Original Article

Diabetic Nephropathy Is Associated With Gene Expression Levels of Oxidative Phosphorylation and Related Pathways

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The in vitro behavior of skin fibroblasts from patients with or without diabetic nephropathy is associated with diabetic nephropathy risk. Here we compared skin fibroblast gene expression profiles from two groups of type 1 diabetic patients: 20 with very fast ("fast-track") versus 20 with very slow ("slow-track") rates of development of diabetic nephropathy lesions. Gene expression profiles of skin fibroblasts grown in 25 mmol/l glucose for 36 h were assessed by Affymetrix HG-U133A GeneChips to determine the proportion of genes in a given biological pathway that were directionally consistent in their group differences. Five pathways reached statistical significance. All had significantly greater proportions of genes with higher expression levels in the fast-track group. These pathways, the first four of which are closely related and have overlapping genes, included oxidative phosphorylation (P < 0.001), electron transport system complex III (P = 0.017), citrate cycle (P = 0.037), propanoate metabolism (P = 0.044), and transcription factors (P = 0.046). These results support the concept that oxidative phosphorylation and related upstream pathways may be important in the pathogenesis of diabetic nephropathy. Whether these findings reflect inherent genetic cellular characteristics, "cell memory," or both requires further study. *Diabetes* 55:1826–1831, 2006

Oxidative stress represents overproduction of reactive oxygen species (ROS) relative to antioxidant defenses (1). Mitochondria, through the oxidative phosphorylation pathway, are the major cellular source of ROS (1). It has been hypothesized, based on indirect evidence, that increased oxidative stress contributes to diabetic nephropathy development (2–4). Mesangial cells incubated in high glucose had increased mitochondrial membrane potential and ROS production (5). Renal proximal tubular cells cultured in high glucose had increased H_2O_2 and lipid peroxide production and reduced antioxidant defenses, including decreased catalase activity and glutathione and endogenous antioxidants production (6). Pretreatment of these cells with rotenone (an inhibitor of the mitochondrial electron transport chain) and diphenylene iodonium (a flavoprotein enzyme inhibitor) blocked these in vitro effects of high glucose (6). Increased mitochondrial ROS production was also found in bovine aortic endothelial cells grown in high glucose, and blocking this increase prevented the downstream changes in several major biological pathways associated with the pathogenesis of diabetic nephropathy (4). Recently, these studies have been modeled into an unified hypothesis linking increased mitochondrial superoxide production to major important pathways in diabetic nephropathy pathogenesis (7). In addition, studies of superoxide dismutase-1 (SOD-1) transgenic *db/db* diabetic mice provided in vivo support for the role of oxidative stress in the pathogenesis of diabetic nephropathy (8). More direct evidence supporting the above hypothesis comes from studies that found increased oxidative stress and impaired antioxidant enzyme responses to high glucose in cultured skin fibroblasts from type 1 diabetic patients with diabetic nephropathy (9,10). These studies (9,10) are consistent with earlier work linking behaviors of cultured skin fibroblasts to diabetic nephropathy risk (11–16).

Microarray analysis can be a powerful tool to explore gene expression profiles. However, it can also produce multiple false-positive or -negative findings. Breaking through this methodological background noise to recognize gene expression patterns related to disease has been challenging. This study compared gene expression profiles in skin fibroblasts from type 1 diabetic patients with very fast versus very slow diabetic nephropathy development by testing whether there were directionally consistent group differences in expression levels within defined biological pathways.

RESEARCH DESIGN AND METHODS

Participants in this study were selected from a cohort of 125 patients with >8 years of diabetes duration. This cohort, described in detail elsewhere (17), had a wide range of kidney involvement (11,12,17). Briefly, skin and kidney biopsies were obtained from each patient, and patients were ranked based on their mesangial expansion score, an estimate of the rate of development of mesangial expansion ascertained by electron microscopy morphometric analysis (11,17). The 25 patients with the highest mesangial expansion score and microalbuminuria or proteinuria were allocated to the "fast-track" and the 25 with lowest mesangial expansion score and normoalbuminuria to the "slow-track" group (11,12). Of these, 20 fast-track (all proteinuric) and 20 slow-track patients were used for the current study. Demographic characteristics were

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ROS, reactive oxygen species.

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TABLE 1
Demographic, clinical, and renal structural characteristics of type 1 diabetic patients

	Slow track	Fast track	*P*
M/F	8/12	9/11	NS
Age (years)	36 ± 10	38 ± 8	NS
Diabetes duration (years)	20 ± 8	24 ± 7	NS
A1C (%)	8.4 ± 1.2	9.4 ± 1.5	0.027
Blood pressure (mmHg)	117 ± 9 / 69 ± 7	135 ± 11 / 77 ± 7	<0.001
Hypertension (yes/no)	1/19	19/1	<0.001
Antihypertensive treatments (yes/no)	0/20	18*/2	<0.001
Albumin excretion rate (μg/min)	5.3 (0.4–15.9)	599.6 (229–4630)	NA
GFR (ml/min per 1.73 m^2)	115 ± 13	72 ± 20	<0.001
GBM width (nm)	415 (329–737)	768 (515–1,249)	<0.001
Vv(Mes/glom)	0.20 ± 0.02	0.56 ± 0.10	<0.001
Mesangial expansion score	−0.012 ± 0.114	1.54 ± 0.490	NA

Data are the means ± SD or median (range), unless otherwise indicated. *Diuretic: $n = 8$; α-blocker: $n = 4$; β-blocker: $n = 2$; calcium channel blocker: $n = 4$; ACE inhibitor: $n = 7$; angiotensin II receptor blocker: $n = 3$. GBM, glomerular basement membrane (normal value = 332 ± 46); GFR, glomerular filtration rate; NA, not applicable, different by study design; Vv(Mes/glom), mesangial fractional volume (normal value = 0.20 ± 0.03).

similar between the groups (Table 1). As expected, glycemia and blood pressure were higher and the glomerular filtration rate was lower in the fast-track patients. A total of 18 of 20 fast-track but none of the slow-track patients were receiving antihypertensive drugs. Glomerular structure was markedly abnormal in the fast-track group, whereas it was normal or only mildly abnormal in the slow-track group (Table 1).

Cell culture and RNA isolation. The methods for cell culture and RNA isolation have been previously detailed (12). Briefly, skin fibroblasts were cultured in Dulbecco's modified Eagle's medium with 25 mmol/l glucose and 10% FCS for 36 h after synchronization for 24 h. Total RNA was isolated and its integrity evaluated, using the RNA 6000 LabChip kit and Agilent 2100 bioanalyzer (Hewlett Packard, Palo Alto, CA).

Microarray: target preparation, hybridization, and data filtering. Biotinylated cRNA was generated from total RNA according to Affymetrix protocols (Santa Clara, CA). A hybridization mixture containing 20 μg biotinylated cRNA from each patient was hybridized to individual Affymetrix HG-U133A GeneChips. The chips were then washed, scanned, and analyzed by GeneChip Microarray Suite V.5.0. Global scaling was used on all probe sets to standardize the average signal of each array to a 500 target value, and the software provided values of expression levels of each gene relative to this average signal (18). Thus, these data are provided in relative value units. The gene expression data set was selected for further analysis as follows. First, genes that meet the criterion of at least 50% present calls in all array experiments were included in the data set. Present, absent, and marginal designations were replaced in an Excel spreadsheet with present = 1, absent = 0, and marginal = 0.2. The values for a given gene in all of the arrays were then summed, and a PAM (present, absent, and marginal) score was obtained. A score of at least 20 was required for inclusion. Second, the previously excluded data with scores <20 and >5 were analyzed, using a χ^2 test to discover any asymmetric distribution in the present calls between groups for any given gene. The significance level was set at $P < 0.2$, Fisher's exact test (two-tailed) was used for an entry <5, and Yates corrected test was used for an entry >5. The selected gene expression levels data were then used for directional pathway analysis.

Directional pathway analysis. The Exploratory Visual Analysis system, which has been previously described (19,20), was modified to test directionally consistent group differences in expression levels within biological pathways defined by the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway database (available online from http://www.genome.jp/kegg/pathway.html). This system uses a 1,000-fold permutation test on each pathway to evaluate whether the occurrence of the proportion of statistically significantly (Student's *t* test, $P \leq 0.05$) increased/decreased genes in a biological pathway is higher than would be expected by chance (19,20). For the *P*-plus test in a given biological pathway, the null hypothesis is that the number of genes with increased expression levels is the same or lower than that found in a "pseudo-pathway" with an identical number of genes randomly selected from the entire database. For the *P*-minus test, the number of significantly decreased genes is tested. A total of 1,000 pseudo-pathway replications were performed, and the final *P* value was calculated by dividing the total counts by 1,000. Thus, the *P* value represents the probability of obtaining the observed number of genes with higher (*P*-plus tests) or lower (*P*-minus test) expression levels in a given biological pathway than by chance alone.

Real-time RT-PCR. The methods for RT-PCR have been described previously (11,12). The sequence of the probe and the forward and reverse primers used for NDUFS1 (NADH dehydrogenase [ubiquinone] Fe-S protein 1) were: 5′-FAM-CAAAGCTGTCACAGAGGGTGCCCA-TAMRA-3′, 5′-CACAGACAATGGCCAAATGTG-3′, and 5′-CAGCATATGGATGGTTCCTCTACTG-3′, respectively. The sequence of the probe and the forward and reverse primers used for COX 7B (cytochrome c oxidase subunit VIIb) were: 5′-FAM-CGCACTAAATCGTCTCCAAGTTCGAAGC-TAMRA-3′, 5′-CGATGTTTCCCTTGGTCAAAA-3′, and 5′-CCTTGCCATTGTTTGCTGAA-3′, respectively. These probes were labeled with 6-FAM (6-carboxyfluorescein) at the 5′ end and TAMRA (6-carboxy-tetramethyl-rhodamine) at the 3′ end.

RESULTS

Skin fibroblasts from 20 patients with a very rapid development of diabetic nephropathy (fast-track group) and 20 with a very slow rate of diabetic nephropathy development (slow-track group) were cultured in Dulbecco's modified Eagle's medium with 25 mmol/l glucose for 36 h, and their gene expression profiles were assessed by Affymetrix HG-U133A GeneChips. At the time of skin biopsy, HbA_{1c} (A1C) was higher in fast-track (9.4 ± 1.5%) than in slow-track (8.4 ± 1.2%, $P = 0.027$) patients. A total of 456 of ∼22,000 genes showed significant group differences

TABLE 2
Pathways with directionally consistent group differences in gene expression levels in the fast- versus slow-track groups

Pathways	T	↑	*P*+	↓	*P*−
Oxidative phosphorylation	127	10	<0.001	0	0.099
Electron transport system, complex III	11	2	0.017	0	0.82
Citrate cycle	35	3	0.037	1	0.493
Propanoate metabolism	40	3	0.044	1	0.554
Transcription factors	36	3	0.046	0	0.516

T, total number of genes in the pathway; ↑, number of genes with higher expression levels in the fast-track versus slow-track groups; *P*+, *P* value for *P*-plus test; ↓, number of genes with lower expression levels in the fast-track versus slow-track groups; *P*−, *P* value for *P*-minus test.

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TABLE 3
Differentially expressed genes in the five pathways with directionally consistent group differences in gene expression levels

Gene ID	Gene name (symbol)	Gene expression levels (relative value)		P	Δ
		Fast track	Slow track		
Oxidative phosphorylation					
522	ATP synthase, H + transporting, mitochondrial F0 complex, subunit F6 (ATP5J)	2,806 ± 419	2,516 ± 254	0.012	+
528	ATPase, H + transporting, lysosomal 42 kDa, V1 subunit C, isoform 1 (ATP6V1C1)	503 ± 69	450 ± 90	0.045	+
1349	Cytochrome c oxidase subunit VIIb (COX7B)	2,762 ± 596	2,321 ± 324	0.007	+
4719	NADH dehydrogenase (ubiquinone) Fe-S protein 1 (NDUFS1)	812 ± 118	724 ± 91	0.012	+
4707	NADH dehydrogenase (ubiquinone) 1β subcomplex 1 (NDUFB1)	2,977 ± 739	2,569 ± 454	0.044	+
4714	NADH dehydrogenase (ubiquinone) 1β subcomplex 8 (NDUFB8)	2,233 ± 570	1,854 ± 573	0.042	+
7385	Ubiquinol-cytochrome c reductase core protein II (UQCRC2)	1,329 ± 138	1,196 ± 126	0.003	+
7381	Ubiquinol-cytochrome c reductase binding protein (UQCRB)	3,913 ± 833	3,306 ± 709	0.018	+
2110	Electron-transferring-flavoprotein dehydrogenase (ETFDH)	236 ± 31	217 ± 28	0.047	+
6391	Succinate dehydrogenase complex subunit C (SDHC)	1,090 ± 173	980 ± 140	0.033	+
Electron transport system, complex III					
7381	Ubiquinol-cytochrome c reductase binding protein (UQCRB)	3,912 ± 833	3,306 ± 709	0.018	+
7385	Ubiquinol-cytochrome c reductase core protein II (UQCRC2)	1,329 ± 138	1,196 ± 126	0.003	+
Citrate cycle (TCA cycle)					
2271	Fumarate hydratase (FH)	1,543 ± 432	1,278 ± 273	0.027	+
6391	Succinate dehydrogenase complex subunit C (SDHC)	1,090 ± 173	980 ± 140	0.033	+
8803	Succinate-CoA ligase, ADP-forming, beta subunit (SUCLA2)	912 ± 93	842 ± 105	0.031	+
1743	Dihydrolipoamide S-succinyltransferase (DLST)	1,049 ± 144	1,231 ± 194	0.002	−
Propanoate metabolism					
34	Acyl-CoA dehydrogenase, C-4 to C-12 straight chain (ACADM)	707 ± 167	583 ± 116	0.01	+
39	Acetyl-CoA acetyltransferase 2 (ACAT2)	616 ± 79	560 ± 67	0.022	+
8803	Succinate-CoA ligase, ADP-forming, β-subunit (SUCLA2)	912 ± 93	842 ± 105	0.031	+
31	Acetyl-CoA carboxylase-α (ACACA)	490 ± 129	581 ± 151	0.048	−
Transcription factors					
2958	General transcription factor IIA, 2 (GTF2A2)	2,059 ± 411	1,835 ± 251	0.045	+
2963	General transcription factor IIF, polypeptide 2 (GTF2F2)	241 ± 83	194 ± 62	0.049	+
6880	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor (TAF9)	1,138 ± 174	980 ± 134	0.003	+

Gene expression levels data are the means ± SD, expressed as values relative to the standardized average signal of 500 on each microarray chip after global scaling. Δ, direction of gene expression level difference between the fast-track versus slow-track group; +, higher expression levels in the fast-track group; −, lower expression levels in the fast-track group.

($P < 0.05$) in mRNA expression levels, with 237 higher and 219 lower in the fast- versus slow-track patients.

There was an increased proportion of genes with higher expression levels in the oxidative phosphorylation pathway (P-plus test, $P < 0.001$) in the fast-track compared with the slow-track group: expression levels of 10 of 127 genes on the U133A GeneChip belonging to the oxidative phosphorylation pathway were higher in the fast-track patients (Tables 2 and 3). No genes in this pathway had lower expression levels in the fast-track group. The expression levels of two of these genes, NDUFS1 and COX 7B, were confirmed by RT-PCR to be higher in the fast-track than in the slow-track patients (Table 4).

Four additional pathways, three closely related to the oxidative phosphorylation pathway, also had increased proportion of genes with higher expression levels in the fast- versus the slow-track group (Tables 2 and 3). These pathways included electron transport system complex III, citrate cycle, and propanoate metabolism. Group directional differences in transcription factors pathway were

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TABLE 4
Gene expression levels of NDUFS1 and COX 7B measured by RT-PCR

Genes	Fast track	Slow track	P
NDUFS1	1.39 ± 0.12	1.31 ± 0.17	0.026
COX 7B	1.50 ± 0.12	1.35 ± 0.13	0.0007

Data are the means ± SD. Relative values of target mRNA in samples with 0.1 μg total RNA are expressed as fold changes to the target mRNA expression in 0.1 μg reference standard total RNA. NDUFS1, NADH dehydrogenase (ubiquinone) Fe-S protein 1; COX 7B, cytochrome c oxidase subunit VIIb.

also seen (Tables 2 and 3). None of the pathways represented on the Affymetrix GeneChip had a greater proportion of genes with lower expression levels in the fast- versus the slow-track group. General cell regulation pathways, such as pyrimidine metabolism (P-plus test: P = 0.106; P-minus test: P = 0.188), lysine degradation (P-plus test: P = 0.62; P-minus test: P = 0.211), and cell cycle (P-plus test: P = 0.61; P-minus test: P = 0.136) were not different between the groups.

DISCUSSION

Several studies using single cell lines (3–6) have led to the hypothesis that oxidative phosphorylation pathway alterations may be important in diabetic nephropathy pathogenesis (2,7). The current study found directional differences in skin fibroblast gene expression levels in oxidative phosphorylation and related pathways. This is consistent with relatively greater oxidative stress in type 1 diabetic patients with diabetic nephropathy. Intracellular ROS are produced through the oxidative phosphorylation pathway by the mitochondrial electron transport chain in response to hyperglycemia (2). High glucose may result in overproduction of electron donors in the citrate cycle, which in turn generates a high mitochondrial membrane potential (2,7). It has been proposed that once a certain threshold is exceeded, high membrane potential inhibits electron transfer at mitochondrial respiratory chain complex III and increases the half-life of superoxide-generating intermediates such as ubisemiquinone (2,3,21). Thus, as a consequence of overall higher gene expression in the oxidative phosphorylation pathway, it is possible that mitochondrial membrane potential may exceed this threshold, resulting in decreased electron transfer at respiratory chain complex III and overproduction of superoxide and ROS.

Four of the five pathways with directionally consistent group differences in gene expression levels were highly clustered among >200 known biological pathways in the genome (Fig. 1). This is, to our knowledge, the first application of this new method of analysis. However, statistical methods are not yet fully developed that would allow calculation of the likelihood that these four related pathways could show directional consistent group differences by chance alone, in part because of the presence of genes that overlap in these related pathways. Nonetheless, it is intuitively very unlikely that these findings are coincidental. The propanoate metabolism pathway is connected to the citrate cycle through acetyl-CoA. Increased citrate cycle activity could further augment the oxidative phosphorylation pathway by increasing production of NADH and $FADH_2$, causing increased production of electron donors and increased mitochondrial membrane potential. These, in turn, could inhibit electron transfer in the oxidative phosphorylation chain, resulting in increased ROS production (3,21). Importantly, the downstream con-

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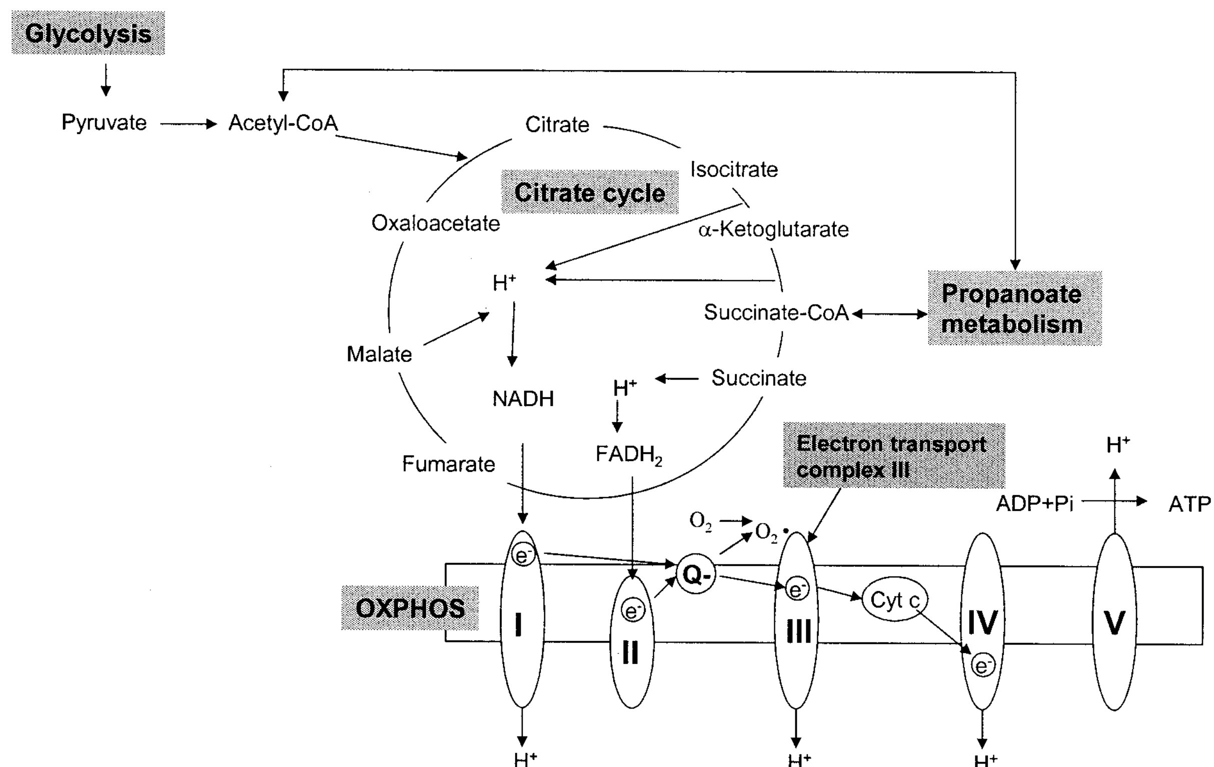

FIG. 1. Pathways that are interrelated and that show directionally consistent group differences in pathway gene expression levels. OXPHOS, oxidative phosphorylation.

sequence of these perturbations on several pathways independently linked to diabetic nephropathy risk was abrogated in experiments that blocked the oxidative stress consequences of increased mitochondrial ROS production (2,3,21). If the functional consequence of the current findings were increased ROS production, this, coupled with the already demonstrated antioxidant enzyme defects in skin fibroblasts from diabetic nephropathy patients (9,10), could result in marked oxidative stress in these cells. Because this and other skin fibroblast behaviors are related to diabetic nephropathy (9–13,15,16), it is tempting to hypothesize that similar processes may be occurring in the kidney.

Skin fibroblasts from the patients in this study were cultured under identical conditions for several passages before gene expression profiling was performed. However, high-glucose–induced overexpression of fibronectin and collagen IV in human cultured endothelial cells persisted after several subsequent passages in the normal glucose condition (22). The fast-track patients had higher A1C than the slow-track patients. Thus, the current study cannot definitely differentiate whether genetic or environmental ("cell memory") effects, or both, may be operative. If the current findings represent cell memory phenomena reflecting in vivo environmental differences, there are several possible candidate influences. Glycemic control, as expected, was worse in the fast-track group. Systemic blood pressure was higher in the fast-track group. Although it is difficult to imagine how higher systemic blood pressure could influence skin fibroblast behavior, this possibility cannot be eliminated. Also, antihypertensive drugs were usually used by the fast-track but not by the slow-track patients. Drugs blocking the renin-angiotensin system have been shown to have effects in rats that persisted (23,24) far beyond any reasonable possibility that these agents had remained in these animals' tissues (25). Although similar studies have not been performed for other antihypertensive agents, drug environmental cell memory phenomena are possible. Other environmental influences are also possible. However, our previously published data showed that there are, at least in part, heritable regulators of in vitro skin fibroblast behaviors that are associated with diabetic nephropathy risk (14). Further studies of cellular behaviors of skin fibroblasts from identical twins discordant for type 1 diabetes could help to dissect the genetic versus cell memory questions raised here. Moreover, the current findings cannot determine whether group differences in gene expression levels represent an increase in the fast-track patients, a decrease in the slow-track patients, or both. Further studies on cells from normal control subjects would be required to answer this question.

Finally, the pathway differences reported here probably do not fully explain the altered cellular phenotype that characterizes diabetic nephropathy risk (26,27). In fact, individual genes in upstream, downstream, or unrelated pathways may be equally or more important. Thus, directional pathway analysis strategies should probably not preclude other approaches that could be applied to the understanding of the cellular basis of diabetic nephropathy risk.

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REFERENCES

1. Betteridge DJ: What is oxidative stress? *Metabolism* 49 (2 Suppl. 1):3–8, 2000
2. Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001
3. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M: Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. *J Clin Invest* 108:1341–1348, 2001
4. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404:787–790, 2000
5. Kiritoshi S, Nishikawa T, Sonoda K, Kukidome D, Senokuchi T, Matsuo T, Matsumura T, Tokunaga H, Brownlee M, Araki E: Reactive oxygen species from mitochondria induce cyclooxygenase-2 gene expression in human mesangial cells: potential role in diabetic nephropathy. *Diabetes* 52:2570–2577, 2003
6. Han HJ, Lee YJ, Park SH, Lee JH, Taub M: High glucose-induced oxidative stress inhibits Na+/glucose cotransporter activity in renal proximal tubule cells. *Am J Physiol Renal Physiol* 288:F988–F996, 2005
7. Brownlee M: The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 54:1615–1625, 2005
8. DeRubertis FR, Craven PA, Melhem MF, Salah EM: Attenuation of renal injury in db/db mice overexpressing superoxide dismutase: evidence for reduced superoxide-nitric oxide interaction. *Diabetes* 53:762–768, 2004
9. Ceriello A, Morocutti A, Mercuri F, Quagliaro L, Moro M, Damante G, Viberti GC: Defective intracellular antioxidant enzyme production in type 1 diabetic patients with nephropathy. *Diabetes* 49:2170–2177, 2000
10. Chiarelli F, Di Marzio D, Santilli F, Mohn A, Blasetti A, Cipollone F, Mezzetti A, Verrotti A: Effects of irbesartan on intracellular antioxidant enzyme expression and activity in adolescents and young adults with early diabetic angiopathy. *Diabetes Care* 28:1690–1697, 2005
11. Huang C, Kim Y, Caramori ML, Fish AJ, Rich SS, Miller ME, Russell GB, Mauer M: Cellular basis of diabetic nephropathy. II. The transforming growth factor-beta system and diabetic nephropathy lesions in type 1 diabetes. *Diabetes* 51:3577–3581, 2002
12. Huang C, Kim Y, Caramori ML, Fish AJ, Rich SS, Miller ME, Russell GB, Mauer M: Cellular basis of diabetic nephropathy. III. In vitro GLUT1 mRNA expression and risk of diabetic nephropathy in type 1 diabetic patients. *Diabetologia* 47:1789–1794, 2004
13. Trevisan R, Li LK, Messent J, Tariq T, Earle K, Walker JD, Viberti G: Na+/H+ antiport activity and cell growth in cultured skin fibroblasts of IDDM patients with nephropathy. *Diabetes* 41:1239–1246, 1992
14. Trevisan R, Fioretto P, Barbosa J, Mauer M: Insulin-dependent diabetic sibling pairs are concordant for sodium-hydrogen antiport activity. *Kidney Int* 55:2383–2389, 1999
15. Iori E, Marescotti MC, Vedovato M, Ceolotto G, Avogaro A, Tiengo A, Del Prato S, Trevisan R: In situ protein kinase C activity is increased in cultured fibroblasts from type 1 diabetic patients with nephropathy. *Diabetologia* 46:524–530, 2003
16. Podesta F, Meregalli G, Ghelardi R, Del Giudice R, Asnaghi V, Maestroni A, Zerbini G: Low Ca(2+) pump activity in diabetic nephropathy. *Am J Kidney Dis* 38:465–472, 2001
17. Caramori ML, Kim Y, Huang C, Fish AJ, Rich SS, Miller ME, Russell G, Mauer M: Cellular basis of diabetic nephropathy. 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. *Diabetes* 51:506–513, 2002
18. Affymetrix Microarray Suite User's Guide. Version 5.0. Santa Clara, CA, Affymetrix, 2001, p. 412–415

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19. Reif DM, Dudek SM, Schaffer CM, Wang J, Moore JH: Exploratory visual analysis of pharmacogenomic results. *Pac Symb Biocomput* 296–307, 2005
20. Reif DM, Moore JH: Visual analysis of statistical results from microarray studies of human breast cancer. *Oncol Rep* 15 (Spec. no.):1043–1047, 2006
21. Korshunov SS, Skulachev VP, Starkov AA: High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Lett* 416:15–18, 1997
22. Roy S, Sala R, Cagliero E, Lorenzi M: Overexpression of fibronectin induced by diabetes or high glucose: phenomenon with a memory. *Proc Natl Acad Sci U S A* 87:404–408, 1990
23. Bergstrom G, Johansson I, Wickman A, Gan L, Thorup C: Brief losartan treatment in young spontaneously hypertensive rats abates long-term blood pressure elevation by effects on renal vascular structure. *J Hypertens* 20:1413–1421, 2002
24. Nagai Y, Yao L, Kobori H, Miyata K, Ozawa Y, Miyatake A, Yukimura T, Shokoji T, Kimura S, Kiyomoto H, Kohno M, Abe Y, Nishiyama A: Temporary angiotensin II blockade at the prediabetic stage attenuates the development of renal injury in type 2 diabetic rats. *J Am Soc Nephrol* 16:703–711, 2005
25. Soldner A, Spahn-Langguth H, Mutschler E: HPLC assays to simultaneously determine the angiotensin-AT1 antagonist losartan as well as its main and active metabolite EXP 3174 in biological material of humans and rats. *J Pharm Biomed Anal* 16:863–873, 1998
26. Lurbe A, Fioretto P, Mauer M, LaPointe MS, Batlle D: Growth phenotype of cultured skin fibroblasts from IDDM patients with and without nephropathy and overactivity of the Na+/H+ antiporter. *Kidney Int* 50:1684–1693, 1996
27. Ng LL, Davies JE, Siczkowski M, Sweeney FP, Quinn PA, Krolewski B, Krolewski AS: Abnormal Na+/H+ antiporter phenotype and turnover of immortalized lymphoblasts from type 1 diabetic patients with nephropathy. *J Clin Invest* 93:2750–2757, 1994

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