Renal mass reduction results in accumulation of lipids and dysregulation of lipid regulatory proteins in the remnant kidney

Hyun Ju Kim,1 Hamid Moradi,1 Jun Yuan,1 Keith Norris,2 and Nosratola D. Vaziri1

1Division of Nephrology and Hypertension, University of California, Irvine; and 2Charles Drew University, Los Angeles, California

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Kim HJ, Moradi H, Yuan J, Norris K, Vaziri ND. Renal mass reduction results in accumulation of lipids and dysregulation of lipid regulatory proteins in the remnant kidney. Am J Physiol Renal Physiol 296: F1297–F1306, 2009. First published April 8, 2009; doi:10.1152/ajprenal.90761.2008.—A significant reduction of renal mass results in proteinuria, glomerulosclerosis, and tubulointerstitial injury, culminating in end-stage chronic renal failure (CRF). The accumulation of lipids in the kidney can cause renal disease. Uptake of oxidized lipoproteins via scavenger receptors, reabsorption of filtered protein-bound lipids via the megalin-cubulin complex, and increased glucose load per nephron can promote lipid accumulation in glomerular, tubular, and interstitial cells in CRF. Cellular lipid homeostasis is regulated by lipid influx, synthesis, catabolism, and efflux. We examined lipid-regulatory factors in the remnant kidney of rats 11 wk after ± nephrectomy in animals or by kidney diseases in humans results in proteinuria, glomerulosclerosis, and tubulointerstitial injury, and progressive deterioration of kidney function and structure. This process is mediated by a constellation of hemodynamic events, namely, glomerular hypertension and hyperfiltration, and non-hemodynamic events including oxidative stress, inflammation, and dyslipidemia. The prevailing oxidative stress in humans and animals with chronic renal insufficiency leads to oxidation of proteins, carbohydrates, nucleic acids, lipids, and lipoproteins and accumulation of their harmful by-products in various tissues and body fluids (19, 50, 51). In this context, the uptake of oxidized lipids and lipoproteins by macrophages and resident cells in the artery wall and kidney plays a critical role in foam cell formation, atherosclerosis, and glomerulosclerosis (1). In addition, reabsorption of filtered lipid-binding proteins such as albumin and apolipoprotein A-I by the megalin-cubulin complex in proximal tubular epithelial cells can lead to cellular lipid engorgement and tubulointerstitial injury (34).

There is growing evidence that dysregulation of sterol-responsive element binding proteins (SREBPs) contribute to the pathogenesis of nephropathy in diabetes and obesity, aging nephrosclerosis, and nephrotic syndrome (22–26, 28, 42, 47, 57). Accumulation of excess lipids in the nonadipose tissues can lead to lipotoxicity and cellular dysfunction which is caused by the direct toxic effect of fatty acids, by-products of their interaction with reactive oxygen species, ATP depletion, and fatty acid-induced apoptosis (45). In fact, accumulation of lipids in the renal tissue has been implicated in the progression of glomerular and tubulointerstitial lesions in metabolic syndrome (1, 38) and chronic glomerulopathies (24, 48). Similarly, accumulation of cholesterol in proximal tubular epithelial cells participates in the pathogenesis of acute kidney injury in animals with experimental rhabdomyolysis (60).

Cellular lipid homeostasis is regulated by influx, synthesis, catabolism, and efflux of lipids. An imbalance in these processes can result in conversion of macrophages, mesangial cells, and vascular smooth muscle cells into foam cells. An influx of lipid into macrophages is mediated by several independent pathways, including scavenger receptor class A (SR-A1), class B (CD36), and class E (lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1)), whereas cholesterol efflux is primarily mediated by liver X receptor α/β (LXRα/β), which serves as an intracellular cholesterol sensor and regulates expression of its target genes, ATP-binding cassette transporter A1 (ABCA1), and the scavenger receptor class B type 1 (SR-B1), among others (1, 16, 31).

SREBPs and carbohydrate-responsive element binding protein (ChREBP) serve as master regulators of cellular lipid synthesis. For instance, SREBP-1c and ChREBP independently regulate fatty acid synthesis, whereas SREBP-2 regulates cholesterol synthesis (8, 21). SREBPs are synthesized and embedded in the endoplasmic reticulum (ER) as inactive transcription factors. Activation and translocation of SREBPs to the nucleus requires their transport to the Golgi compartment for proteolysis. The proteolytic activation of SREBPs is inhibited by elevated intracellular free cholesterol concentration, thereby limiting production of cholesterol through feedback repression (7, 56). This process is regulated by a sterol-sensing polyclonal membrane protein called SRBP cleavage-activating protein (SCAP), which binds to SREBPs via its regulatory domain. Elevation of cholesterol/oxysterols in the ER membranes promotes binding of the SCAP-SREBP complex to a
class of ER-embedded proteins known as Insig (insulin-inducible gene) -1 or -2. Binding to Insigs causes retention of the SCAP-SREBP complex in the ER. In contrast, sterol depletion leads to detachment and ubiquitination of Insigs, release of the SCAP-SREBP complex, its packaging in coat protein complex II (COPII)-coated vesicles for transport to the Golgi complex, and proteolytic activation of SREBP (43, 59).

Peroxisome proliferator-activated receptor (PPAR) α is highly expressed in the liver, muscle, kidney, and heart, where it regulates expression of genes involved in uptake, binding, transport, cellular retention, and catabolism of fatty acids (3, 17, 18). PPARα deficiency has been shown to accelerate dyslipidemia, glomerular matrix expansion, inflammatory cell infiltration, and proteinuria in animal models with diabetic nephropathy (36, 41).

The effect of chronic kidney disease caused by renal mass reduction on proteins involved in lipid metabolism in the remnant kidney has not been investigated. We believe that uptake of oxidized lipids/lipoproteins via scavenger receptors, reabsorption of filtered lipid-carrying proteins, and increased filtered glucose load in the remaining nephrons can lead to accumulation of lipids in glomerular, tubular, and interstitial cells in the diseased kidney. This study was designed to test the hypothesis that renal mass reduction may result in accumulation of lipids and alterations of scavenger receptors, lipid-regulatory nuclear transcription factors, enzymes, and transporters involved in lipid metabolism in the diseased/remnant kidney.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, weighing 225–250 g, were purchased from Harlan Sprague Dawley (Indianapolis, IN). They were housed in a climate-controlled, light-regulated facility with 12:12-h light-dark cycle. The animals were fed regular rat chow (Purina Mills, Brentwood, MO) and water ad libitum and randomly assigned to chronic renal failure (CRF) and normal control groups. The animals assigned to the CRF group were subjected to 5% nephrectomy by surgical resection using a dorsal incision, as described previously (52). The animals assigned to the control group were subjected to sham operation. All surgical procedures were carried out under general anesthesia (Nembulkat, 50 mg/kg ip). Strict hemostasis and aseptic techniques were observed. The animals were observed for 11 wk. At the conclusion of the 11-wk observation period, the animals were placed in metabolic cages for a 24-h urine collection. They were then anesthetized (pentobarbital sodium, 50 mg/kg ip) and euthanized by exsanguinations using cardiac puncture. The kidneys were immediately removed, frozen in liquid nitrogen, and stored at −70°C until processed. All experiments were approved by the University of California, Irvine Institutional Committee for the Use and Care of Experimental Animals. Serum creatinine, total cholesterol, triglyceride, HDL, and LDL cholesterol concentrations, and urinary protein excretion were measured as described in our previous studies (52).

The serum thiobarbituric acid (TBA)-reactive substance levels were measured using the method of Naito and Yamanaka (39).

Tissue lipid contents. Total lipids were extracted from 100 mg of tissue by the method of Folch et al. (15). Briefly, samples were homogenized in 6 ml chloroform-methanol (2:1). The mixture stood for 1 h, after which, 1.5 ml water was added, and the mixture was centrifuged for 10 min at 4,000 g. The organic phase was evaporated to dryness under a nitrogen stream and taken up in chloroform. Fifty-microliter aliquots of this organic phase were solubilized by adding a drop of Triton X-100, and total cholesterol and triglyceride contents were determined using enzymatic kits from Stanbio Labora-

tory (Boerne, TX). Data are expressed as the amount of given lipids per gram of original kidney mass.

Lipid staining. After the animals were euthanized, the kidneys were rapidly removed, sliced longitudinally, and immersed in 4% PFA/PBS (at 4°C) overnight. Subsequently, the tissue was cryoprotected in 30% sucrose at 4°C and then frozen using liquid nitrogen. Frozen sections were then cut using a Leica CM 1900 UV (Leica) at 10 μm. Each section was air dried for 1 h and fixed in 10% formalin for 10 min. Subsequently, the tissue was rinsed with distilled water and the sections were stained with oil red O (Sigma-Aldrich, St. Louis, MO) per the manufacturer’s protocol.

Immunohistochemical analyses. Paraffin sections (4 μm) of formalin-fixed kidney tissues were deparaffinized with xylene, and antigens were unmasked using sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) in a pressure cooker (high power) for 10 min and then cooled down to room temperature. After a rinsing with PBS, the sections were treated for 10 min with 1% H2O2 and blocked with donkey serum, followed by incubation with avidin D and biotin-blocking solution (Vector Laboratories). The sections were incubated 25 μl with goat anti-cubilin or rabbit anti-megalin (Santa Cruz Biotechnology, Santa Cruz, CA) at 4°C overnight. The slides were rinsed with PBS, incubated with goat biotinylated IgG and then with avidin-biotin complex (ABC kit, Vector Laboratories). Immunoreactivity was detected by incubation with 3,3′-diaminobenzidine (DAB kit, Vector Laboratories) and counterstaining with Mayer hematoxylin. Sections incubated with 10% nonimmune goat serum instead of the primary antiserum were used as negative controls.

Preparation of kidney homogenates and nuclear extracts. All solutions, tubes, and centrifuges were maintained at 0–4°C. The nuclear extract was prepared as described previously (44). Briefly, 100 mg of kidney cortex was homogenized in 0.5 ml buffer A containing 10 mM HEPES, pH 7.8, 10 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.1 mM EDTA, 0.1 mM PMSF, 1 μM pepstatin, and 1 mM P-amino-benzamidine using a tissue homogenizer for 20 s. Homogenates were kept on ice for 15 min, 25 μl of a 10% Nonidet P-40 (NP-40) solution was added and mixed for 15 s, and the mixture was centrifuged for 2 min at 12,000 rpm. The supernatant containing cytosolic proteins was collected. The pelleted nuclei were washed once with 200 μl of buffer A plus 25 μl of 10% NP-40, centrifuged, then suspended in 50 μl of buffer B (50 mM HEPES, pH 7.8, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mM PMSF, 10% (vol/vol) glycerol), mixed for 20 min, and centrifuged for 5 min at 12,000 rpm. The supernatant containing nuclear proteins was stored at −80°C. The protein concentration in tissue homogenates and nuclear extracts was determined by Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

Western blot analyses. Target proteins in the cytoplasmic and/or nuclear fractions of the kidney tissue were measured by Western blot analysis using the following antibodies. Rabbit antibodies against rat SREBP-1, SREBP-2, SCAP, Insig-1, Insig-2, PPARα, liver-type fatty acid binding protein (L-FABP), and LXRα/β antibodies were purchased from Santa Cruz Biotechnology. Antibodies against ChREBP, ABCA-1, SR-B1, and SR-A1 were obtained from Novus Biologicals (Littleton, CO). Antibodies against acyl-CoA:cholesterol acyltransferase-1 (ACAT1) obtained from GenScript (Piscataway, NJ), the antibody against 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) from Upstate (Billerica, MA), anti-LOX-1 from Abcam (Cambridge, MA), and anti-CD36 antibody from Cayman Chemical (Ann Arbor, MI) were used for Western blot analysis of the corresponding proteins. Antibodies to histone H1 (Santa Cruz Biotechnology) and β-actin (Sigma) were used for measurements of histone and β-actin, which served as housekeeping proteins for nuclear and cytosolic target proteins.

Briefly, aliquots containing 50 μg of protein were fractionated on 8 and 4–20% Tris-glycine gels (Novex, San Diego, CA) at 120 V for 2 h and transferred to Hybond-ECL membrane (Amersham Life Science, Arlington Heights, IL). The membrane was incubated for 1 h

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Table 1. Biochemical data in the control and chronic renal failure groups

<table>
<thead>
<tr>
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<th>CTL</th>
<th>CRF</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>71.2 ± 9.8</td>
<td>221.2 ± 20.5‡</td>
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<tr>
<td>Total/HDL cholesterol ratio</td>
<td>1.5 ± 0.2</td>
<td>2.2 ± 0.2†</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>19.1 ± 6.0</td>
<td>95.9 ± 14.1‡</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>45.8 ± 18.3</td>
<td>90.7 ± 3.6‡</td>
</tr>
<tr>
<td>Free fatty acid, meq/l</td>
<td>0.192 ± 0.074</td>
<td>0.220 ± 0.068</td>
</tr>
<tr>
<td>Urine volume, ml</td>
<td>18.3 ± 5.1</td>
<td>42.5 ± 5.5‡</td>
</tr>
<tr>
<td>Urine protein, mg/24 h</td>
<td>6.7 ± 1.3</td>
<td>80.3 ± 7.3‡</td>
</tr>
<tr>
<td>Ccr, ml/min</td>
<td>5.62 ± 1.18</td>
<td>1.74 ± 0.38‡</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>25.4 ± 2.1</td>
<td>53.6 ± 5.0‡</td>
</tr>
<tr>
<td>Scr, mg/dl</td>
<td>0.50 ± 0.1</td>
<td>1.56 ± 0.5‡</td>
</tr>
<tr>
<td>Ucr, mg/dl</td>
<td>116.3 ± 25.8</td>
<td>38.8 ± 6.0‡</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123.5 ± 13.4</td>
<td>161.4 ± 10.9†</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>87.5 ± 7.4</td>
<td>117.0 ± 4.5</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>459.8 ± 21.5</td>
<td>435.6 ± 17.3</td>
</tr>
<tr>
<td>Kidney weight, g</td>
<td>1.45 ± 0.16</td>
<td>2.38 ± 0.38‡</td>
</tr>
<tr>
<td>Plasma TBA-reactive substance, nmol/ml</td>
<td>0.39 ± 0.07</td>
<td>2.63 ± 1.01†</td>
</tr>
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</table>

Values are given as mean ± SD. CTL, control; CRF, chronic renal failure; Ccr, creatinine clearance; Scr and Ucr, serum and urine creatinine, respectively; BUN, blood urea nitrogen; SBP and DBP, systolic and diastolic blood pressure, respectively; TBA, thiobarbituric acid. *P < 0.05, †P < 0.01, ‡P < 0.001 vs. CTL group.

RESULTS

General data. Data are shown in Table 1. The CRF group exhibited heavy proteinuria, marked elevation of plasma total cholesterol, LDL cholesterol, triglyceride, and free fatty acid concentrations and increased total cholesterol-to-HDL-cholesterol ratio. This was associated with a marked increase in serum concentration of the TBA-reactive substances, pointing to increased production and accumulation of lipid peroxidation products in the CRF animals.

Tissue lipid contents. Data are shown in Fig. 1. Staining (oil-red O) of the frozen kidney tissue revealed marked accumulation of neutral lipids in the glomerular and tubulointerstitial regions of the remnant kidneys in the CRF group. In contrast, little lipid staining was found in the kidneys of normal control rats. Similarly, cholesterol and triglyceride contents of the kidney tissue were markedly elevated in the CRF group compared with those found in the controls.

Fig. 1. Top: representative photomicrographs of the oil red O-stained glomerular (G) and tubulointerstitial (D) regions of the kidney in the chronic renal failure (CRF; C and D) and the control (CTL; A and B) groups. Bottom: bar graphs depicting tissue cholesterol and triglyceride contents expressed per gram of the original kidney mass in the CRF and the control groups. ***P < 0.001.
SR-A1, CD36, and LOX-1 data. Data are shown in Fig. 2. Compared with the control group, the CRF group exhibited a marked increase in SR-A1 and LOX-1 protein abundance in the remnant kidney. Although the mean value for CD36 abundance was greater in the CRF group, the difference did not reach statistical significance. These findings point to upregulation of proteins involved in the influx of modified lipids and lipoproteins and provide a potential mechanism for the observed increase in lipid deposition in the remnant/diseased kidney.

SREBP-2, SCAP, HMG-CoA reductase, and ACAT1 data. Data are illustrated in Fig. 3. The mRNA and protein abundance of the 125-kDa SREBP-2 precursor molecule were unchanged in the remnant kidneys of the CRF group. However, the active 68-kDa SREBP-2 in the nuclear fraction was significantly lower in the renal tissue of the CRF group than that in the control group. This was associated with a significant reduction of SCAP and HMG-CoA reductase and marked increase in ACAT1 abundance in the renal tissues from the CRF rats compared with the corresponding values in the control group.
group. These findings point to reduced cholesterol production capacity and enhanced cholesterol esterification capacity in the remnant kidney.

**SREBP-1, ChREBP, fatty acid synthase, and acetyl-CoA carboxylase.** Data are shown in Fig. 4. The CRF group exhibited a marked reduction of SREBP-1c mRNA and nuclear SREBP-1 protein abundance and an insignificant reduction of the inactive SREBP-1 protein abundance in the remnant kidney. In contrast ChREBP abundance in the nuclear fraction was significantly increased of the remnant kidney of the CRF group. This was associated with a significant elevation of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) protein abundance in the remnant kidney of the CRF group compared with those found in the control group. Upregulation of FAS and ACC (the key enzymes in fatty acid synthesis) and activation of ChREBP, which is known to stimulate fatty acid production (21), point to increased lipogenic capacity of the remnant kidney.

**Insig-1 and Insig-2 data.** Data are depicted in Fig. 5. Insig-1 and Insig-2 mRNA abundance were significantly lower in the remnant kidney of the CRF group than those found in the control kidneys. Similarly, Insig-2 protein abundance was significantly lower while Insig-1 protein abundance was insignificantly lower in the remnant kidneys of the CRF group compared with that found in the control kidneys.

**PPARα, L-FABP, and ACO.** Data are illustrated in Fig. 6. L-FABP protein abundance and the ACO mRNA level were significantly lower in the kidneys of the CRF groups compared with those found in the control group. This was associated with a significant downregulation of gene and protein expressions of PPARα, the key regulator of fatty acid oxidation. These findings point to the contribution of depressed lipid catabolism in the pathogenesis of lipid accumulation in the remnant kidney.

**Megalin and cubilin data.** Data are illustrated in Fig. 7. Immunohistological examination revealed a marked increase in megalin and cubilin abundance in the proximal tubules of the remnant kidneys in the CRF group. Megalin and cubilin play a central role in reabsorption of lipid-binding (e.g., ApoA-1 and albumin) and other proteins by proximal tubular epithelial cells.

**DISCUSSION**

The present study revealed that induction of progressive kidney disease by subtotal nephrectomy in genetically normal, otherwise intact animals, results in lipid accumulation in the tubular and glomerular cells of the remnant kidney. Cellular lipid homeostasis is regulated by influx, synthesis, catabolism...
and efflux of lipids. An imbalance among the influx, synthesis, catabolism, and efflux of cholesterol can result in intracellular accumulation of lipids. To gain insight into the mechanisms responsible for accumulation of lipids in the remnant kidney, we examined expression of key proteins involved in cellular influx, synthesis, catabolism, and efflux of lipids in this tissue.

Lipid accumulation in the remnant kidney of the CRF animals was associated with marked upregulation of SR-A1 and LOX-1, which are the major mediators for uptake of oxidized or otherwise modified lipids and lipoproteins. Inflammatory cytokines and growth factors enhance cholesterol influx via upregulation of SR-A1 and CD36 (29, 30). Similarly, oxidized LDL induces SR-A1 in the circulating monocytes and renal interstitial macrophages in animals and humans with chronic kidney disease (6, 9–11, 27, 32). CRF is associated with oxidative stress, inflammation, elevation of proinflammatory cytokines, and accumulation of oxidized lipids and lipoproteins (19, 50, 51), which can collectively stimulate expression of these receptors. Scavenger receptors including SR-A1 and CD36 (SR-BII) and LOX-1 internalize oxidized and otherwise modified LDL. It is of note that unlike the LDL receptor, SR-A1 expression is not regulated by intracellular cholesterol content (13) and, as such, irrepresible uptake of modified lipoproteins via these receptors results in foam cell formation, tissue lipid accumulation, and glomerulosclerosis (14, 35). Therefore, upregulation of SR-A1 and LOX-1 can, in part, contribute to the observed lipid accumulation by increased uptake of modified lipids in the remnant kidney of the CRF animals.

Filtered proteins are reabsorbed by the megalin-cubilin complex and degraded by proximal tubular epithelial cells. In particular, the apical membrane in proximal tubular epithelial cells has a high capacity for uptake of filtered lipid-binding proteins such as albumin and apolipoprotein A-I via the megalin and cubilin complex (34). As expected, proteinuria greatly heightens the burden of reabsorbed filtered proteins and their lipid and other cargos in proximal tubular cells. In fact, proteinuria and heavy lipid accumulation in the tubular epithelial cells in our CRF animals was coupled with an intense staining for cubilin and megalin. It is of interest that uptake of protein-bound lipids by the megalin-cubilin complex leads to activation of numerous signal transduction pathways that promote apoptosis of tubular epithelial cells and production of inflammatory and profibrotic mediators, culminating in tubular atrophy, interstitial fibrosis, and inflammation (2).

To address the potential impact of renal mass reduction on cellular lipid production machinery, we next examined key factors involved in cholesterol and fatty acid biosynthesis. The study revealed a significant reduction of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis. In contrast, ACAT1 abundance was markedly elevated in the remnant kidneys. ACAT catalyzes esterification of free cholesterol, a process which leads to sequestration of esterified cholesterol in cytoplasmic vesicles and foam cell formation (5). We have found a similar phenomenon in the liver of rats with CRF (54). The contribution of elevated ACAT to the pathogenesis of lipid disorders and progression of renal disease in this model is supported by amelioration of proteinuria and preservation of residual renal function in response to pharmacological inhibition of ACAT in this model (55). The observed downregulation of the cholesterol biosynthetic pathway in the remnant kidney reflects feedback repression of the system by increased influx of cholesterol contained in the oxidized lipoproteins through scavenger receptors.
Downregulation of HMG-CoA reductase in the remnant kidney in CRF animals was accompanied by diminished activity (nuclear translocation) of SREBP-2, which is the master regulator of cholesterol synthesis (8). Activation and eventual translocation SREBPs to the nucleus in response to cellular sterol depletion is mediated by detachment and ubiquitination of Insigs from the SCAP-SREBP complex. In contrast, inhibition of SREBP activation by excess cellular sterol is mediated by binding of the SCAP-SREBP complex to Insigs and the consequent retention of the complex in the ER (4, 43). In addition to their role in the regulation of SREBP activity, Insigs participate in posttranslational regulation of HMG-CoA reductase. In this context, accumulation of sterols leads to conformational modification of HMG-CoA reductase, which triggers its binding of to Insig 1 and Insig-2. This results in recruitment of a membrane-associated ubiquitin ligase, gp78, which initiates ubiquitination of HMG-CoA reductase, thereby the reduction of cholesterol biosynthesis (12). In view of their critical role in the regulation of SREBP activity and HMG-CoA reductase, we examined the expression of SCAP and Insigs in the kidney tissues of the animals studied. The results showed significant downregulations of SCAP and Insig-2 and a mild reduction of Insig-1 in the remnant kidneys of the CRF animals. Downregulation of SCAP and Insig-1 and -2 in the remnant kidney is consistent with the general suppression of the sterol-regulated lipogenic pathway occasioned by the heavy influx of lipid from the extracellular compartments.

Genes involved in fatty acid synthesis are independently regulated by SREBP-1c and ChREBP, which are activated by a reduction of cellular sterol and increased cellular glucose loads, respectively (21, 56). The present study revealed marked upregulation of FAS and ACC, the key enzymes in fatty acid synthesis. This was associated with downregulation of SREBP-1 and marked upregulation of ChREBP in the remnant kidney. The observed upregulation of lipogenic enzymes in the face of the divergent regulatory pathways, which are independently driven by cellular sterol and glucose loads, points to the dominant influence of the latter in the diseased kidney. It is of note that ACC links fatty acid and carbohydrate metabolism through the shared intermediate metabolite, acetyl-CoA, which is the by-product of pyruvate dehydrogenase. We speculate that the observed upregulation of ChREBP and the consequent upregulation of fatty acid-producing enzymes in the remnant kidney are driven by increased filtered glucose load in the remaining nephrons undergoing compensatory hyperfiltration. Further studies are planned to determine the effect of interventions aimed at limiting glomerular hyperfiltration in the remnant kidney on lipid metabolism in this model.

The CRF animals employed in the present study exhibited significant downregulation of L-FABP and ACO, the key enzyme involved in cellular oxidation and catabolism of fatty acids in the remnant kidney. This was associated with a marked reduction of PPARα, which is the master regulator of fatty acid catabolism. PPARα is predominantly expressed in tissues with high fatty acid catabolic rates, such as the liver, kidney, heart, and muscle. In the kidney, PPARα is highly abundant in the proximal tubules and medullary thick ascending limb of the loop of Henle, with much lower levels in glomerular mesangial cells (18). PPARα promotes fatty acid catabolism by promoting L-FABP and ACO expression, leading to stimulation of...
mitochondrial and peroxisomal β-oxidation. FABP serves as the vehicle for delivery of fatty acids to intracellular sites of utilization and as such plays an important role in cellular fatty acid metabolism (46). Thus downregulation of PPARα and its target genes contributes to accumulation of lipid in the remnant/diseased kidney. In addition to causing lipotoxicity, impaired fatty acid oxidation can contribute to cellular injury and dysfunction by limiting lipid-derived energy production in the remnant kidney, which faces increased energy demand by the remaining nephrons occasioned by compensatory hypertrophy and hyperfiltration.

The CRF group exhibited a marked upregulation of LXR in the remnant kidney. LXRs is a member of the nuclear receptor and transcription factor superfamily, which exists as LXRα and LXRβ isoforms. LXRα is abundantly expressed in the liver, intestine, and the kidney, while LXRβ is present in most tissues ubiquitously. They form heterodimers with the retinoic acid receptor RXRα, which bind to the LXR-response elements (LXRE) in the 5′-flanking region of target genes. LXR serves as an intracellular sterol sensor which regulates expression of genes involved in absorption, excretion, catabolism, and cellular efflux of cholesterol. In addition, LXR regulates expression of genes involved in fatty acid metabolism. In this context, LXRs regulate expression ABCA1, ABCG5, ABCG8, apolipoprotein E (ApoE), cholesterol ester transfer protein, lipoprotein lipase, FAS, and SREBP-1c. These observations illustrate the critical role of LXRs in the regulation of lipid and cholesterol metabolism. LXRα is expressed in mesangial cells, where its activation mediates cholesterol efflux via ABCA1 (58).

![Fig. 7. Representative photomicrographs depicting cubilin and megalin immunostaining in the kidney tissues from a CRF and CTL rat.](image)

![Fig. 8. Representative Western blots and group data depicting protein abundance of renal tissue ATP-binding cassette transporter A1 (ABCA1), SR-B1, and nuclear liver X receptor (LXR) α/β in the CRF and CTL groups. *P < 0.05, **P < 0.001.](image)
The ABCA1 transporter is the gatekeeper of the reverse cholesterol transport pathway, which mediates transfer of cellular cholesterol and phospholipids to lipid-poor HDL for disposal in the liver (40). As noted above, expression of ABCA1 is regulated by LXRα/β, which serves as a cholesterol sensor (49). The CRF rats employed in the present study exhibited marked upregulation of ABCA1 in the remnant kidney. Similarly, protein abundance of SR-B1 which mediates bidirectional flux of cellular cholesterol and phospholipids was significantly increased in the remnant kidney tissue of the CRF animals. This was associated with increased nuclear translocation of LXRA/β in the remnant kidney. Upregulation of ABCA1 and SR-B1, which are the primary pathways of cholesterol efflux and activation of their master regulator, LXR, points to the cellular response to an increased cholesterol burden in the remnant kidney. It should be noted, however, that despite upregulation of cellular efflux pathways, HDL-mediated reverse cholesterol and phospholipid transport may be impaired in CRF. Several factors can contribute to defective reverse cholesterol transport in CRF. First, CRF significantly lowers hepatic production of ApoA-I, which is the principal constituent of HDL (53). Second, CRF significantly lowers hepatic production and reduces plasma concentration of lecithin:cholesterol acyltransferase (54), which is essential for efficient HDL-mediated cholesterol uptake and maturation of HDL. Finally, CRF results in oxidative modification of HDL (37), which can impair reverse cholesterol transport by interfering with HDL binding to the ABCA1 transporter (40). Accumulation of lipids despite upregulation of reverse cholesterol transport and downregulation of cholesterol biosynthetic capacity noted above points to the predominance of cholesterol influx in the remnant kidney.

In conclusion, chronic renal failure induced by 5⁄6 nephrectomy in genetically normal, otherwise intact, rats leads to the accumulation of lipids in the remnant kidney. The accumulation of lipids in the remnant kidney is associated with and largely due to upregulation of receptors involved in the influx of oxidized lipids and lipoproteins, activation of fatty acid biosynthetic machinery, and inhibition of pathways involved in fatty acid oxidation in the diseased kidney.

GRANTS
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