Adipose differentiation-related protein and regulators of lipid homeostasis identified by gene expression profiling in the murine db/db diabetic kidney

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Mishra, Rangnath, Steven N. Emancipator, Casey Miller, Timothy Kern, and Michael S. Simonson. Adipose differentiation-related protein and regulators of lipid homeostasis identified by gene expression profiling in the murine db/db diabetic kidney. Am J Physiol Renal Physiol 286: F913–F921, 2004.—We investigated the molecular basis of progressive diabetic renal injury in db/db mice by profiling kidney gene expression. Using high-density microarrays, we identified 482 RNA transcripts differentially expressed in 8-wk db/db vs. nondiabetic db/m kidneys, a time characterized by hyperglycemia but by little renal histopathology. By 16 wk significant mesangial expansion had developed. Sixteen-week db/db kidneys differentially expressed 639 RNA transcripts. Diabetic kidneys specifically expressed several genes normally found in adipocytes, including adipocyte differentiation-regulated protein (ADRP; or adipophilin in humans). ADRP mRNA was specifically upregulated 5.4-fold in 16-wk db/db kidneys. This finding was confirmed at the protein level by Western blotting, and immunohistochremistry localized ADRP diffusely to tubular epithelium throughout the cortex. ADRP is a perilipin family protein that forms lipid storage vesicles and controls triglyceride utilization; we showed that accumulation of lipid storage droplets correlated with the magnitude and localization of ADRP in db/db kidneys. Other genes involved in lipid transport, oxidation, and storage were differentially regulated in db/db kidneys, and peroxisome proliferator-activated receptor-α (PPARα) has been shown to regulate their expression in adipocytes. In our experiments, PPARα mRNA was elevated in db/db diabetic kidneys, and PPARα protein was upregulated in glomeruli, cortical tubules, and renal arterial vessels of db/db mice. In conclusion, these studies furnish new RNA-based data for mechanistic investigation into renal injury in the diabetic kidney and identify a switch of kidney phenotype in favor of lipid accumulation in diabetic kidney.

transcriptome; diabetes; microarray; peroxisome proliferator-activated receptor-α

KIDNEY FAILURE SECONDARY TO diabetes is the most prevalent cause of end-stage renal disease in most developed countries. The etiology of type 2 diabetic nephropathy is complex and involves both environmental and genetic factors (7, 19, 27, 28, 36). A commonly evoked model for the etiology of nephropathy in type 2 diabetes divides the disease into incipient and overt responses to injury (7, 28). In the incipient phase, the kidney is exposed to chronic hyperglycemia, hyperinsulinemia, and hyperlipidemia but has little in the way of significant histopathology. In the overt injury phase, the kidney develops glomerulosclerosis with mesangial expansion, basement membrane thickening, and tubulointerstitial fibrosis. Litte is known about changes in gene expression that underlie the incipient and overt injury phases of diabetic nephropathy.

To better understand the mechanisms underlying diabetic nephropathy, the tools of functional genomics have recently been applied to profile changes in renal gene expression in uninephrectomized streptozotocin-induced mice (47, 48). These studies identified 81 genes that were differentially regulated in an early phase of renal injury, including several known genes related to glucose or lipid metabolism (48). Suppression-subtractive hybridization has been used to identify 200 genes regulated by high glucose in human mesangial cells in vitro (5). These studies were not designed to profile expression of genes in progressive renal injury. Moreover, gene profiles have not been assembled in models of type 2 diabetic nephropathy, which accounts for the majority of diabetic patients with nephropathy compared with type 1 diabetes.

In this study, we used high-density oligonucleotide microarrays to profile changes in kidney gene expression in a mouse model of progressive renal injury in type 2 diabetes, db/db mice (2, 15, 24, 35). The db/db congenic strain develops type 2 diabetes due to a loss-of-function mutation in the leptin receptor gene that causes abnormal splicing (4, 23). These mice develop a progressive form of renal injury that recapitulates aspects of diabetic nephropathy in humans, including albuminuria and glomerular injury with mesangial expansion (2, 6, 18, 51). Changes in gene expression were analyzed in the incipient and overt injury phases to uncover genes potentially involved in this critical transition.

MATERIALS AND METHODS

Assessment of diabetes and renal structure/function. Male db/db (BKS.Cg-m+/+Leprdb) and db/m mice were used for all studies (Jackson Laboratories, Bar Harbor, ME). Mice were housed in a sterile environment with 12:12-h light-dark cycles and had free access to food and water. Manipulation and experiments were done in a barrier facility with gowning. To measure 24-h albumin excretion and creatinine clearance, mice were placed in individual mouse diuresis cages (Nalgene, Rochester, NY) with access to water but not food for 24 h. After 24-h ad libidum access to food and water, the mice were anesthetized and serum was collected and frozen at −40°C. Kidney tissue was rapidly collected and snap-frozen in liquid nitrogen for subsequent extraction of RNA. Blood glucose was monitored using an Accu-Chek meter (Roche Diagnostics, Indianapolis, IN), and the percent glycated hemoglobin was measured after separation on a cation exchange resin (Sigma, St. Louis, MO). Serum and urinary creatinine were measured using a kinetic micro-Jaffe reaction (10-μl sample) with care taken to measure only the initial reaction rate to minimize interference by glucose, although these reactions suffer
from measurement of noncreatinine chromagens in mouse blood (35). Urinary albumin concentration was measured with an ELISA specific for mouse albumin (Exocell, Philadelphia, PA). At the time of death, sagittal sections of kidney were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 5 μm. Morphometric analysis of renal structure was conducted using previously established techniques (6, 8). The mesangial matrix fraction, which is highly correlated with renal function in humans with diabetic nephropathy (8), was analyzed blindly in coded sections stained with periodic acid-Schiff reagent. Forty glomeruli from each of the three sections were imaged.

**Microarray measurement of transcript expression.** Total RNA was extracted from db/db and db/m kidneys (n = 3 from each group), and target RNA was labeled and hybridized to high-density oligodeoxynucleotide microarrays from Affymetrix (Santa Clara, CA) as previously described (29) with modifications. Briefly, biotin-labeled cRNA (15 μg) was hybridized to murine genome U74v2 microarrays for 16 h at 45°C, and after stringent washing in a microfluidics station, bound cRNA was stained with R-phycocerythrin streptavidin (Molecular Probes, Eugene, OR) and scanned before and after antibody amplification. Fluorescence intensities were measured with a laser confocal scanner (Hewlett Packard). Image output files were inspected for hybridization artifacts, and based on fluorescence intensity differences between perfect match and mismatch probes, a hybridization intensity was calculated for each transcript (Microarray Suite 5.0, Affymetrix). Default parameters optimized by the manufacturer for this microarray chip were used for all signal analyses. The U74v2A murine microarray represents gene sequences derived from expressed sequence tag (EST) clusters in build 74 of Unigene. Of these sequences, ~6,000 are annotated genes (either GenBank or TIGR) and ~6,000 are unannotated ESTs.

**Analysis of RNA expression data.** The transcript signal, which represents mRNA transcript abundance, was used for all subsequent analysis of expression data. Scanned images were globally scaled to a target intensity of 1,500 to facilitate comparison of transcript levels from different mice. Gene transcripts with a high probability of target intensity of 1,500 to facilitate comparison of transcript levels from different mice. Gene transcripts with a high probability of being differentially expressed were identified using the permutation-based significance analysis of microarrays (SAM v1.2) algorithm on the log2 transcript signal (40, 41, 45). The q value, a P value-like measure of each of many tests performed simultaneously, was also calculated (40, 41). Transcript data were visualized and grouped by hierarchical clustering using pairwise average-linkage and the Pearson correlation coefficient as the metric of similarity in Cluster v2.2 (10). Annotations and gene ontology were from the National Cancer Institute’s Database for Annotation, Visualization, and Integrated Discovery (9). The list of significantly changed genes with identifiers is available at www.cwru.edu/med/simonson/Link2MiceData.htm, as is a complete file of all transcript levels from each mouse. All microarray data in a MIAME-compliant format have been deposited to the Gene Expression Omnibus at NCBI (accession no. GSE642).

**RT-PCR analysis of adipose differentiation-related protein and peroxisome proliferator-activated receptor-α mRNA.** Isolation of total RNA from mouse kidney and cDNA synthesis were carried out as described above for microarray analysis. cDNA synthesized from 5 μg of RNA was used for quantification of mRNA by real-time PCR using the ABI Prism Sequence Detection System 7000 (Applied Biosystems, Foster City, CA). We designed primers for mouse peroxisome proliferator-activated receptor-α (PPARα) and adipose dif-
differentiation-related protein (ADRP) using Primer Express (Applied Biosystems): PPAR
/H9251-upstream: GCA GTG CCC TGA ACA TCG A; downstream: TCG CCG AAA GAA GCC CTT A; ADRP-upstream: CTG GAC CGT GCC GAC TTG; downstream: GCT CTG TTG GGG ATC CAC TAC; GAPD upstream: TCA ACG ACC CCT TCA TTG AC; downstream: ATG CAG GGA TGA TGT TCT GG. PCR amplification was performed in 25 μl of reaction mixture containing 0.5 μl of cDNA, 1 μl of each up- and downstream primer, and 12.5 μl of SYBR Green PCR master mix (Applied Biosystems) with denaturation at 94°C for 10 min, followed by 45 two-temperature cycles (15 s at 94°C and 1 min at 60°C). A melting curve was recorded at the end of PCR, and 1.5% agarose gel electrophoresis was performed to ensure that the correctly sized amplicon was present. Relative quantification of the mRNA between db/db and db/m mice

Fig. 2. Patterns of gene expression identified in db/db and db/m kidneys. A: transcripts identified by significance analysis of microarrays (SAM) as having a high likelihood of differential expression were clustered to group genes with similar expression profiles. Expression values for each gene in individual mice (n = 3) were calculated as log2 of the fold-change relative to the mean expression value in 8-wk db/m mice (n = 3), which is represented as 0-fold in the black, leftmost column. The intensity of induction or repression is signified by the saturation of green or red, respectively (key beneath D). Genes were functionally annotated, and transcripts involved in cell signaling and growth (B), oxidative stress (C), and lipid homeostasis (D) were clustered as in A.
was computed using the comparative Ct method, and GAPD served as the reference gene (25).

Western blotting in db/db and db/m kidney. Kidney homogenates (15% wt/vol) were prepared in extraction buffer, composed of 50 mM PIPES/HCl, pH 6.5, 2 mM EDTA, 0.1% 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonate, 20 μg/ml aprotinin, 5 mM DTT, 2 mM Na pyrophosphate, 1 mM NaVO₄, and 1 mM NaF, and centrifuged at 2,000 g for 10 min at 4°C. Protein content in the supernatant was assayed with a DC protein assay (Bio-Rad Labs, Hercules, CA). An aliquot of the lysate (25 μg protein) was boiled with SDS sample buffer, resolved on a 4–12% SDS-PAGE gradient gel, and transferred to a 0.2-μm nitrocellulose membrane. After blocking in 5% nonfat dry milk in TBS-T (20 mM Tris·HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20) for 1 h, the membrane was washed three times with TBS-T for 5 min each and incubated overnight at 4°C with guinea pig polyclonal anti-ADRP antibody (1:1,000, Research Diagnostic, Flanders, NJ) or rabbit polyclonal anti-murine PPARγ that does not recognize PPARγ or 6 (1:1,000, Research Diagnostic) in 3% BSA in TBS-T. After incubating with a suitable HRP-labeled secondary antibody (1:2,000) and extensive washing, the membrane was incubated with a chemiluminescence mixture and exposed to film with an average exposure duration ranging from 10 to 30 s. Statistically significant between different groups of mice was calculated by ANOVA with a Bonferroni posttest.

Localization of ADRP and PPARγ by immunohistochemistry and of lipid storage droplets by oil red-O staining. Protocols for immunohistochemistry have been previously published (37, 38). Briefly, small blocks of kidney were immediately fixed in 10% buffered formalin for 24 h before being embedded in paraffin. Five-micrometer-thick sections were deparaffinized, washed with Dulbecco’s phosphate buffered saline (DPBS), and incubated with 1.5% H₂O₂ in methanol to block endogenous peroxidase activity. Nonspecific binding was blocked with 10% normal goat serum in DPBS. Sections were incubated overnight with the anti-ADRP or anti-PPARγ antibody (1:500 in blocking solution) in a humidified chamber at 4°C. The anti-ADRP antiserum has been extensively characterized for immunohistochemistry (17). Nonimmune antibodies at the same concentration of IgG were used in negative controls. For immunohistochemical staining of PPARγ, tissue sections were exposed to antigen unmasking in 10 mM Na citrate, pH 6.0, and 0.05% Tween 20 was added to the blocking buffer. Antibodies were localized with the ABC technique (Vector Labs, Burlingame, CA) and 3,3’-diaminobenzidine substrate solution with nickel chloride enhancement. Sections were then dehydrated in ethanol, cleared in xylene, and mounted in Eukitt without counterstaining. To localize lipid droplets, 5-μm-thick frozen sections were stained with oil red-O. Images were acquired with a SPOT CCD camera (Diagnostic Instruments, Sterling Heights, MI) using the same exposure to facilitate semiquantitative comparisons of staining intensity.

RESULTS

Differential gene expression in the incipient and overt injury phases of db/db kidneys. Our initial goal was to identify genes whose differential regulation correlated with the early response to injury in diabetic kidney. Previous studies (6, 35, 51) showed that 8-wk db/db kidneys demonstrate little histopathology despite the presence of hyperglycemia; in contrast, 16-wk db/db kidneys elaborate significant structural and functional damage. We therefore chose to profile mRNA levels in 8- and 16-wk db/db kidneys compared with age-matched db/m littermates. As shown in Table 1, blood glucose and hemoglobin were significantly elevated at 8 wk in db/db mice, and the hemoglobin was further increased at 16 wk. Albuminuria was also elevated in 8-wk db/db mice, but the mesangial matrix fraction and the tubulointerstitium were unchanged compared with the 8-wk db/m controls (Fig. 1). At 16 wk, the obese, hyperglycemic db/db mice showed evidence of renal injury including increments in albuminuria, kidney weight, glomerular cross section, serum creatinine (Table 1), and mesangial matrix fraction (Fig. 1). These results confirm that 8-wk db/db mice have relatively normal renal histology despite hyperglycemia and albuminuria and that 16-wk db/db mice elaborate significant renal injury.
To identify changes in gene expression that potentially contribute to early kidney injury in diabetes, three mice were chosen at random from the four groups described above (i.e., 8-wk db/db and db/m; 16-wk db/db and db/m). RNA was isolated, labeled, and hybridized to high-density oligonucleotide microarrays. Genes with a high likelihood of differential expression in the 8- and 16-wk mice were identified using SAM. Using this approach, 482 genes were identified as significantly changed in 8-wk db/db vs. 8-wk db/m mice (437 induced and 45 repressed); of these genes, the least significant (thyroid peroxidase) exhibited a 3.1-fold change and a q value of 6.20. The 16-wk db/db vs. db/m kidneys had 639 significantly changed genes (533 induced and 86 repressed) with the least significant gene (an unknown EST), demonstrating a 4.5-fold induction and a q value of 6.85. The combined list of genes (1,016 distinct transcripts) was clustered to detect patterns in the expression profile (Fig. 2A). The first cluster contained genes that were repressed in 8- and 16-wk db/db mice, whereas the second and largest cluster had genes induced at 8 and 16 wk. The third and fourth clusters contained genes induced predominantly at 8 and 16 wk, respectively. The fifth cluster consisted of genes repressed in 16-wk db/m mice with variable patterns of expression in db/db. We also clustered genes by functional classification using GO gene annotations and found that the gene profile was enriched in genes that regulate cell signaling and growth, oxidative stress, and lipid homeostasis (Fig. 2, B–D). Some of these genes, such as those encoding transforming growth factor (TGF)-β and IGF-1, have been previously identified in diabetic kidneys (27); most have not been previously associated with renal injury in diabetes.

A program of genes directing lipid homeostasis in db/db kidneys. One of the most striking changes revealed by gene expression profiling was the induction of a group of genes that control aspects of lipid homeostasis (Fig. 2D). This profile included several genes induced exclusively in 16-wk diabetic kidneys: acyl-CoA synthetase, apolipoprotein CII, PPARα, lipin-1, C/EBPβ, and ADRP. ADRP is transcriptionally upregulated in differentiating adipocytes and is a member of the perilipin family of lipid storage proteins, with the human ortholog being adipophilin (26). Because ADRP is necessary for the formation and function of lipid storage droplets, we asked whether db/db kidneys contained these cytoplasmic storage vesicles for neutral lipids. Lipid storage droplets, analyzed by oil red-O staining, were undetectable in 8-wk db/m kidneys and only rarely observed in 16-wk db/m kidneys (Fig. 3, A and bottom right). In contrast, 8-wk db/db kidneys had abundant lipid storage droplets in the cortical tubules, and the number of droplets increased 2.3-fold at 16 wk (Fig. 3, B and bottom right). The lipid droplets were diffusely associated with cortical tubules (Fig. 3C) and were less often identified in the glomeruli and medulla (data not shown). The abundance of lipid storage droplets represented a major difference in the renal phenotype of diabetic and nondiabetic kidneys.

Further investigation of ADRP expression in db/db kidney showed specific upregulation of ADRP protein in the diabetic kidneys. ADRP mRNA was elevated 5.4-fold in 16-wk db/db vs. db/m kidneys (Fig. 4A), and the increase in ADRP mRNA was confirmed by real-time RT-PCR (Fig. 4A, inset). ADRP mRNA was not elevated in 8-wk db/db kidneys compared with the db/m controls. In contrast to ADRP mRNA levels, ADRP protein detected by Western blotting was elevated in both 8- and 16-wk db/db kidneys but was undetectable in db/m controls (Fig. 4, B and C), suggesting a complex mechanism of ADRP expression in the diabetic kidney. Localization of ADRP by immunohistochemistry showed that ADRP was undetectable even in 16-wk nondiabetic kidneys (Fig. 5A). In 16-wk diabetic kidneys, ADRP immunoreactivity was localized to the cytoplasm of cortical tubules in a pattern consistent with the lipid storage vesicles (Fig. 5B). Nonspecific staining...
was minimal (Fig. 5C), and specific ADRP staining was occasionally observed in glomeruli (data not shown). Higher-magnification staining reveals the essentially dropletlike pattern of ADRP protein localization in db/db kidneys (Fig. 5D).

Taken together, these results demonstrate that ADRP mRNA and protein are specifically upregulated in db/db diabetic kidneys in a manner consistent with the presence of lipid storage droplets.

Because PPARα has previously been shown to be important for the gene regulatory network in response to lipid accumulation, we next sought to determine whether PPARα expression in diabetic kidney was accurately predicted by the gene expression profile. Transcript levels for PPARα were elevated in 16-wk db/db kidneys compared with 16-wk db/m and with 8-wk db/m and db/db kidneys (Fig. 6A). RT-PCR confirmed the induction of PPARα mRNA in db/db kidney (Fig. 6A, inset). Western blotting of kidney extracts revealed a trend toward increased PPARα protein in 8-wk db/db kidneys and a marked increase in 16-wk db/db kidneys (Fig. 6, B and C). By immunohistochemistry, sparse staining for PPARα was observed in cortical tubules of 16-wk db/m kidneys (Fig. 7A). No significant staining for PPARα was observed in the glomerulus and the renal vasculature in 16-wk db/m kidneys. In 16-wk db/db kidneys, nuclear staining for PPARα was elevated in cortical tubules and in the vasculature (Fig. 7, B and C). Occasional sparse staining was observed in the glomerulus (not shown). Sparse cytoplasmic staining in the tubules was also observed in 16-wk db/db kidneys (Fig. 7B). Vascular staining was greatly elevated in the endothelium and less so in the medial layer (Fig. 7C). These results demonstrate that upregulation of PPARα mRNA and protein is associated with progressive renal injury in db/db mice.

**DISCUSSION**

Whereas sustained hyperglycemia drives renal injury in diabetes, the genes that mediate the renal response are incompletely understood. The data presented here represent a large-scale gene expression profile in db/db kidneys as they progress from an early stage of diabetes to a stage characterized by mesangial expansion and tubulointerstitial fibrosis. Analysis of this expression profile, particularly in the context of previously published results using db/db mice (35), should provide a foundation on which to build further mechanistic investigations regarding the function of specific genes or groups of genes that mediate renal damage in diabetes.

Perhaps the most striking change in diabetic kidneys was the induction of genes normally expressed in adipocytes and related to lipid homeostasis. This adipose-selective group included genes that regulate lipid transport, β- or α-oxidation, and lipid accumulation in cortical tubules. Lipid storage droplets, which consist of triglycerides and esterified cholesterol, were undetectable in normal db/m kidneys at 8 wk and were only rarely detected at 16 wk. In marked contrast, small lipid storage droplets were present in cortical tubules of 8-wk db/db mice, and the droplets increased in size and number at 16 wk. Abnormal lipid deposits have been observed in tubules of human diabetic kidney (30, 32, 44) and have been proposed to participate in the pathogenesis of diabetic nephropathy and other proteinuric renal diseases (21, 33, 34). Lipid droplets were also detected in the cortical tubules of the streptozotocin-induced diabetic rat (42). A direct link between lipid accumulation and kidney injury has not been established directly. However, excess intracellular lipid in other nonadipose tissues has convincingly been linked to insulin resistance and cellular damage (i.e., lipotoxicity). Several mechanisms have been proposed to explain lipotoxicity in nonrenal tissues, including increased oxygen consumption costs, elevated reactive oxygen species due to fatty acid oxidation, dysregulation of lipid signaling, and apoptosis (11, 46, 49, 50). Collectively, our results suggest that diabetes induces a fundamental change in the phenotype of db/db kidneys in favor of utilizing and storing lipids. From our experiments, we cannot determine whether hyperglycemia, hyperlipidemia, or both mediate the differential expression of these genes. However, we speculate that
increased deposition of lipid in the kidney contributes to cellular damage and to the progression of diabetic kidney disease.

Induction of the ADRP gene is apparently an important aspect of the response to diabetes and formation of lipid storage droplets in the \textit{db/db} kidney. ADRP, also called adipophilin in humans, is a \textasciitilde{50-kDa fatty acid binding protein that is transcriptionally activated when preadipocytes differentiate into mature adipocytes (20). ADRP belongs to the perilipin family of lipid storage proteins (26), and similar to other perilipin members, localizes to the cytoplasmic surface of lipid storage droplets in distinct cell types (3, 17). We found that the ADRP gene and protein were robustly induced in \textit{db/db} vs. \textit{db/m} kidneys. The discordance between the kinetics of ADRP mRNA and protein induction reflects a complex mode of regulation that might involve posttranscriptional control. Indeed, posttranscriptional control has been observed for perilipin A, presumably involving protein stabilization secondary to lipid binding (26), and a similar mechanism might be operative in \textit{db/db} diabetic kidney for ADRP. In \textit{db/db} kidneys, ADRP protein was present in a spherical pattern in cortical tubules and corresponded with the presence of oil red-O-positive lipid droplets. Induction of kidney ADRP by diabetes might simply be a marker of diabetic injury, but recent evidence from other sources suggests that ADRP could play a regulatory role in lipid accumulation in nonadipose tissues. For example, ADRP facilitates uptake and transport of long-chain fatty acids in a dose-dependent manner (13), and fatty acids are a robust stimulus of ADRP gene transcription (14). In addition, deletion of the gene encoding perilipin in mice blocks diet-induced lipid uptake and obesity (43), which suggests that other perilipin family proteins might help regulate lipid uptake in addition to

![Fig. 6](http://ajprenal.physiology.org/)

**Fig. 6. Elevated expression of peroxisome proliferator-activated receptor-\(\alpha\) (PPAR\(\alpha\)) protein in \textit{db/db} diabetic kidney.** A: PPAR\(\alpha\) transcript microarray signal from \(n = 3\) independent mouse kidney RNA preparations. Inset: data from RT-PCR. **\(P < 0.01\) vs. corresponding \textit{db/m} by unpaired \(t\)-test. B: Western blots for PPAR\(\alpha\) protein in 2 independent mouse kidney preparations (lanes 1 and 2) from \textit{db/db} and \textit{db/m} mice. C: densitometric analysis of PPAR\(\alpha\) expression in Western blots from 4 different mice/condition presented as in Fig 4C. **\(P < 0.01\) vs. corresponding \textit{db/m} mice. * \(P < 0.05\) by ANOVA.

![Fig. 7](http://ajprenal.physiology.org/)

**Fig. 7. Localization of PPAR\(\alpha\) to multiple nephron segments and to the vasculature.** A: PPAR\(\alpha\) immunoreactivity was sparse in 16-wk \textit{db/m} kidneys. B: intense nuclear and less cytoplasmic staining for PPAR\(\alpha\) in cortical tubules of 16-wk \textit{db/db} mice. C: vascular reactivity for PPAR\(\alpha\) in the endothelium of 16-wk \textit{db/db} mice.
forming lipid storage droplets. Additional experiments are required to test the functional role of ADRP in the kidney’s response to diabetic injury.

Our experiments with db/db kidneys demonstrated marked induction by diabetes of the gene encoding PPARα, which we propose might help direct the transcriptional response of db/db kidneys in diabetes. PPARα belongs to a family of nuclear hormone receptors that bind fatty acid ligands (16). In concert with specific coactivators, the liganded PPARα binds to DNA as a heterodimer with the 9-cis retinoic acid receptor and regulates transcription of genes containing a cognate PPAR response element. PPARα is expressed in muscle, liver, heart, and kidney cortex, where it regulates fatty acid catabolism (1, 22). PPARα activates transcription of numerous genes involved in fatty acid uptake (fatty acid binding proteins), β-oxidation (acyl-CoA oxidase), ω-oxidation (cytochrome P-450 4-family), and lipoprotein assembly (apolipoprotein C-II), many examples of which reside in the list of genes differentially regulated by diabetes in our experiments with db/db kidneys. Thus we speculate that PPARα is a diabetes-induced transcription factor that helps control the kidney’s response to lipids. In the normal kidney, PPARα expression is highest in the proximal tubule and thick limb where it contributes to lipid-induced gene expression (31). Although PPARα induction in response to fasting and the initial response to hyperlipidemia appears beneficial, the longer-term effect of PPARα in the kidney is difficult to predict. Indeed, cardiac-specific PPARα overexpression increases fatty acid oxidation, elevates lipid storage droplets, and augments cardiomyopathy in diabetes compared with wild-type controls (11). PPARα null mice do not develop this phenotype. These results suggest that by sensing fatty acids and stimulating their uptake, PPARα can contribute to tissue damage in diabetic target organs like the heart. On the other hand, oral fibrates, PPARα agonists that lower serum triglycerides, reduce proteinuria in a subset of diabetic patients (12, 39). Thus perhaps the net effect of PPARα induction is beneficial because a systemic reduction in triglycerides results in less renal lipid accumulation. Therefore, additional studies in db/db mice with PPARα activators will be needed to clarify the role of this transcription factor in diabetic renal injury.

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