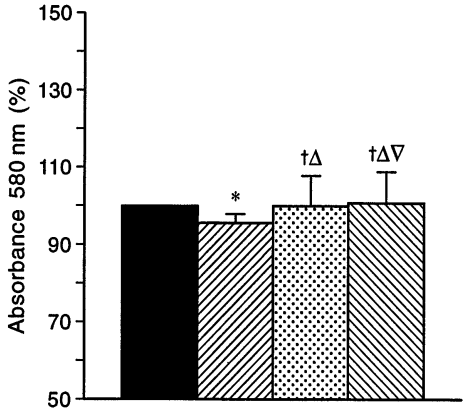

Fig. 3. Proliferation assays of HUVEC after culture in glucose 5.6 ■ and 28.0 ▨ mmol/l, with □ or without ▨ 150 μmol/l thiamine, expressed as percentages of the absorbances at 580 nm of glucose 5.6 mmol/l. * $p < 0.01$ vs 5.6 glucose; † NS vs 5.6 glucose; △, $p < 0.05$ vs 28.0 glucose; ▽, NS vs 5.6 glucose + thiamine

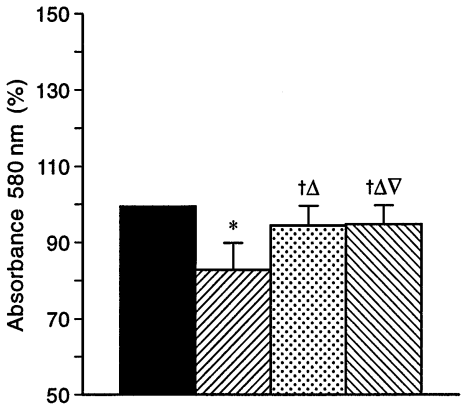

Fig. 4. Proliferation assays of BREC after culture in glucose 5.6 ■ and 28.0 ▨ mmol/l, with □ or without ▨ 150 μmol/l thiamine, expressed as percentages of the absorbances at 580 nm of glucose 5.6 mmol/l. * $p < 0.01$ vs 5.6 glucose; † NS vs 5.6 glucose; △, $p < 0.01$ vs 28.0 glucose; ▽, NS vs 5.6 glucose + thiamine

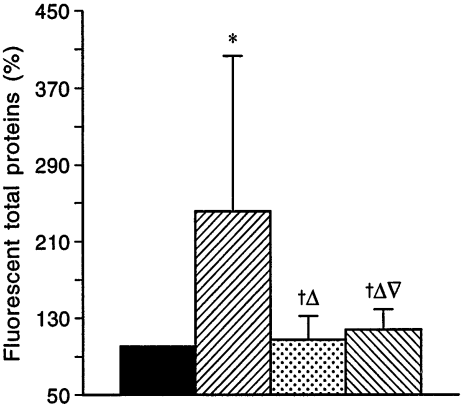

Fig. 5. AGE production in HUVEC after culture in glucose 5.6 ■ and 28.0 ▨ mmol/l, with □ or without ▨ 150 mmol/l thiamine, expressed as percentages of the fluorescence/total protein ratio of glucose 5.6 mmol/l. * $p < 0.05$ vs 5.6 glucose; † NS vs 5.6 glucose; △, $p < 0.05$ vs 28.0 glucose; ▽, NS vs 5.6 glucose + thiamine

The proliferation assay results in HUVEC ($n = 10$) were as follows. High glucose: 95.4 ± 2.3 % ($p < 0.01$ vs physiological glucose). Physiological glucose plus thiamine: 100.4 ± 7.4 % (NS vs physiological glucose alone, $p < 0.05$ vs high glucose alone). High glucose plus thiamine: 100.7 ± 8.1 % (NS vs physiological glucose alone, $p < 0.05$ vs high glucose alone, NS vs physiological glucose plus thiamine) (Fig. 3).

The proliferation assay in BREC ($n = 12$) was as follows. High glucose: 83.2 ± 6.8 % ($p < 0.01$ vs physiological glucose). Physiological glucose plus thiamine: 94.6 ± 5.2 % (NS vs physiological glucose alone, $p < 0.01$ vs high glucose alone). High glucose plus thiamine: 94.8 ± 5.4 % (NS vs physiological glucose, $p < 0.01$ vs high glucose, NS vs physiological glucose plus thiamine) (Fig. 4).

The results of lactate measurements ($n = 6$) are shown in Table 1 (HUVEC) and Table 2 (BREC).

Finally, fluorescent AGE production in HUVEC seemed to be prevented by thiamine. High glucose 241.5 ± 163.2 % ($p < 0.05$ vs 5.6 glucose), physiological glucose plus thiamine 106.9 ± 24.5 % (NS vs 5.6 glucose, $p < 0.05$ vs high glucose), high glucose plus thiamine 117.5 ± 21.2 (NS vs 5.6 glucose and 5.6 plus thiamine, $p < 0.05$ vs 28.0 glucose) (Fig. 5).

Measurements of glucose concentrations and pH in the media at the time of exchange and at the end of the experiments did not show any relevant modification from the starting conditions (data not shown). Osmotic pressure after addition of glucose was 334 vs 311 mosmol/l of standard medium; addition of thiamine had no relevant effect on osmotic pressure.

Table 1. Lactate concentrations in HUVEC (mmol · l · 10^5 cells · h), expressed as percentages of those produced in physiological glucose alone ($n = 6$)

Day	Glucose 5.6 mmol/l	Glucose 28.0 mmol/l	Glucose 5.6 mmol/l + thiamine	Glucose 28.0 mmol/l + thiamine
2	100.0 ± 0.0	163.5 ± 33.1 ($p < 0.05$ vs 5.6 glucose)	130.7 ± 28.3 (NS vs 5.6 and 28.0 glucose)	134.5 ± 33.1 (NS vs 5.6 glucose and 5.6 + thiamine, $p < 0.05$ vs 28.0 glucose)
7	100.0 ± 0.0	209.2 ± 83.2 ($p < 0.05$ vs 5.6 glucose)	112.8 ± 40.0 (NS vs 5.6 glucose, $p < 0.05$ vs 28.0 glucose)	113.2 ± 20.5 (NS vs 5.6 glucose and 5.6 + thiamine, $p < 0.05$ vs 28.0 glucose)

Table 2. Lactate concentrations in BREC (mmol · l · 10^5 cells · h), expressed as percentages of those produced in physiological glucose alone ($n = 6$)

Day	Glucose 5.6 mmol/l	Glucose 28.0 mmol/l	Glucose 5.6 mmol/l + thiamine	Glucose 28.0 mmol/l + thiamine
2	100.0 ± 0.0	149.3 ± 47.8 ($p < 0.05$ vs 5.6 glucose)	102.2 ± 12.2 (NS vs 5.6 glucose and $p < 0.05$ vs. 28.0 glucose)	97.0 ± 11.6 (NS vs 5.6 glucose and 5.6 + thiamine, $p < 0.05$ vs 28.0 glucose)
7	100.0 ± 0.0	158.0 ± 32.5 ($p < 0.05$ vs 5.6 glucose)	97.5 ± 23.9 (NS vs 5.6 glucose, $p < 0.05$ vs 28.0 glucose)	84.8 ± 19.0 (NS vs 5.6 glucose and 5.6 glucose + thiamine, $p < 0.05$ vs 28.0 glucose)

Discussion

It has not been possible up to now to identify the mechanisms through which high glucose concentration delays endothelial cell replication in culture. This paper was aimed at testing the hypothesis that damage by high glucose concentration is produced via direct stimulation of the glycolytic pathway. The results suggest that thiamine (vitamin B_1), a coenzyme of transketolase [6], pyruvate-dehydrogenase [7] and α-ketoglutarate-dehydrogenase [8], prevents endothelial cell damage due to high glucose [1–4], by re-directing the glycolytic flux.

Upon entering endothelial cells, glucose is either shuttled to the surrounding tissues by GLUT1 and GLUT3 glucose transporters [14, 15], or phosphorylated to glucose 6-phosphate, by the enzyme hexokinase, to enter the cell metabolism. A relatively constant amount enters the pentose phosphate shunt [6] while, in the presence of supraphysiological concentrations, aldose reductase may activate the polyol pathway [16, 17]. Thiamine reduces anaerobic glycolysis and increases the aerobic pentose phosphate pathway by helping transketolase to transform glyceraldehyde-P into ribulose-P [6]. Increased lactate production, as shown in this paper (Tables 1 and 2), suggests that glycolysis was indeed potentiated by high glucose in the cells tested, whereas its reduction after addition of thiamine suggests that the co-enzyme managed to deviate the flux towards the pentose-phosphate shunt and/or to re-direct pyruvate towards the Krebs cycle.

Increased glycolysis suggests that hexokinase had been activated by high glucose. Hexokinase is the first key enzyme of glycolysis and has a low K_m for glucose [18], which means that it is maximally functional for physiological substrate concentrations. To account for the finding of increased lactate production, it must be assumed that the enzyme was further activated by high glucose. Some authors have reported that hexokinase activity is indeed modified by high glucose in the diabetic rat kidney cortex [19] and in the *rete mirabilis* of the eel swimbladder [20], but did not establish whether this is due to over-expression or post-translational modifications of the enzyme.

Both cell counts and mitochondrial dehydrogenase activity assay confirmed the damage induced by high glucose to the cell replication machinery (Figs. 1–4). When thiamine was added, the number of surviving cells was significantly increased. Hence, delayed cell replication induced by high glucose seems to be corrected by thiamine.

Potentiation of glycolysis may lead to increased production of some metabolites that are very active in non-enzymatic binding of the NH_2-*epsilon*-terminal lysine groups of proteins. In particular, glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-bisphosphate and G3P may be orders of magnitude more active than glucose itself in glycating proteins [5, 21]. After 20 days of culture in high glucose, HUVEC proteins were glycated to a greater extent than those of the cells grown in physiological glucose. Thiamine appeared to prevent AGE formation (Fig. 5), possibly by shifting excess G3P towards the pentose phosphate cycle.

Thiamine had no stimulating or inhibiting effects on cell growth and lactate or fluorescent AGE

production, when added to physiological (5.6 mmol/l) glucose concentrations. The data on cell counts in BREC and lactate measurement in HUVEC at day 2 cannot be fully accounted for, but do not appear to disprove the protective effects of the coenzyme on glucose-produced damage.

The 150-μmol/l thiamine concentration used in the experiments is higher than the concentrations found in serum (about 150 nmol/l). However, circulating thiamine is promptly taken up and stored in tissues, where higher, though unknown, concentrations are present. The amount used in this study, though presumably higher than physiological, was definitely effective in preventing damage caused by high glucose through putative mechanisms (activation of metabolic pathways alternative to glycolysis and removal of excess damaging metabolites, such as G3P) which are not sufficiently activated in the presence of the thiamine concentrations contained in the culture media (about 15 μmol/l, depending on the batch of serum added).

Alternative mechanisms of high glucose-induced damage to cell replication may be related to excess acetyl-CoA production, as derived from enhanced glycolysis. Acetyl-CoA binds to tubulin subunits and stabilizes the microtubular network, thus impairing the formation of the mitotic spindle [22]. We were previously able to show colchicine resistance, a sign of tubulin stabilization, in HUVEC maintained in high glucose [23] and correction of delayed HUVEC replication following the addition of L-tyrosine [24], which is involved in tubulin depolymerization [25]. By activating α-ketoglutarate-dehydrogenase, thiamine might facilitate the utilization of excess acetyl-CoA in the Krebs cycle.

The possibility that glucose exerts merely osmotic damage, rather than direct toxic effects, is ruled out by previous reports that equiosmolar concentrations of mannitol do not impair cell growth [2, 3]. Aldose reductase has been described in HUVEC [16], whereas doubts remain about its relevance to the pathophysiology of retinal capillaries [26]. High glucose might upregulate the expression of the enzyme in chronic hyperglycaemia [16] but addition of specific inhibitors of aldose-reductase to the culture media could not correct the antimitotic effect of high glucose on HUVEC [27, 28].

In conclusion, thiamine corrects delayed endothelial replication. Reduced lactate production and AGE formation may be among the mechanisms involved in this effect.

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References

1. Stout RW (1982) Glucose inhibits replication of cultured human endothelial cells. Diabetologia 23: 436–439
2. Lorenzi M, Cagliero E, Toledo S (1986) Glucose toxicity for human endothelial cells in culture: delayed replication, disturbed cell cycle, and accelerated death. Diabetes 34: 621–627
3. Porta M, La Selva M, Bertagna A, Molinatti GM (1988) High glucose concentrations inhibit DNA synthesis and replication, without causing death or impairing injury repair, in cultured human endothelial cells. Diabetes Res 7: 59–63
4. Porta M, Molinatti PA, Dosso A, Williams FMK, Brooks RA, Kohner EM (1994) Growth of bovine retinal pericytes and endothelial cells in high hexose concentrations. Diabète Métab 20: 25–30
5. Brownlee M (1994) Glycation and diabetic complications. Diabetes 43: 836–841
6. Racker E, De La Haba G, Leder IG (1953) Thiamine pyrophosphate, a coenzyme of transketolase. J Am Chem Soc 75: 1010–1015
7. Fernandez-Moran A, Reed LJ, Koike M, Willms CR (1964) Electron microscopy and biochemical studies of pyruvate-dehydrogenase complex of *E. coli.* Science 145: 939–932
8. Koike M, Reed LJ (1960) α-keto acid dehydrogenation complexes. J Biol Chem 235: 1931–1935
9. Jaffe EA, Nachman RL, Becker CG, Minick CR (1973) Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. J Clin Invest 52: 2745–2756
10. Wong HC, Boulton M, Marshall J, Clark P (1987) Growth of retinal capillary endothelial cells using pericyte conditioned medium. Invest Ophtalmol Vis Sci 28: 1767–1775
11. McIntosh LC, Muckersie L, Forrester JV (1988) Retinal capillary endothelial cells prefer different substrates for growth and migration. Tissue Cell 20: 193–209
12. Scudiero DA, Shoemaker RH, Paull KD et al. (1988) Evaluation of a soluble tetrazolium/formazan assay for cell growth and drug sensitivity in culture using human and other tumor cell lines. Cancer Res 48: 4827–4833
13. Monnier VM, Kohn RR, Cerami A (1984) Accelerated age-related browning of human collagen in diabetes mellitus. Proc Natl Acad Sci USA 81: 583–587
14. Corkey RF, Corkey BE, Gimbrone Jr M (1981) Hexose transport in normal and SV-40-transformed human endothelial cells in culture. J Cell Physiol 106: 425–434
15. Knott RM, Robertson M, Forrester AB (1993) Regulation of glucose transporter (GLUT3) and aldose reductase mRNA in bovine retinal endothelial cells and retinal pericytes in high galactose culture. Diabetologia 36: 808–812
16. Hawthorne GC, Bartlett K, Hetherington CS, Alberti KGMM (1989) The effect of high glucose on polyol pathway activity and myo-inositol metabolism in cultured human endothelial cells. Diabetologia 32: 163–166
17. Ohtaka M, Tawata M, Hosaka Y, Onaya T (1992) Glucose modulation of aldose reductase mRNA expression and its activity in cultured pulmonary artery endothelial cells. Diabetologia 35: 730–734
18. Grossbard L, Schimke RT (1966) Multiple hexokinases of rat tissues. Purification and comparison of soluble forms. J Biol Chem 241: 3546–3560
19. Sochor M, Baquer NZ, McLean P (1979) Glucose overutilization in diabetes: evidence from studies on the changes in hexokinase, the pentose phosphate pathway and glucuronate-xylulose pathway in rat kidney cortex in diabetes. Biochem Biophys Res Commun 86: 32–39

20. Belfiore F, Iannello S, Campione R, Bombaci M, Gulisano U (1990) Selected aspects of capillary morphology and metabolism in diabetes. In: Molinatti GM, Bar RS, Belfiore F, Porta M (eds) Endothelial cell function in diabetic microangiopathy: problems in methodology and clinical aspects. Karger, Basel pp 57–63
21. Swamy MS, Tsai C, Abraham A, Abraham EC (1993) Glycation mediated lens cristallin aggregation and cross-linking by various sugars and sugar phosphates in vitro. Exp Eye Res 56: 177–185
22. Falconer M, Vielkind U, Brown D (1989) Establishment of a stable, acetylated microtubule bundle during neuronal committment. Cell Motil Cytoskeleton 12: 169–180
23. Porta M, La Selva M, Molinatti P (1991) von Willebrand factor abnormalities in diabetic microangiopathy. Diabetes Care 14 [Suppl 1]: 152–155
24. La Selva M, Chiara P, Muccini E et al. (1992) Delayed replication of human umbilical vein endothelial cells in high glucose is corrected by L-tyrosine. Diabetes Res 19: 87–90
25. Bulinski JC, Richards JE, Piperno F [1988] Post-translational modification of *alpha*-tubulin: detyrosination and acetylation differentiate populations of interphase microtubules in cultured cells. J Cell Biol 106: 1213–1230
26. Akagi Y, Yajima Y, Kador PF, Kuwabara T, Kinoshita JH (1984) Localization of aldose reductase in human eye. Diabetes 33: 562–566
27. Lorenzi M, Toledo S, Boss GR, Lane MJ, Montisano DF [1987] The polyol pathway and glucose 6-phosphate in human endothelial cells cultured in high glucose concentrations. Diabetologia 30: 222–227
28. Molinatti PA, La Selva M, Porta M, Molinatti GM (1990) Effects of an aldose-reductase inhibitor on replicating cultured human endothelial cells subjected to increasing glucose concentrations. Diabetes Nutr Metab 3: 153–155