Altered renal lipid metabolism and renal lipid accumulation in human diabetic nephropathy

M. Herman-Edelstein, P. Scherzer, A. Tobar, M. Levi, and U. Gafter

Abstract: Animal models link ectopic lipid accumulation to renal dysfunction, but whether this process occurs in the human kidney is uncertain. To this end, we investigated whether altered renal TG and cholesterol metabolism results in lipid accumulation in human diabetic nephropathy (DN). Lipid staining and the expression of lipid metabolism genes were studied in kidney biopsies of patients with diagnosed DN (n = 34), and compared with normal kidneys (n = 12). We observed heavy lipid deposition and increased intracellular lipid droplets. Lipid deposition was associated with dysregulation of lipid metabolism genes. Fatty acid β-oxidation pathways including PPAR-α, carnitine palmitoyltransferase 1, acyl-CoA oxidase, and L-FABP were downregulated. Downregulation of renal lipoprotein lipase, which hydrolyzes circulating TGs, was associated with increased expression of angiopoietin-like protein 4. Cholesterol uptake receptor expression, including LDL receptors, oxidized LDL receptors, and acetylated LDL receptors, was significantly increased, while there was downregulation of genes effecting cholesterol efflux, including ABCA1, ABCG1, and apoE. There was a highly significant correlation between glomerular filtration rate, inflammation, and lipid metabolism genes, supporting a possible role of abnormal lipid metabolism in the pathogenesis of DN. These data suggest that renal lipid metabolism may serve as a target for specific therapies aimed at slowing the progression of chronic kidney disease. NA

Supplementary key words: lipotoxicity • lipid droplets • cholesterol metabolism

Diabetic nephropathy (DN) is an increasing cause of morbidity and mortality worldwide and the leading cause of chronic kidney disease (CKD). Dyslipidemia in patients with type 2 diabetes is a reversible risk factor for the progression of kidney disease and cardiovascular mortality (1, 2). Sustained hyperglycemia in diabetes promotes FA synthesis and TG accumulation. Elevated serum TGs, FFA s, and modified cholesterol cause ectopic lipid accumulation in nonadipose tissues, including the pancreas, heart, liver, and blood vessel walls (3–6); this process, termed lipotoxicity, seems to play a role in the pathogenesis of DN (7–11) and other diabetic complications.

The effect of lipid accumulation in the kidney is well described in animal models of DN and obesity, where lipids accumulate in the kidney and are associated with glomerulosclerosis and tubulointerstitial injury (9, 10). Although lipid deposits in glomeruli were part of Kimmelstiel and Wilson’s original pathological description of nodular sclerosis (12, 13), renal lipotoxicity or “fatty kidney” is an unappreciated and not well documented clinical entity, partly due to the confounding multiple coexisting risk factors in DN (14).

Lipotoxicity and lipid accumulation cause podocyte dysfunction and apoptosis (7–11, 15–17). The podocyte uptake of oxidized LDL (oxLDL) is mediated mainly by the scavenger chemokine receptor CXCL16 (18).

FA synthesis is catalyzed through fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), while stearoyl-CoA desaturase 1 (SCD1) is a rate-limiting enzyme that converts saturated FAs to monounsaturated FAs. FAs undergo trans-saturase 1 (SCD1) is a rate-limiting enzyme that converts saturated FAs to monounsaturated FAs. FAs undergo trans-saturase 1 (SCD1) is a rate-limiting enzyme that converts saturated FAs to monounsaturated FAs. FAs undergo trans-saturase 1 (SCD1) is a rate-limiting enzyme that converts saturated FAs to monounsaturated FAs, modulated by carnitine palmitoyltransferase 1 (CPT1) and acyl-CoA oxidase (ACO) (19–21).
Dysregulation of PPAR activity is a potential cause of metabolic syndrome-related disorders, such as insulin resistance and hyperlipidemia. PPARα and PPARδ regulate expression of genes involved in β-oxidation of FAs. PPARα deficiency has been shown to accelerate dyslipidemia, proteinuria, and renal failure in animal models with DN (16, 22).

There is growing evidence that dysregulation of sterol regulatory element binding proteins (SREBPs) contributes to the pathogenesis of DN (23). SREBPs serve as the master regulators of cellular FA and cholesterol synthesis; SREBP-1 regulates FA synthesis, whereas SREBP-2 regulates cholesterol synthesis (14). In addition, the carbohydrate-responsive element binding protein (ChREBP) regulates the expression of ACC and FAS, and also regulates lipogenesis by induction of the glycolytic enzyme L-pyruvate kinase (L-PK) (24).

Dysregulation of cholesterol metabolism has also been linked to lipotoxicity and lipid accumulation in diabetes. Cholesterol influx into cells is mediated by several independent receptors, including scavenger receptor class A (SR-A1), class B (CD36), lectin-like oxLDL receptor-1 (LOX-1 or OLR-1) (25), and LDL receptor (LDLR) (15). Cholesterol efflux that prevents cholesterol accumulation is primarily mediated by ATP-binding cassette transporters (ABCA1 and ABCG1). Renal lipoprotein receptor expression correlates with kidney damage (26, 27).

The aim of this study was to test whether renal dysfunction in human DN is associated with renal lipid accumulation, lipotoxicity, and dysregulation of renal FA and cholesterol metabolism.

**MATERIALS AND METHODS**

**Kidney biopsy case selection and histological assessment**

The study was approved by the Institutional Ethics Committee.

Kidney samples were obtained from leftover portions of diagnostic kidney biopsies of patients with DN (n = 34) and normal kidneys (n = 12) from the pathological archives of the Department of Pathology at Rabin Medical Center.

All patients had T2D. DN was histologically confirmed by hematoxylin and eosin, periodic acid-schiff, Masson’s trichrome staining, immunofluorescence microscopy for immunoglobulin, and complement and electron microscopy (EM) analysis.

Criteria for DN included glomerular hypertrophy, diffuse mesangial and focal nodular glomerulosclerosis, arteriolar hyalinosis, focal and segmental glomerulosclerosis, the presence of hyaline drops between Bowman’s capsule and epithelial cells, and interstitial fibrosis (28).

In the DN group, renal biopsy was performed according to clinical indications and in order to exclude the presence of other types of kidney disease due to the presence of atypical features, including short duration between the diagnosis of diabetes and the onset of nephropathy and/or the absence of concomitant diabetic retinopathy. Cases were defined by the presence of diabetic histological changes consistent with DN and the absence of other potential causes of glomerulonephritis in the pathology evaluation.

The degrees of sclerosis, thickening, and fibrosis were evaluated on the basis of an arbitrary scale (0 = normal = 0%, 1 = mild = 20%, 2 = moderate = 20–40%, and 3 = severe = >40%) and were taken from the original pathological evaluations following biopsies. Vascular sclerosis: 0 = normal; 1 = mild; 2 = moderate; 3 = severe. Lumen cells and lumen thrombi: 0 = normal; 1 = increased. Mesangial matrix: 0 = normal; 1 = focal; 2 = increased, mild; 3 = increased, nodular. Bowman’s capsule thickening: 0 = normal; 1 = focal; 2 = circumferential. Interstitial fibrosis: 0 = no inflammation; 1 = mild; 2 = severe.

Clinical information about the diabetic patients and controls was collected from the patients’ files. The glomerular filtration rate (eGFR) was estimated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula (29, 30). Twenty-four hour urine albumin, protein, and creatinine were available to all patients in the last month prior to biopsy. Data on blood pressure were collected from the patients’ files on the day of hospitalization for kidney biopsy.

As a control group (n = 12), we used diagnostic kidney biopsies from living kidney donors (n = 6) and nonaffected parts of tumor nephrectomy samples (n = 6). Control subjects were defined as having an eGFR >60 (ml/min) and <10% glomerulosclerosis and tubulointerstitial fibrosis.

**Electron microscopy**

Kidney biopsy tissue was immediately fixed in Karnovsky’s fixative and then 1% buffered osmium tetroxide (method that preserved lipids). The sample was dehydrated in a graded series of alcohols and propylene oxide, then embedded in an epoxy resin (Technovit 7100, Heraeus, Germany). Five-micrometer sections were cut with glass knives and stained with 2% toluidine blue in 1% sodium borate. Thin sections were cut with a diamond knife, mounted on 200-mesh copper grids, and double-stained with uranyl acetate and lead citrate. They were examined with an electron microscope (Jeol 2100F, Japan).

**Table 1. Clinical and biochemical characteristics of patients with DN and normal controls evaluated in this study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Kidney (n = 12)</th>
<th>DN (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex [n (%)]</td>
<td>7 (58)</td>
<td>14 (41)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.3 ± 21.0</td>
<td>58.6 ± 18.3</td>
</tr>
<tr>
<td>HTN [n (%)]</td>
<td>5 (42)</td>
<td>35 (97)*</td>
</tr>
<tr>
<td>DM [n (%)]</td>
<td>6 (50)</td>
<td>18 (100)*</td>
</tr>
<tr>
<td>ACE inhibitor or ARB [n (%)]</td>
<td>4 (33)</td>
<td>14 (41)*</td>
</tr>
<tr>
<td>Statin [n (%)]</td>
<td>7 (58)</td>
<td>19 (56)*</td>
</tr>
<tr>
<td>Fibrinogen [mg/dl]</td>
<td>3.2 ± 1.1</td>
<td>4.1 ± 1.8</td>
</tr>
<tr>
<td>BP (mmHg) on admission</td>
<td>126/75 ± 26/12</td>
<td>154/83 ± 18/11</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.3</td>
<td>2.7 ± 1.8</td>
</tr>
<tr>
<td>Serum urea (mg/dl)</td>
<td>39.2 ± 14.7</td>
<td>101.5 ± 56.6*</td>
</tr>
<tr>
<td>Estimated GFR (ml/min/1.73 m²)</td>
<td>97.4 ± 29.5</td>
<td>40.6 ± 30.5*</td>
</tr>
<tr>
<td>Glycated hemoglobin (%)</td>
<td>8.7 ± 2.6</td>
<td>8.7 ± 2.6</td>
</tr>
<tr>
<td>Plasma LDL-c (mg/dl)</td>
<td>115.2 ± 27.2</td>
<td>115.8 ± 66.4</td>
</tr>
<tr>
<td>Plasma HDL-c (mg/dl)</td>
<td>51.7 ± 17.1</td>
<td>49.4 ± 15.9</td>
</tr>
<tr>
<td>Plasma TGs (mg/dl)</td>
<td>154.0 ± 66.4</td>
<td>267.6 ± 309.3</td>
</tr>
<tr>
<td>Serum albumin (mg/dl)</td>
<td>4.1 ± 0.5</td>
<td>3.3 ± 0.6*</td>
</tr>
<tr>
<td>Proteinuria (mg/day) (median)</td>
<td>0</td>
<td>424.5*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>62.2 ± 17.5</td>
<td>85.2 ± 22.1</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomeruli number</td>
<td>29.4 ± 8.9</td>
<td>24.1 ± 17.3</td>
</tr>
<tr>
<td>Globally sclerotic glomeruli (%)</td>
<td>1.9 ± 3.0</td>
<td>34.3 ± 27.3*</td>
</tr>
<tr>
<td>Segmentally sclerotic</td>
<td>0.0</td>
<td>20.7 ± 23.1*</td>
</tr>
<tr>
<td>glomeruli (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increased mesangial matrix</td>
<td>0.3 ± 0.4</td>
<td>1.9 ± 0.3*</td>
</tr>
<tr>
<td>Bowman’s capsule thickening</td>
<td>0</td>
<td>1.9 ± 0.4*</td>
</tr>
<tr>
<td>Tubular atrophy (%)</td>
<td>0</td>
<td>1.7 ± 0.6*</td>
</tr>
<tr>
<td>Interstitial fibrosis (%)</td>
<td>0</td>
<td>1.5 ± 0.6*</td>
</tr>
<tr>
<td>Vascular sclerosis (%)</td>
<td>0.1 ± 0.35</td>
<td>2.2 ± 0.8*</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>0</td>
<td>1.5 ± 0.9*</td>
</tr>
</tbody>
</table>

*P < 0.05 normal versus DN.

†P < 0.001 normal versus DN.
Lipid staining

4,4-Difluoro-1,3,5,7-tetramethyl-4-Bora-3a,4a-diaza-s-indacene (BODIPY) staining (which stains neutral lipids) was performed on 4 μm-thick sections of fixed frozen kidney DN biopsies and on leftover frozen tissue designated for immunofluorescent microscopy. BODIPY (Molecular Probes, Invitrogen) was diluted in DMSO at a concentration of 1 mg/ml, applied to the kidney section in OCT for 30 min and costained with membranes using a series of ethanol and embedded in an epoxy resin. Tissue was surveyed with a series of 1 μm sections for a representative sample. The selected specimens were thin sectioned, viewed, and photographed with an electron microscope (transmission electron microscope, JEOL 1010) from the Rabin Medical Center pathology department. The sections were read by a nephropathologist to determine kidney morphology and lipid droplets (LDs).
normal kidneys. Total RNA was isolated using RNAeasy mini columns (Qiagen, Valencia, CA). The manufacturer’s protocol was followed with the exception of increased proteinase K digestion time. RNA quantity and quality were determined by optical density 260:280 nm ratio on a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). RNA was converted to cDNA using RevertAid First Strand cDNA synthesis kit (Fermentas); cDNA was then amplified using TaqMan PreAmp Master Mix (Applied Biosystems) for 14 cycles of preamplification according to the manufacturer’s protocol using target gene assays. Candidate lipid and glucose metabolic gene expression were analyzed by real-time RT-PCR, performed as described previously (14), using the TaqMan and Syber system based on real-time detection of accumulated fluorescence (ABI Prism Step One; Perkin-Elmer, Foster City, CA). Fluorescence for each cycle was quantitatively analyzed by an ABI Step One sequence detection system (Perkin-Elmer). In order to control for variation in the amount of DNA that was available for PCR in the different samples, gene expression of the target sequence was normalized in relation to the expression of an endogenous control, 18S rRNA or RPLPO (large ribosomal protein). Primer sequences and assay details are available upon request.

Statistical analysis

Values are shown as mean ± SEM unless otherwise specified. Statistical analysis was performed using two-tailed Student’s t-test for independent data. Pearson’s correlations were calculated using GraphPad software. $P < 0.05$ was considered significant.
Renal lipid accumulation and lipotoxicity in human DN

The diabetic group were older and consisted of a higher percentage of women, but these differences did not reach statistical significance. Three control patients were diagnosed with T2D without renal involvement. Histological evaluation showed severe glomerulosclerosis: global sclerosis of 34 ± 17% and segmental sclerosis of 20 ± 23%, tubular atrophy, interstitial fibrosis, vascular sclerosis, mesangial matrix expansion, and marked interstitial inflammation.

The phenotype analysis represents all clinical and pathological diversity of DN from early CKD (CKD 1) to advanced CKD (CKD 4–5).

Patients with DN showed a progressive decline in kidney function with eGFR deterioration at a rate of 1.0 ± 0.9 ml/min/1.73 m²/month (excluding patients who started dialysis in the 2 months after biopsy). Patients reached dialysis within 29.7 ± 23.6 months after kidney biopsy.

RESULTS

Clinical characteristics of the research participants

RNA samples were prepared from kidney biopsy samples from 34 DN patients and 12 normal controls. The clinical characteristics of the study participants are summarized in Table 1. The DN patients had a higher prevalence of hypertension and obesity. DN patients were more likely to have dyslipidemia and to be on active statin (HMG-CoA reductase inhibitor) therapy. LDL levels were the same in both groups, but diabetics had higher levels of serum TGs. DN patients had decreased eGFR, increased proteinuria, hypoalbuminemia, and increased serum creatinine and urea. Patients in the diabetic group were older and consisted of a higher percentage of women, but these differences did not reach statistical significance. Three control patients were diagnosed with T2D without renal involvement.

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The phenotype analysis represents all clinical and pathological diversity of DN from early CKD (CKD 1) to advanced CKD (CKD 4–5).

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We further found extensive accumulation of intracellular LDs in podocyte cells, tubular epithelial cells, and mesangial cells, and also in fenestrated endothelial cells (Fig. 2). LDs are round membrane-coated organelles filled with inert lipids that can be well observed by EM in adipocytes and fatty liver. LDs in DN showed different electron densities depending on the type of lipid and were in different sizes. LDs appeared in clusters mostly seen in podocytes, both in the podocyte cell body and in the major foot processes. Some podocytes were loaded with LDs, while others had no lipid deposits.

Increased lipid staining in human kidney

We studied renal lipid accumulation by using different staining methods on six frozen DN kidney tissue biopsies: Oil Red O, BODIPY staining, and filipin staining (Fig. 3A). We found marked neutral lipid accumulation in both glomeruli and tubulointerstitium, as determined by Oil Red O staining and by BODIPY staining in diabetic kidneys. We also found evidence for cholesterol accumulation by filipin staining (Fig. 3). In the absence of unfixed normal frozen kidney samples and due to the fact that lipids dissolve during routine fixation methods, we used immunostaining of adipophilin (ADRP), a LD-associated protein, to quantify LDs in fixed processed tissue (16, 17). ADRP staining (Fig. 3) showed increased LD protein expression in DN compared with normal kidneys. Furthermore, ADRP (PLIN2) mRNA expression was increased in DN kidneys in the early stages of disease progression (Fig. 3).

Regulation of FA metabolism enzymes in the diabetic kidney

To determine the possible mechanism of the lipid accumulation in the kidney of the DN group, we measured the expression of several key genes involved in FA synthesis, catabolism, uptake, and oxidation in DN patients and compared them to normal controls (Fig. 4). The DN group had increased SCD1 mRNA abundance, which was associated with increased TG content. In addition, there was increased FA transporter CD36 mRNA, indicative of increased FA uptake (Fig. 4).

Most of the lipogenic genes that we studied, including SREBP-1c, FAS, ACC, ChREBP, and L-PK were all decreased. In addition, expression of diacylglycerol O-acyltransferase 1 did not change. These results suggest that accumulation of TGs is not due to increased active synthesis in the stage of long-standing fibrotic diabetic kidney injury.

The most significant changes were found in the expression of genes and enzymes that control FA oxidation. ACO1 and CPT1 were significantly lower in the DN kidneys (Fig. 4). This was also associated with a significant downregulation of PPARα and PPARδ, the key transcriptional regulators of FA oxidation (Fig. 4). L-FABP (FA binding protein), which can be used as a biomarker in the urine of DN patients (33) and is a lipid-binding protein responsible for transporting FAs to intracellular sites of oxidation, was also downregulated. The expression of renal LPL mRNA, which hydrolyzes circulating TGs, was significantly decreased in DN. Downregulation of renal LPL.
may increase TG levels in kidney tissue (34). Angiopoietin-like protein 4 (Angptl4), which has recently been shown to play a major role in mediating hypertriglyceridemia in nephrotic syndrome (35) and also to inhibit LPL (36), was found to play a major role in mediating hypertriglyceridemia in the diabetic kidney. Furthermore, upregulation of lipoprotein receptors can also result in the increased delivery of TGs to the cell by uptake of TGs in VLDLs and chylomicron remnants.

**Regulation of cholesterol metabolism enzymes in the diabetic kidney**

To gain further insight into the mechanisms responsible for accumulation of cholesterol in the kidney, we analyzed proteins and enzymes involved in cholesterol synthesis, uptake, and efflux.

The expression of LOX-1 (OLR-1) was very low in normal kidneys and significantly upregulated in the diabetic kidneys. We also found upregulation of LDLR, acetylated LDLR-NR-A1, and CD36 (SR-BI), which is involved in the uptake of oxLDL, as well as TG uptake (Fig. 5). The expression of CXCL16, the main chemokine receptor in podocytes mediating uptake of oxLDL (37), was decreased, possibly as part of decreases in the mRNA of all other podocyte markers (Fig. 1).

In DN, we also found decreased expression of the cholesterol efflux genes ATP-binding cassette transporters (ABCA1, ABCG1) and apoE (Fig. 5). The expression of liver X receptor α (30, 38), the nuclear receptor which regulates these cholesterol efflux genes, was also downregulated.

No significant differences in HMG-CoA reductase and synthase or SREBP-2 mRNA levels were seen between the groups, suggesting no change in cholesterol synthesis (Fig. 5).

Taken together, these data suggest that increased cholesterol uptake and decreased FA oxidation may lead to increased FA oxidation, but decreased FA oxidation may lead to TG and neutral lipid accumulation in the diabetic kidney. Furthermore, upregulation of lipoprotein receptors can also result in the increased delivery of TGs to the cell by uptake of TGs in VLDLs and chylomicron remnants.

**Correlation between lipid metabolic genes and renal dysfunction**

To determine whether the accumulation of lipids within the kidney is associated with alterations in kidney function, we studied the correlation between eGFR, proteinuria, and genes of lipid metabolism. We found highly significant correlations between increased expression of LDLR, oxLDL, acetylated LDL (acLDL), and CD-36 versus the progression of DN and deterioration of eGFR. On the other hand, there was a highly significant negative correlation between genes that regulate FA oxidation, including PPARα, ACO, CPT1, and eGFR. There was also a highly significant correlation between decreased mRNA expression of genes that regulate cholesterol efflux, ABCA1 and ABCG1, and eGFR and proteinuria (Fig. 6; Tables 2, 3).

**DISCUSSION**

Ectopic lipid accumulation in the liver, heart, and pancreas is associated with lipotoxicity, inflammation, and fibrosis. Although animal studies suggest that lipotoxicity also contributes to renal dysfunction, its importance for human DN has not been established. The aim of this work was to determine whether lipids accumulate in the human diabetic kidney and to elucidate the alterations in lipid metabolism that may be associated with lipotoxicity and deterioration of renal function (eGFR).

In this study, we showed that heavy lipid deposition is a common feature of human DN. These results are mostly consistent with the findings in animal models of diabetic kidney in which accumulation of lipid, lipotoxicity, and lipid metabolism dysregulation are associated with renal damage (39).

This phenomenon most likely has been previously underestimated, probably because diabetic patients rarely undergo renal biopsy once proteinuria and renal failure develop, and furthermore, lipids and cholesterol are not preserved in routine pathology tissue processing and in EM processing.

TABLE 2. Pearson’s correlation between TNFα and genes of lipid metabolism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PPARα</th>
<th>ACO</th>
<th>CPT1</th>
<th>SCD</th>
<th>LOX-1</th>
<th>SR-A</th>
<th>LDLR</th>
<th>CD36</th>
<th>ABCA1</th>
<th>ABCG1</th>
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<tbody>
<tr>
<td>Number of XV pairs</td>
<td>41</td>
<td>36</td>
<td>33</td>
<td>35</td>
<td>33</td>
<td>45</td>
<td>35</td>
<td>39</td>
<td>37</td>
<td>38</td>
</tr>
<tr>
<td>Pearson r</td>
<td>-0.3254</td>
<td>-0.4958</td>
<td>-0.4437</td>
<td>0.4602</td>
<td>0.6261</td>
<td>0.3160</td>
<td>0.3416</td>
<td>0.2082</td>
<td>-0.4499</td>
<td>-0.3534</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.0196 to 0.1184</td>
<td>0.6880</td>
<td>0.7979</td>
<td>0.5886</td>
<td>0.0901</td>
<td>0.0001</td>
<td>0.0339</td>
<td>0.1106</td>
<td>0.2035</td>
<td>0.0052</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>0.0379</td>
<td>0.0021</td>
<td>0.0097</td>
<td>0.0054</td>
<td>&lt;0.0001</td>
<td>0.0339</td>
<td>0.1106</td>
<td>0.2035</td>
<td>0.0052</td>
<td>0.0295</td>
</tr>
<tr>
<td>P value summary</td>
<td>*</td>
<td>**</td>
<td>**</td>
<td>**</td>
<td>***</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Is the correlation significant? (α = 0.05)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<td>Yes</td>
</tr>
<tr>
<td>R square</td>
<td>0.1059</td>
<td>0.2458</td>
<td>0.1969</td>
<td>0.2118</td>
<td>0.392</td>
<td>0.1004</td>
<td>0.1167</td>
<td>0.0433</td>
<td>0.2024</td>
<td>0.1249</td>
</tr>
</tbody>
</table>

*The r is Pearson’s correlation coefficient.
**Correlation is significant at the 0.05 level (2-tailed).
***Correlation is significant at the 0.01 level (2-tailed).
****Correlation is significant at the 0.0001 level (2-tailed).

Fig. 6. Correlations between the eGFR and expression of different genes in lipid metabolism: LDLR (n = 35) (A); LOX-1 (OLR-1) (n = 44) (B); SR-A (n = 56) (C); CD36 (N = 48) (D); ABCG1 (n = 54) (E); ABCA1 (n = 51) (F); SC1 (n = 48) (G); PPARα (n = 52) (H); ACO (n = 49) (I); and CPT1a (n = 45) (J). Filled circles represent individual patients.

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Renal lipid accumulation and lipotoxicity in human DN

Development of a new technique to extract mRNA from pathology archives and departmental policy to perform kidney biopsy on diabetic patients in order to reject other differential diagnoses allowed us to collect, over the years, a large group of DN biopsies that represent different stages of DN. By using a large number of biopsies, we overcame the within-subject biological variation and bias that result from choosing a selected group of patients for kidney biopsy. Using clinical data, eGFR, and the rate of renal progression, and correlating these data with gene expression, further helped us overcome the fact that we tend to perform biopsies when there is atypical presentation of DN: rapid kidney deterioration, severe proteinuria, short duration of diagnosed diabetic, or AKI events.

Kidney tissue can also be purchased from tissue banks, but the advantage of this work is that all the patients were followed-up and treated in our department before and after the biopsy until dialysis or transplantation. We have all clinical records and information on medication that are not available in tissue banks.

Lipids are not visible after normal processing of kidney biopsies for routine study. As seen in the case of cholesterol emboli, all lipids dissolved in the process of preparing biopsies. LDs are cytosolic structures in which lipids are stored as a central core of TGs and cholesterol esters surrounded by LD-associated protein (25). These organelles allow storage of potentially toxic TGs, which can then be broken down by LPL into FFAs for mitochondrial oxidation to supply the cell with ATP (41). Staining for adipophilin (ADRP), which is the protein component of LDs, is possible even in formalin embedded kidney tissue and can be used to quantify lipid accumulation.

A novel finding of our study was the demonstration of markedly increased lipid accumulation in renal tissues from diabetic patients, as shown by different lipid staining methods including BODIPY, Oil Red O, fl uorescein, and ADRP immunofluorescence. Furthermore, we showed the presence of LDs by EM in glomerular and tubular cells. LDs can be seen in different stages of progression of DN kidneys and are mostly prominent in podocytes. We found upregulation of ADRP staining and Oil Red O staining in diabetic kidneys, when compared with normal kidneys. However, in advanced fibrotic kidneys the amount of LDs tends to decrease. This process may be similar to the disappearance of LDs in “burnt out” cirrhotic liver, secondary to ‘non-alcoholic steatohepatitis (NASH) (42).

Different animal models for kidney disease, including metabolic syndrome (43), chronic glomerulopathy, nephrotic syndrome (44), chronic renal insufficiency, DN, obesity-associated renal disease, aging nephrosclerosis, and acute kidney injury (44), also showed accumulation of lipids in the renal tissue, which plays an important part in the progression of renal disease.

Lipids are not visible after normal processing of kidney biopsies for routine study. As seen in the case of cholesterol emboli, all lipids dissolved in the process of preparing biopsies. LDs are cytosolic structures in which lipids are stored as a central core of TGs and cholesterol esters surrounded by LD-associated protein (25). These organelles allow storage of potentially toxic TGs, which can then be broken down by LPL into FFAs for mitochondrial oxidation to supply the cell with ATP (41). Staining for adipophilin (ADRP), which is the protein component of LDs, is possible even in formalin embedded kidney tissue and can be used to quantify lipid accumulation.

TABLE 3. Pearson’s correlation between log of proteinuria and key genes in lipid metabolism

<table>
<thead>
<tr>
<th>Parameter</th>
<th>eGFR</th>
<th>Nephrin</th>
<th>Podocine</th>
<th>PPARa</th>
<th>ACO</th>
<th>CPT1</th>
<th>SCD</th>
<th>LOX-1</th>
<th>SR-A</th>
<th>CD36</th>
<th>ABCA1</th>
<th>ABGC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of XV pairs</td>
<td>48</td>
<td>34</td>
<td>41</td>
<td>48</td>
<td>42</td>
<td>48</td>
<td>40</td>
<td>38</td>
<td>49</td>
<td>42</td>
<td>47</td>
<td>44</td>
</tr>
<tr>
<td>Pearson r</td>
<td>-0.5624</td>
<td>-0.732</td>
<td>-0.7271</td>
<td>-0.5537</td>
<td>-0.495</td>
<td>-0.1878</td>
<td>0.289</td>
<td>0.3577</td>
<td>0.4391</td>
<td>0.3808</td>
<td>-0.2393</td>
<td>-0.3751</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.7190 to 0.1135</td>
<td>-0.8579 to -0.3513</td>
<td>-0.8456 to -0.00001</td>
<td>-0.6269 to 0.0028</td>
<td>-0.4818 to 0.05402</td>
<td>-0.02177 to 0.1919</td>
<td>0.4881 to 0.5150</td>
<td>0.04285 to 0.6079</td>
<td>0.1800 to 0.6411</td>
<td>0.08687 to 0.0128</td>
<td>-0.4854 to 0.06194</td>
<td>-0.6047 to 0.1194</td>
</tr>
<tr>
<td>P value (two-tailed)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.1860</td>
<td>0.02013</td>
<td>0.0705</td>
<td>0.0275</td>
<td>0.0016</td>
<td>0.0138</td>
<td>0.06100</td>
<td>0.0121</td>
</tr>
<tr>
<td>P value summary</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>**</td>
<td>NS</td>
<td>NS</td>
<td>*</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>*</td>
</tr>
<tr>
<td>Is the correlation significant? (α = 0.05)</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>R square</td>
<td>0.3162</td>
<td>0.5359</td>
<td>0.5286</td>
<td>0.5066</td>
<td>0.2021</td>
<td>0.03526</td>
<td>0.08355</td>
<td>0.128</td>
<td>0.1928</td>
<td>0.145</td>
<td>0.05892</td>
<td>0.1407</td>
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</table>

*The r is Pearson’s correlation coefficient.
**Correlation is significant at the 0.05 level (2-tailed).
Fig. 7. Lipid metabolism in the kidney. Intracellular lipid accumulation is governed by a balance between the influx, syntheses, and oxidation or efflux of TGs and cholesterol. In this cartoon, we illustrate lipid metabolism. Our results provide insights into the expression of key genes of lipid metabolism in the DN human kidney compared with a normal kidney. Arrows represent changes that we showed in DN.

To clarify possible mechanisms of TG accumulation in the kidney, we examined the expression of enzymes that are key regulators of FA metabolism (SCD1) (19–21), increased uptake (CD36), and decreased expression of enzymes of fat β-oxidation (ACO and CPT1). The expressions of PPARα and PPARδ and their downstream enzymes ACO and CPT1 were decreased and correlated with the decline in eGFR (Fig. 6). Taken together these alterations in lipid metabolism clarify possible mechanisms for intracellular TG accumulation in diabetic kidneys.

To clarify mechanisms of cholesterol accumulation in the kidney, we examined genes that are master regulators of cholesterol synthesis, cholesterol efflux, and cholesterol uptake (different lipoprotein receptors). We found upregulation in most lipoprotein receptors: LOX-1 (OLR-1), LDLR, acLDL scavenger receptor SR-A1, and CD36 (Fig. 5). These receptors were negatively correlated with eGFR. CCL16, the main cholesterol receptor in podocytes, was downregulated, possibly secondary to podocytopathy. There was marked downregulation of cholesterol efflux genes, the ATP-binding cassette transporters (ABCA1, ABCG1) and apoE that also correlated with eGFR. Taken together, these alterations in cholesterol metabolism gene expression that can mediate increased cholesterol influx and impaired cholesterol efflux may suggest possible mechanisms for intracellular cholesterol accumulation in the diabetic kidney.

Expression of mRNA of SREBP-1c, SREBP-2, and ChREBP and their target lipogenesis enzymes was downregulated in human DN, unlike the findings in rodent models (39), where only early manifestations of DN occur (45). Changes that we observed in the diabetic human kidney did not always parallel data from animal models; these differences could be due to the fact that changes seen in the human kidney represent much prolonged exposure to the diabetic environment, but it is still possible that in the early stage of hyperglycemia there is increased denovo lipogenesis in the kidney. Our findings parallel data processed from other human diabetic kidney gene expression profiling (31, 32, 40) and also described in human fibrotic liver as a result of NASH (42).

Angptl4 is an inhibitor of LPL and expressed in the podocyte. Angptl4 secreted by the podocyte to the circulation was recently suggested as a mechanism of hypertriglyceridemia in nephrotic syndrome (35). We found upregulation of Angptl4 in DN kidneys that could contribute to serum hypertryglyceridemia, as well as LPL inhibition (Fig. 4).

The mechanism of lipotoxicity directly or indirectly involved activation of inflammatory and profibrotic responses. We found a correlation between patient’s eGFR and expression of lipid metabolic genes and inflammatory cytokines TNFa (Figs. 1, 6; Tables 2, 3). Inflammation plays a central role in the progression of DN (Fig. 1) and induces marked changes in lipid and lipoprotein metabolism (46–50) in animal models that resemble the changes we found in human kidneys, suggesting a possible role for inflammation in causing altered lipid metabolism, even when the serum lipid profile is well controlled (Table 1).

In summary, we showed evidence for lipid accumulation and lipotoxicity in human kidneys. Accumulation of TGs and cholesterol in human kidney in diabetes can result from a mismatch between enhanced lipid uptake versus decreased lipid oxidation versus decreased catabolism and decreased efflux; these changes may result in alterations in lipid metabolism and lipid accumulation (Fig. 7). We showed significant correlation between dysregulated lipid metabolism, inflammation, podocyte dysfunction, fibrosis, and eGFR. Understanding the mechanisms of lipid accumulation in human kidney and possible lipotoxicity may serve as a target for specific therapies aimed to slow the progression of glomerulosclerosis in DN.

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REFERENCES


deficiency and inhibited by farnesoid X receptor activation in a type 1 diabetes model. *Diabetes.* **59:** 2916–2927.


