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Niacin improves renal lipid metabolism and slows progression in chronic kidney disease

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ABSTRACT

Background: Mounting evidence points to lipid accumulation in the diseased kidney and its contribution to progression of nephropathy. We recently found heavy lipid accumulation and marked dysregulation of lipid metabolism in the remnant kidneys of rats with chronic renal failure (CRF). Present study sought to determine efficacy of niacin supplementation on renal tissue lipid metabolism in CRF.
Methods: Kidney function, lipid content, and expression of molecules involved in cholesterol and fatty acid metabolism were determined in untreated CRF (5/6 nephrectomized), niacin-treated CRF (50 mg/kg/day in drinking water for 12 weeks) and control rats.
Results: CRF resulted in hypertension, proteinuria, renal tissue lipid accumulation, up-regulation of scavenger receptor A1 (SR-A1), acyl-CoA cholesterol acyltransferase-1 (ACAT1), carbohydrate-responsive element binding protein (ChREBP), fatty acid synthase (FAS), acyl-CoA carboxylase (ACC), liver X receptor (LXR), ATP binding cassette (ABC) A-1, ABCG-1, and SR-B1 and down-regulation of sterol responsive element binding protein-1 (SREBP-1), SREBP-2, HMG-CoA reductase, PPAR-α, fatty acid binding protein (L-FABP), and CPT1A. Niacin therapy attenuated hypertension, proteinuria, and tubulo-interstitial injury, reduced renal tissue lipids, CD36, ChREBP, LXR, ABCA-1, ABCG-1, and SR-B1 abundance and raised PPAR-α and L-FABP.
Conclusions and general significance: Niacin administration improves renal tissue lipid metabolism and renal function and structure in experimental CRF.

1. Introduction

Renal mass reduction by subtotal nephrectomy in animals or by disease processes in humans results in progressive glomerulosclerosis, tubulo-interstitial injury and proteinuria, culminating in end-stage renal disease [1]. The decline in renal function and structure following renal mass reduction is mediated by arterial hypertension, glomerular capillary hypertension and hyperfiltration, oxidative stress, inflammation and lipid disorders, among other factors.

Chronic renal insufficiency results in profound alteration of lipid metabolism and plasma lipid profile which is marked by hypertriglyceridemia, diminished HDL cholesterol, impaired HDL maturation and depressed HDL antioxidant and anti-inflammatory activities [2–5]. These alterations are due to diminished production of Apo A-I, Apo A-II, and lecithin-cholesterol acyltransferase (LCAT), down-regulation of lipoprotein lipase and hepatic lipase and up-regulation of acyl-CoA cholesterol acyltransferase (ACAT) [6–10].

The associated dyslipidemia has been shown to contribute to progression of kidney disease [11–14] and lipid lowering strategies have proven effective in retarding progression of renal disease [15,16]. Cellular lipid accumulation in the kidney has been shown to promote or accelerate kidney disease in animal models of diabetic nephropathy, and aging glomerulosclerosis [17–22]. In this context, reabsorption of filtered protein-bound lipids and uptake of oxidized lipid/lipoproteins by tubular and glomerular cells and infiltrating monocytes can potentially lead to accumulation of lipids in the diseased kidney. This can, in turn, cause cytotoxicity and contribute to progression of renal disease.

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. Influx of lipids into macrophages is mediated by several independent pathways including scavenger receptor class A (SR-AI), 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 (LXR α/β) which serves as an intracellular cholesterol sensor and regulates expression of its target genes, adenosine triphosphate-binding cassette A-1 (ABCA-1) and ABCG-1 transporters and the scavenger receptor class B type 1 (SR-B1), among others [11,23,24]. Sterol

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responsive element binding proteins (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 [25,26]. The proteolytic activation of SREBPs and the consequent production of cholesterol and fatty acids is inhibited by elevated intracellular free cholesterol and enhanced by reduced intracellular free cholesterol concentration [27,28]. In addition to sterols, changes in cellular glucose can influence cellular lipid biosynthesis. For instance, elevation of cellular glucose raises fatty acid synthesis via activation of ChREBP which serve as a regulator of cellular lipid synthesis. There is growing evidence that dysregulation of SREBP contributes to the pathogenesis of nephropathy in diabetes and obesity, aging nephrosclerosis and nephrotic syndrome [17–22,29,30].

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 [31–33]. PPAR-α deficiency has been shown to accelerate dyslipidemia, glomerular matrix expansion, inflammatory cell infiltration and proteinuria in animal model with diabetic nephropathy [34,35].

In a recent study we found that glomerulosclerosis and tubulointerstitial injury in rats with CRF induced by 5/6 nephrectomy was associated with heavy accumulation of neutral lipids in the remnant kidney. This was accompanied by marked up-regulation of ChREBP and proteins involved in fatty acid synthesis and down-regulation of PPAR-α and molecules involved in fatty acid catabolism [36]. Niacin is known to raise HDL, lower triglyceride [37–40] and exert potent antioxidant and anti-inflammatory properties [41].

Given the role of dyslipidemia and inflammation in progression of renal disease and the demonstrated efficacy of niacin in ameliorating these abnormalities we designed the present study to test the hypothesis that long-term niacin supplementation may reduce accumulation of lipids in the remnant kidney and retard progression in animals subjected to subtotal nephrectomy.

2. Materials and methods

2.1. Study groups

Male Sprague–Dawley rats with an average body weight of 225–250 g (Harlan Sprague-Dawley, Inc., Indianapolis, IL) were used in this study. Animal were housed in a climate-controlled vivarium with 12-h day and night cycles and were fed a standard laboratory diet (Purina Mills, Brentwood, MO) and water ad libitum. The animals were randomly assigned to the CRF and sham-operated control groups. The CRF group underwent 5/6 nephrectomy (5/6 Nx) by surgical resection of the upper and lower thirds of left kidney, followed by right nephrectomy 7 days later. The control group underwent sham operation. The procedures were carried out under general anesthesia (sodium pentobarbital, 50 mg/kg i.p.) using strict hemostasis and aseptic techniques. The 5/6 nephrectomized animals were randomized to untreated and niacin-treated subgroups. The niacin-treated group was given niacin (50 mg/kg/day, dissolved in the drinking water) for 12 weeks. The untreated group received regular water instead. Six animals were included in each group.

Timed urine collections were carried out at baseline, week 8, and week 12, using metabolic cages. Urine protein concentration (Chondrex, Inc., Redmond, WA) and specific gravity were determined in the 24-h urine samples. Blood pressure was determined by tail cuff plethysmography (CODA2, Kent Scientific Corporation, Torrington, CT). Conscious rats were placed in a restrainer on a warming pad and allowed to rest inside the cage for 15 min before blood pressure measurements. Rat tails were placed inside a tail cuff, and the cuff was inflated and released several times to allow the animal to be conditioned to the procedure.

At the end of the experiment, animals were anesthetized (sodium pentobarbital, 50 mg/kg i.p.) and euthanized by exsanguinations using cardiac puncture. Kidneys were removed and weighted. Hypertrophy of the remnant kidney was estimated assuming that the left kidney mass that remained after 5/6 nephrectomy was equivalent to the combined weight of its excised poles subtracted from the weight of the removed right kidney. A piece of the kidney was separated and fixed in 10% formalin for histological examination; another piece was secured for lipid staining and processed as described below. The remaining tissue was cleaned with phosphate-buffered saline, snap-frozen in liquid nitrogen, and stored at −70 °C until processed.

Plasma total cholesterol (Stanbio Laboratory, Boerne, TX), triglyceride (Stanbio Laboratory, Boerne, TX), HDL cholesterol (Wako Chemicals, Richmond, VA), urea (Bioassay Systems, Hayward, CA), and creatinine (Bioassay Systems, Hayward, CA) were analyzed using the specified products. Creatinine clearance was calculated using standard equation.

The experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of California (Irvine, CA).

2.2. Tissue lipid contents

Total lipids were extracted from 100 mg tissue by the method of Folch et al. [42]. 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 10 min at 2000×*g*. The organic phase was evaporated to dryness under N_2 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 the enzymatic kits from Stanbio Laboratory (Boerne, TX) [42]. Data were expressed as amount of the given lipids per gram of original kidney mass.

2.3. Lipid staining

After euthanizing the animals, the kidneys were rapidly removed, sliced longitudinally, and immersed in 4% PFA/PBS (at 4 °C) overnight. Subsequently the tissue was cryo-protected in 30% sucrose at 4 °C and then frozen using liquid nitrogen. Frozen sections were then cut using Leica CM 1900 UV (Leica, Germany) 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 manufacturer's protocol.

2.4. 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 [43]. 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 $MgCl_2$, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 0.1 mM PMSF, 1 μM pepstatin, and 1 mM *p*-aminobenzamidine using a tissue homogenizer for 20 s Homogenates were kept on ice for 15 min, 125 μl of a 10% Nonidet p40 (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

Table 1
General data in the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats.

	CTL	CRF	CRF+Niacin
Body weight, 12 weeks (g)	459.80 ± 21.51	411.74 ± 55.36	418.92 ± 30.52
Left kidney weight, 12 weeks (g)	1.42 ± 0.03	2.20 ± 0.51*	2.03 ± 0.47
Heart left ventricle weight, 12 weeks (g)	1.27 ± 0.08	1.50 ± 0.10**	1.33 ± 0.05††
Systolic blood pressure, 12 weeks (mmHg)	123.52 ± 13.37	168.80 ± 2.83**	132.52 ± 15.44†
Diastolic blood pressure, 12 weeks (mmHg)	87.50 ± 10.09	117.00 ± 4.53*	92.46 ± 15.11†
Urine output, 12 weeks (ml/day)	18.33 ± 5.10	42.50 ± 5.49**	28.17 ± 6.91*, ††
24-h urine protein, 12 weeks (g/day)	6.70 ± 1.27	80.28 ± 7.31**	53.18 ± 19.68**, †
Urine specific gravity, 12 weeks	1.033 ± 0.01	1.022 ± 0.00**	1.030 ± 0.00†
Hematocrit (%)	48.91 ± 4.43	38.12 ± 9.64*	43.14 ± 5.40
Plasma glucose (mg/dl)	162.47 ± 23.82	168.04 ± 37.88	172.65 ± 25.44
Plasma urea nitrogen (mg/dl)	25.37 ± 2.06	60.04 ± 16.42***	47.44 ± 9.55**
Plasma creatinine (mg/dl)	0.50 ± 0.14	2.22 ± 1.51*	1.44 ± 0.30
Creatinine clearance (ml/min)	5.62 ± 1.18	1.45 ± 0.72***	2.28 ± 0.57***

$n = 6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

PMSF, 10% (v/v) 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 the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA).

2.5. Western blot analyses

Target proteins in the cytoplasmic and/or nuclear fractions of the kidney tissue were measured by Western blot analysis as previously described [30,36] using the following antibodies. Antibodies against rat SREBP-1, SREBP-2, SCAP, Insig-1 and 2, PPAR-α, liver-type fatty acid binding protein (L-FABP), and LXR α/β were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies against ChREBP, ABCA-1, ABCG-1, carnitine palmitoyltransferase 1A (CPT1A), SR-B1 and SR-A1 were obtained from Novus Biologicals, Inc.(Littleton, CO). Antibodies against acyl-CoA: cholesterol acyltransferase-1 (ACAT-1) and CD36 were obtained from Cayman Chemical, Inc. (Ann Arbor, MI). Antibody against 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) was purchased from Upstate, Inc. (Billerica, MA). Antibodies against fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) were purchased from Cell Signaling Technology, Inc. (Denver, CO). Peroxidase-conjugated immunopure goat anti-rabbit IgG antibody and anti-mouse IgG antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Antibodies to histone H1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and β-actin (Sigma, Inc., St. Louis, MO) for measurements of histone and β-actin which served as housekeeping proteins for nuclear and cytosolic target proteins.

Briefly, aliquots containing 50 μg proteins were fractionated on 8% and 4–20% Tris–glycine gel (Novex, Inc., San Diego, CA) at 120 V for 2 h, and transferred to Hybond-ECL membrane (Amersham Life Science, Inc., Arlington Heights, IL). The membrane was incubated for 1 h in blocking buffer (1× TBS, 0.05% Tween-20 and 5% nonfat milk) and then overnight in the same buffer containing the given antibodies. Membrane was washed three times for 5 min in 1× TBS, 0.05% Tween-20 prior to 2-h incubation in a buffer (1× TBS, 0.05% Tween-20 and 3% nonfat milk) containing horseradish peroxidase-linked anti-rabbit IgG and anti-mouse IgG (Amersham Life Science, Inc.) at 1:1000 dilution. The membrane was washed 4 times and developed by autoluminography using the ECL chemiluminescent agents (Amersham Life Science, Inc.).

2.6. Statistical analysis

ANOVA and post hoc Tukey tests (SPSS 13.0, Chicago, IL) were used in statistical evaluation of the data which are presented as mean ± standard deviation. *P* values below equal to or less than 0.05 were considered significant.

3. Results

3.1. General data

Data are summarized in Table 1. The CRF group showed a significant rise in systolic and diastolic arterial pressure and a marked left ventricular hypertrophy. Long-term niacin administration resulted in significant reduction of systolic pressure and prevention of left ventricular hypertrophy. As expected the CRF animals had significant elevation of plasma creatinine and urea nitrogen concentrations and urinary protein excretion. Niacin supplementation did not significantly change plasma creatinine or urea nitrogen concentrations, but significantly lowered urinary protein excretion. Likewise, the CRF animals exhibited marked polyuria and reduced urine specific gravity. Niacin therapy attenuated polyuria and raised urinary concentrating capacity in the study animals.

3.2. Plasma lipid data

Data are shown in Table 2. The CRF group exhibited a significant increase in plasma triglyceride and total cholesterol concentrations and a decrease in plasma HDL cholesterol-to-total cholesterol ratio. Niacin administration resulted in a mild reduction of plasma triglyceride and cholesterol concentrations; however, the difference did not reach statistical significance.

3.3. Tissue lipid contents

The CRF animals had significant elevation of cholesterol and triglyceride in the remnant kidney which was partially reduced by niacin supplementation. Likewise, Oil Red O staining revealed marked accumulation of neutral lipids in the tubular, interstitial and glomerular regions of the remnant kidney in untreated CRF rats. Niacin administration reduced but did not reverse lipid

Table 2
Plasma concentration and renal tissue contents of cholesterols and triglyceride in the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats.

	CTL	CRF	CRF+Niacin
Plasma total cholesterol (mg/dl)	71.19 ± 9.80	221.19 ± 20.53***	197.22 ± 56.93***
Plasma HDL cholesterol/T cholesterol	0.62 ± 0.09	0.39 ± 0.16*	0.50 ± 0.06
Plasma triglyceride (mg/dl)	45.85 ± 18.30	99.72 ± 3.57*	92.69 ± 40.16*
Kidney tissue total cholesterol (mg/g)	4.06 ± 0.35	13.59 ± 2.82**	9.22 ± 2.04**, †
Kidney tissue triglyceride (mg/g)	4.78 ± 0.47	17.17 ± 3.62**	10.82 ± 2.97**, †

HDL cholesterol: high density lipoprotein cholesterol. $n = 6$ in each group. Data are means ± SD. Kidney tissue data were expressed as amount of the given lipids per gram of original kidney mass. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

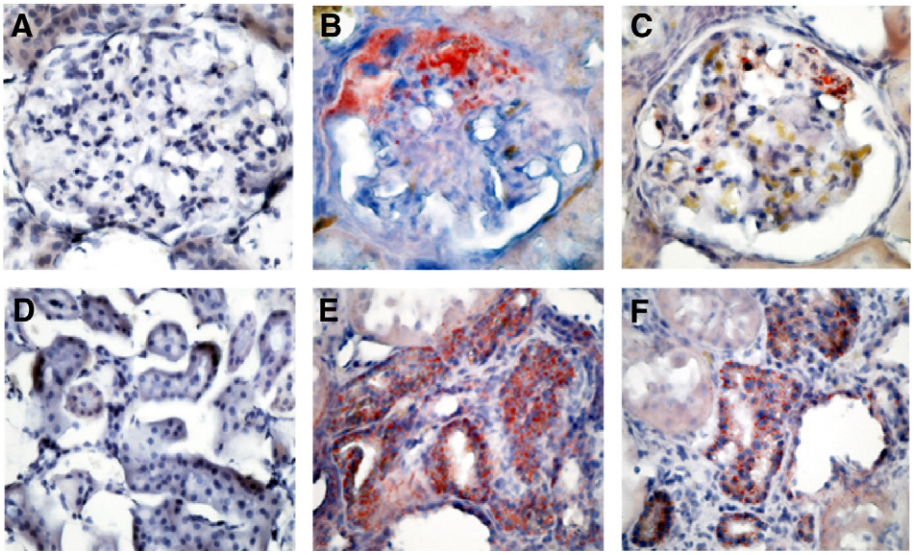

Fig. 1. Representative photomicrographs of the Oil Red O stained renal tissue of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. Examination of the remnant kidney showed neutral lipid accumulation in glomeruli, proximal tubules and interstitial region in untreated CRF rats and their attenuation in niacin-treated animals. (A and D) Control (CTL) rats. (B and E) 5/6 nephrectomized (CRF). (C and F) Niacin-treated CRF (CRF+Niacin).

accumulation in the kidneys of the treated CRF rats (Fig. 1 and Table 2).

3.4. SRA-1 and CD36 data

Data are shown in Fig. 2. Compared to the control group, the CRF group exhibited a marked increase in SRA-1 protein abundance and a mild elevation of CD36 abundance in the remnant kidney. Niacin supplementation significantly reduced CD36 abundance but failed affect SRA-1 expression in the remnant kidney tissue.

3.5. SREBP-2, HMG-CoA reductase and ACAT1 data

Data are illustrated in Fig. 3. In confirmation of our recent study [36], SREBP-2 abundance and activity were significantly lower in the renal tissue of the CRF group than that found in the control group. This was associated with significant reduction of HMG-CoA reductase and marked increase in ACAT1 abundance in the renal tissues from the CRF rats compared to the corresponding values found in the control groups. These findings point to reduced cholesterol production capacity and enhanced cholesterol esterification capacity in the remnant kidney. Administration of niacin normalized nuclear SREBP-2 expression but did not significantly affect HMG-CoA reductase and ACAT1 abundance in the remnant kidney of the CRF animals.

3.6. SCAP, Insig-1 and Insig-2 data

Data are depicted in Fig. 4. SCAP and Insig-2 protein abundance were significantly lower in the remnant kidney of the CRF group. Niacin supplementation partially reversed down-regulation of SCAP but did not significantly affect Insig-1 or Insig-2 abundance.

3.7. ChREBP, SREBP-1, fatty acid synthase (FAS), and acyl-CoA carboxylase (ACC) data

Data are shown in Fig. 5. In confirmation of our recent studies [36], the ACC and FAS protein abundance were significantly higher in the kidney of the untreated CRF groups when compared with that found in the control group. This was accompanied by marked reduction of SREBP-1 and significant elevation of ChREBP in the nuclear extracts. Up-regulation of FAS and ACC (the key enzymes involved in fatty acid synthesis) and activation of ChREBP which is known to stimulate fatty acid production [26] point to increased

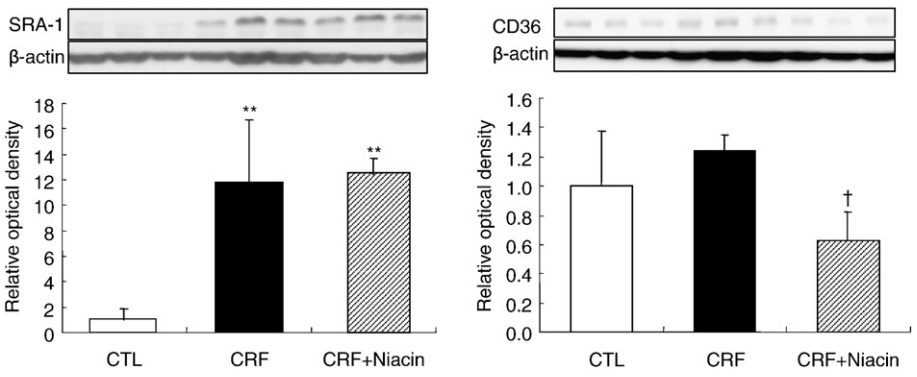

Fig. 2. Representative Western blots and group data depicting protein abundance of the SR-A1 and CD36 in the renal tissues of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

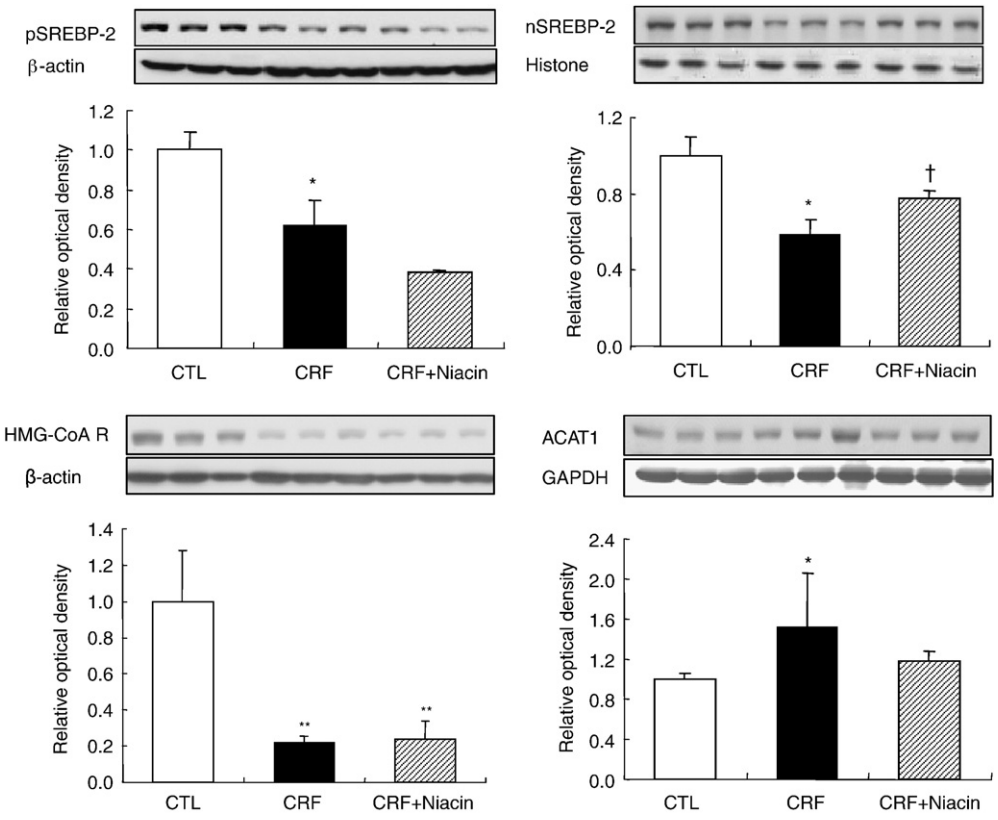

Fig. 3. Representative Western blots and group data depicting protein abundance of SREBP-2 (inactive and nuclear), ACAT1 and HMG-CoA reductase in the renal tissues of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

lipogenic capacity of the remnant kidney. Niacin supplementation normalized SREBP-1 expression and reversed up-regulation/activation of ChREBP in the remnant kidney.

3.8. PPAR-α, L-FABP and carnitine palmitoyltransferase 1A (CPT1A) data

Data are illustrated in Fig. 6. L-FABP and CPT1A protein expression was significantly lower in the kidneys of the CRF groups when compared with those found in the control group. This was associated with significant down-regulation of PPAR-α, the key regulator of fatty acid oxidation. These findings point to the potential role of depressed lipid catabolism as a cause of lipid accumulation in the remnant kidney. Niacin administration resulted in up-regulation of PPAR-α and L-FABP but failed to alter CPT1A abundance in the remnant kidney.

3.9. LXR α/β, ABCA-1, ABCG-1 and SR-B1 data

Data are shown in Fig. 7. ABCA-1, ABCG-1 and SR-B1 which are the main pathways of cholesterol and phospholipid efflux and their master regulator LXR were significantly increased in the remnant kidneys in the untreated CRF group. Niacin administration resulted in significant reductions of ABCA-1, ABCG-1, and SR-B1 abundance and LXR α/β activity toward values found in the control group. This observation reflects the response to niacin-induced reduction of cellular lipid burden in the study animals.

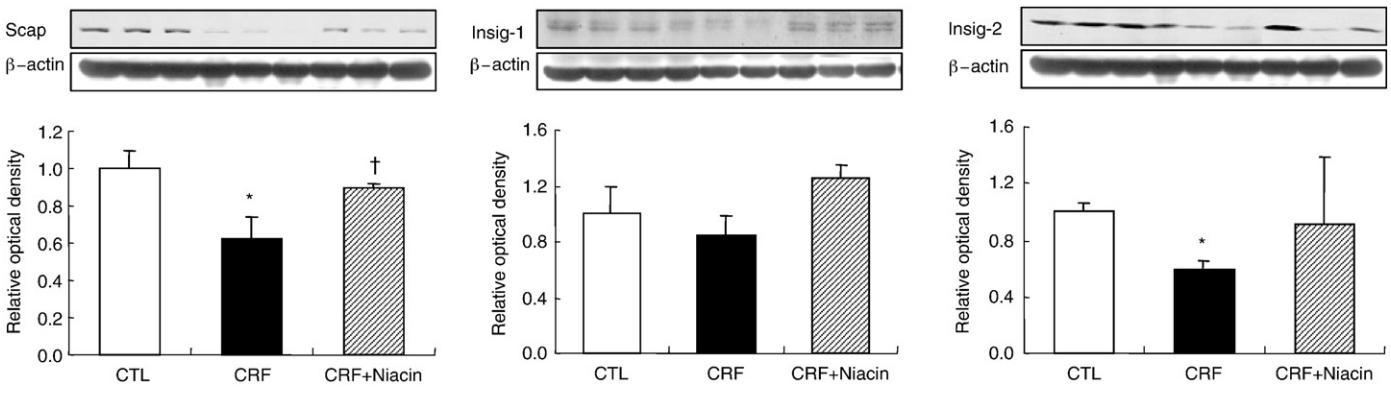

Fig. 4. Representative Western blots and group data depicting protein abundance of SCAP, Insig-1 and Insig-2 in the renal tissues of the untreated 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$ vs. control group; †$p<0.05$ vs. CRF group.

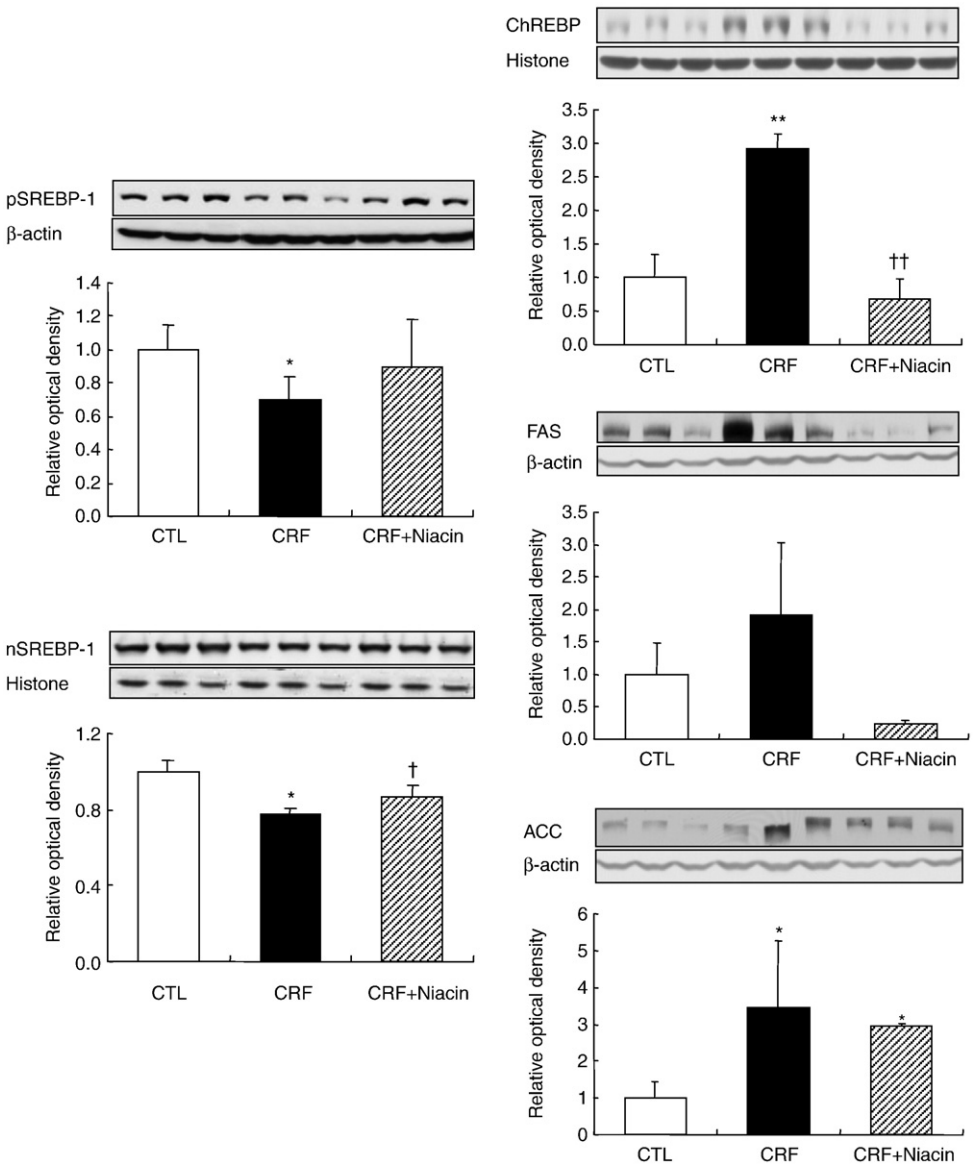

Fig. 5. Representative Western blots and group data depicting protein abundance of ChREBP, SREBP-1 (inactive and nuclear), FAS and ACC in the renal tissues of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

4. Discussion

Pharmacological doses of niacin reduce total cholesterol, triglyceride (TG), very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and lipoprotein(a) levels and increases high density lipoprotein (HDL) levels [38,39]. In addition, niacin inhibits vascular inflammation by lowering production of reactive oxygen species in endothelial cells, reducing oxidation of LDL, limiting vascular cell adhesion molecule-1 (VCAM-1) and MCP-1 expression and monocyte adhesion and infiltration [41].

Cellular lipid homeostasis is regulated by influx, synthesis, catabolism and efflux of lipids. An imbalance between these processes can result in intracellular accumulation of lipids. Lipid accumulation in the remnant kidney of the CRF rats was associated with marked up-regulation of SR-A1 and LOX-1 which mediate uptake of oxidized or otherwise modified lipids and lipoproteins. Inflammatory cytokines and growth factors enhance cholesterol influx via up-regulation of SR-A1, LOX-1 and CD36 [44,45]. Similarly oxidized LDL induces SR-A1, LOX-1 and CD36 expression in the circulating monocytes and renal interstitial macrophages in animals and humans with chronic kidney disease [46–50]. Scavenger receptors including SR-A1 and CD36 internalize oxidized and otherwise modified LDL. It is of note that unlike LDL receptor [25], SR-A1 expression is not regulated by the intracellular cholesterol contents [51] and as such irrepressible uptake of modified lipoproteins via these receptors can result in foam cell formation, tissue lipid accumulation and glomerulosclerosis [52,53]. Therefore, up-regulation of SR-A1 and CD36 can, in part, contribute to the observed lipid accumulation by increased uptake of modified lipids in the remnant kidney of our CRF animals. Recent studies have shown that niacin increases expression of peroxisome proliferator-activated receptor-γ (PPAR-γ) and CD36 in monocyte and macrophages and up-regulates ABCA-1 which is the gatekeeper of reverse cholesterol transport pathway [54]. Interestingly, however, niacin supplementation resulted in a significant reduction of CD36 in the remnant kidneys of the CRF rats. The reason for the observed discordance is unclear. However the decline in renal tissue CD36 abundance with long-term niacin supplementation in the remnant kidneys of our CRF animals may be, in part, due to the reduction of the infiltrating mononuclear leukocytes (which heavily

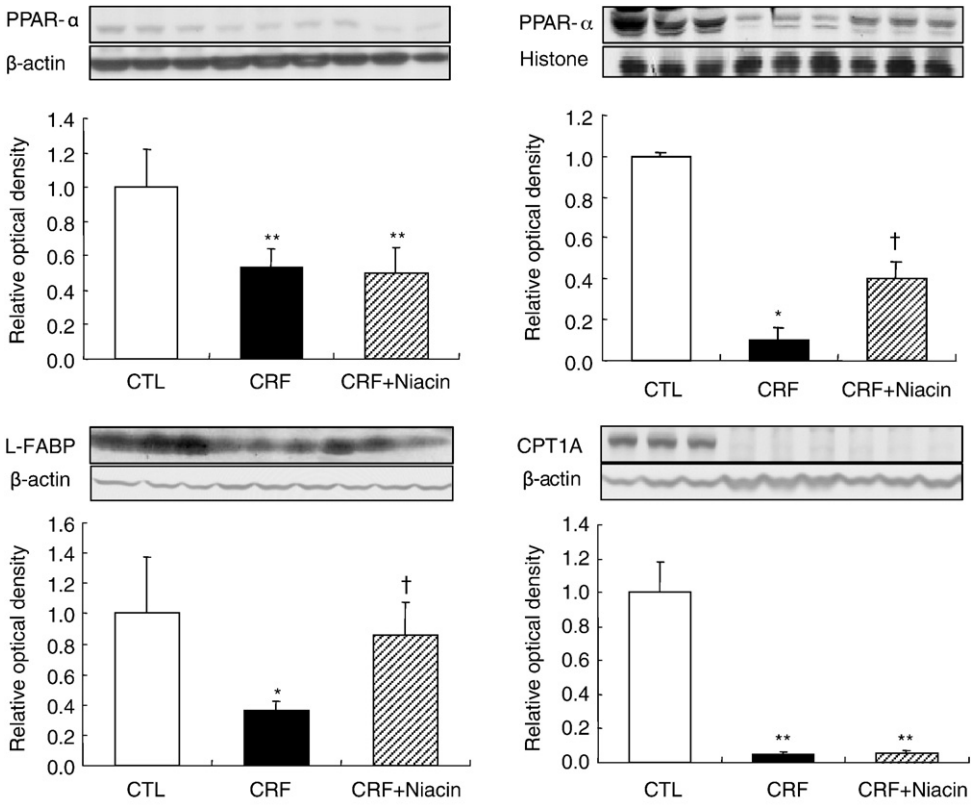

Fig. 6. Representative Western blots and group data depicting protein abundance of PPAR-α (inactive and nuclear), L-FABP and CPT1A in the renal tissues of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

express this receptor) and the fall in oxidized LDL as shown in our recent study [55].

Our untreated CRF animal exhibited significant down-regulations of HMG-CoA reductase, SREBP-2, SCAP and Insig-2 and a mild reduction of Insig-1 in the remnant kidney. In presence of high cellular sterol levels, SREBP–SCAP complexes are bound to the endoplasmic reticulum membrane by Insigs, preventing their migration to and proteolytic activation in the Golgi apparatus and eventual nuclear translocation [56]. Moreover, in response to elevated cellular sterol concentrations, Insigs bind and facilitate ubiquitination of HMG-CoA reductase [57]. Down-regulations of SREBP-2, HMG-CoA reductase, SCAP and Insigs in the remnant kidney point to the general suppression of sterol-regulated lipogenesis by the heavy lipid influx in the CRF animals. Administration of niacin normalized nuclear SREBP-2 expression and partially reversed down-regulation of SCAP but did not significantly affect HMG-CoA reductase, Insig-1 or Insig-2 abundance in the remnant kidney of the CRF animals.

ACAT catalyzes esterification of cholesterol and storage of esterified cholesterol in the cytoplasmic vesicles. Consequently up-regulation of ACAT can potentially contribute to foam cell formation [58] and progression of renal disease. This assertion is supported by amelioration of proteinuria and preservation of residual renal function by pharmacologic inhibition of ACAT in this model [10]. Our CRF animals showed a marked elevation of ACAT1 abundance in the remnant kidney. Niacin supplementation did not significantly affect ACAT1 expression in the remnant kidney tissue of the treated CRF animals.

Cellular fatty acid synthesis is independently regulated by SREBP-1c which is activated by reduction in cellular sterol and ChREBP which is activated by increased cellular glucose load [26,27]. In confirmation of our recent study [36], we found marked up-regulation of FAS and ACC, the key enzymes in fatty acid synthesis in the remnant kidney tissue of CRF animals. This was associated with down-regulation of SREBP-1 and marked up-regulation of ChREBP in the remnant kidney. The observed up-regulation of lipogenic enzymes in the face of the divergent regulatory pathways 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 byproduct of pyruvate dehydrogenase. We speculate that the observed up-regulation of ChREBP and the consequent up-regulation of fatty acid producing enzymes in the remnant kidney are driven by increased filtered glucose load in the remaining nephrons undergoing compensatory hyperfiltration.

The CRF animals employed in the present study exhibited significant down-regulation of L-FABP and CPT1A (the key enzyme involved in cellular oxidation and catabolism of fatty acids) in the remnant kidney. This was associated with marked reduction of PPAR-α which is the master regulator of fatty acids catabolism. PPAR-α promotes fatty acid catabolism by promoting L-FABP and CPT1A 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 [59]. Thus, down-regulation of PPAR-α 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 damage and dysfunction by limiting lipid-derived energy production. Recent studies demonstrated that niacin directly and noncompetitively inhibits hepatocyte microsomal diacylglycerol acyltransferase-2 (DGAT2), a key enzyme in triglyceride synthesis [60]. The inhibition of triglyceride synthesis by niacin results in accelerated intracellular Apo B degradation in the liver and the decreased secretion of VLDL and formation of LDL [61]. Niacin administration resulted in partial but significant restoration of PPAR-α expression and activity and of L-FABP expression in the remnant kidneys of CRF animals. Thus,

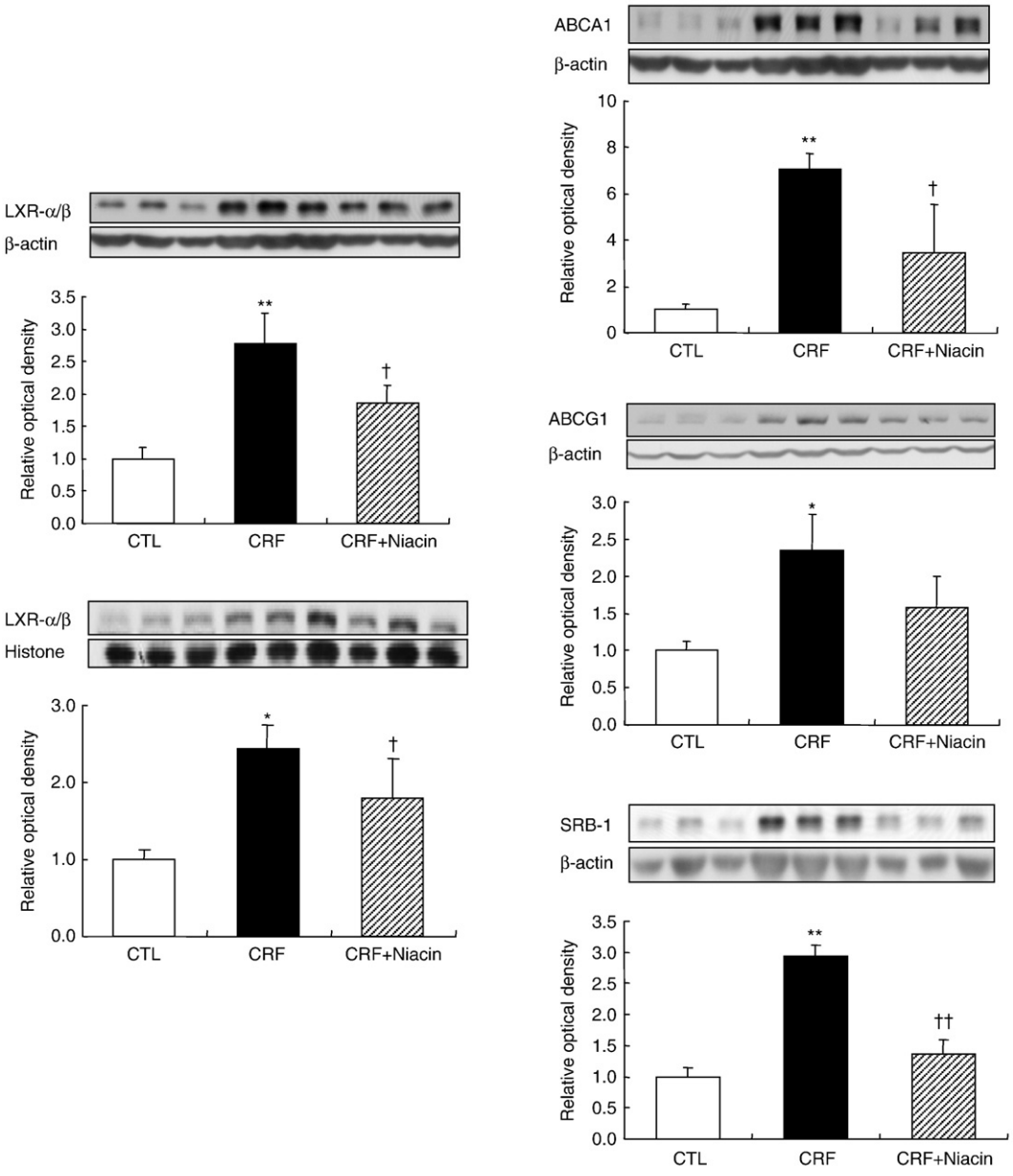

Fig. 7. Representative Western blots and group data depicting protein abundance of LXR α/β (inactive and nuclear), ABCA1, ABCG1 and SR-B1 in the renal tissues of the 5/6 nephrectomized (CRF), niacin-treated CRF (CRF+Niacin), and control (CTL) rats. $n=6$ in each group. Data are means ± SD. *$p<0.05$, **$p<0.01$ vs. control group; †$p<0.05$, ††$p<0.01$ vs. CRF group.

improvement of PPAR-α activity may have, in part, contributed to the reduction of the remnant kidney tissue lipid contents in the study animals.

ABCA1 transporter mediates transfer of cellular cholesterol and phospholipids to lipid-poor HDL for disposal in the liver and as such serves as the gatekeeper of reverse cholesterol transport pathway [62]. Expression of ABCA1 and several other proteins involved in cholesterol absorption, transport, excretion and efflux is regulated by LXR α/β which acts as a cellular cholesterol sensor [63]. Activation of LXR in macrophages leads to the coordinate induction of multiple genes potentially involved in cholesterol efflux, including ABCA1, ABCG1, and Apo E. Induction of these genes in response to lipid loading may serve to limit lipid accumulation and thus protect against development of fatty lesions and atherosclerosis [64,65]. Our untreated CRF rats exhibited a significant increase in ABCA1 abundance in the remnant kidney. Similarly, kidney tissue abundance of SR-B1 which mediates bidirectional flux of cellular cholesterol and phospholipids was significantly increased in the CRF animals. This was associated with increased LXR α/β expression and activity in the remnant kidney. Up-regulation of the lipid efflux pathway in the remnant kidney of the untreated CRF rats reflects the response to increased cellular cholesterol burden. Despite up-regulation of cellular efflux pathways, HDL-mediated reverse cholesterol and phospholipid transport may be impaired in CRF. Several factors contribute to defective reverse cholesterol transport in CRF. These include diminished production and reduced plasma concentration of Apo A-I [66] and LCAT (lecithin-cholesterol acyltransferase) [67], and oxidative modification of HDL (4), which impairs HDL binding to ABCA1 transporter [62]. Accumulation of lipids despite up-regulation of reverse cholesterol transport and down-regulation of cholesterol biosynthetic capacity noted above points to predominance of cholesterol influx in the remnant kidney. Recent in vitro studies have suggested that niacin raises HDL level, in part, by down-regulating hepatic expression of beta chain ATP synthase which binds,

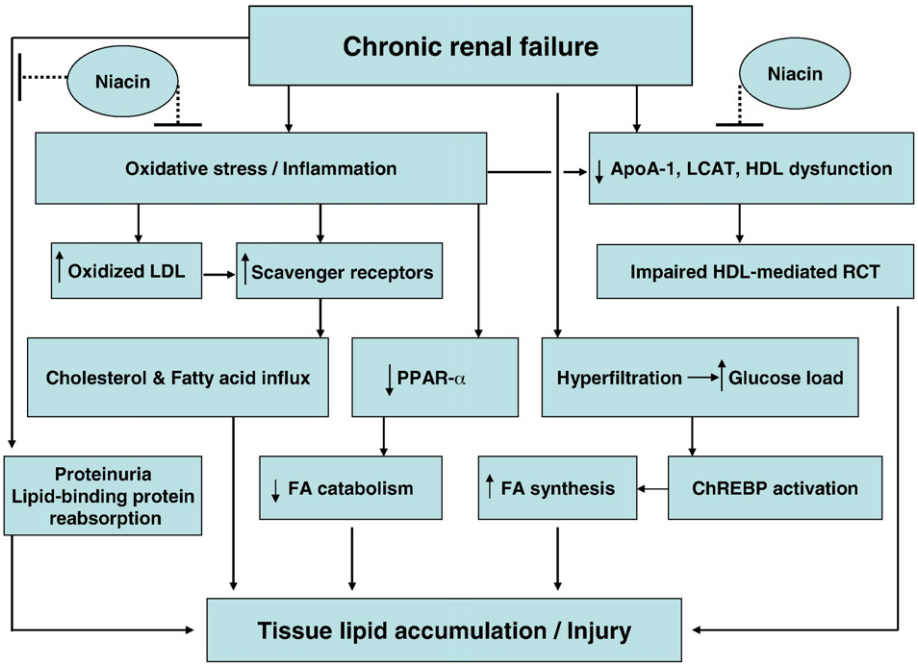

Fig. 8. Diagram depicting the potential effects of CRF and niacin supplementation on renal tissue lipid metabolism. Via induction of oxidative stress, inflammation, proteinuria, glomerular hyperfiltration, ApoA-1 and LCAT deficiency and HDL dysfunction, CRF heightens cellular lipid influx/uptake, increases fatty acid (FA) synthesis, reduces FA catabolism and limits HDL-mediated lipid efflux. The constellation of these events leads to lipid accumulation and lipotoxicity in the remnant/disease kidney. Niacin administration appears to attenuate lipid accumulation by exerting antioxidant and anti-inflammatory actions and reducing proteinuria.

internalizes and degrades Apo A-I. [68,69]. Reduction in HDL and Apo A-I catabolism by niacin accounts for its known ability to increase the half-life of HDL and augment reverse cholesterol transport [70]. Interestingly, niacin administration resulted in partial to complete reversal of up-regulation of LXR α/β, ABCA1, ABCG-1, and SR-B1 in the remnant kidney of the CRF rats, reflecting the reduction of the cellular cholesterol content as seen by tissue lipid staining. The potential effects of CRF and niacin supplementation on pathways of renal tissue lipid metabolism in CRF are illustrated in Fig. 8.

In conclusion, chronic renal failure induced by 5/6 nephrectomy in genetically normal, otherwise intact, rats leads to accumulation of lipids in the remnant kidney. This is associated with and largely due to increased influx of oxidized lipids and lipoproteins, tubular reabsorption of lipid-binding proteins (occasioned by proteinuria), activation of fatty acid biosynthetic pathway and inhibition of fatty acid oxidation machinery in the diseased kidney. Thus long-term niacin administration improves hypertension and proteinuria and attenuates lipid accumulation in the remnant kidneys of animals with CRF induced by subtotal nephrectomy.

Conflict of interest

The authors have no conflict of interest.

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