Renal Lipids in the Pathogenesis of Kidney Disease, and the Role of 2-Hydroxypropyl-β-Cyclodextrin as a Potential Treatment Option

ZyVersa THERAPEUTICS℠

Background

There is increasing evidence that accumulation of intracellular lipids in the kidney contributes to the pathogenesis and progression of kidney disease. Lipid accumulation is observed in both genetic and non-genetic origins of kidney disease.[1]

Data from animal models of three distinct kidney diseases demonstrate that 2-hydroxypropyl-β-cyclodextrin decreases intracellular renal lipids, prevents structural damage to the kidney, and restores function. Zyversa licensed worldwide rights to 2-HPβCD for the treatment of kidney disease based on these results. The research was led by Dr. Alessia Fornoni, Professor of Medicine and Chief, Katz Family Division of Nephrology and Hypertension, University of Miami Miller School of Medicine. A phase 2a study in patients with FSGS is scheduled to begin in 2020.

The purpose of this White Paper is to review the scientific evidence supporting the role of intracellular lipid accumulation in the pathogenesis of kidney disease, and to summarize the data available for 2-HPβCD as a promising therapeutic agent.

Role of Lipids in Normal Kidney Function

The kidneys' filtration system, the nephron, includes a network of small capillaries known as the glomerulus. An important component of the kidneys' filtration barrier is the podocyte. Podocytes have long projections called foot processes that wrap around the capillaries of the glomerulus, with slits between them for filtering blood called a slit diaphragm (Figure 1). The slit diaphragm is a cholesterol-rich raft-like structure critical for size-selective filtration of plasma to ensure that essential proteins are retained in the blood rather than spilled into the urine (proteinuria).

Maintenance of podocyte intracellular cholesterol at appropriate levels (homeostasis) is critical to support the structural integrity and function of podocytes and the cholesterol-rich slit diaphragm.

Glomerular Filtration System

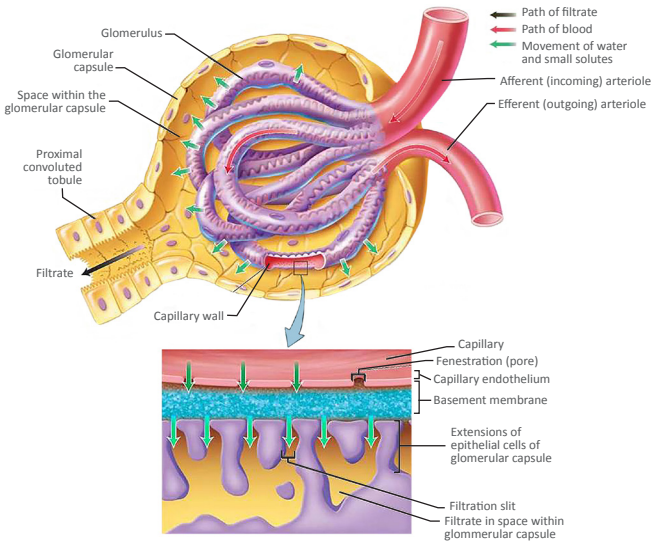


Figure 1.

Role of lipids in the Pathogenesis of Kidney Disease

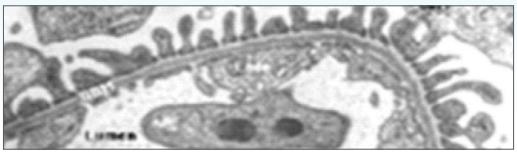
Lipid homeostasis can be disrupted in a number of ways to result in podocyte lipid accumulation. Synthesis or influx into cells may be increased, efflux out of cells may be decreased, or a combination of the 3 may occur. As illustrated in Figures 2 and 3, lipid accumulation results in podocyte distortion and

damaged podocyte foot processes, leading to impaired kidney filtration and protein leakage into the urine (proteinuria).

If lipid homeostasis and podocyte integrity are not restored, ongoing kidney damage leads to kidney failure and the need for dialysis and ultimately transplant.

Lipid abnormalities are common in patients with chronic kidney disease, including high levels of cholesterol in the blood (hypercholesterolemia) and cholesterol accumulation within the kidney.[1]

Normal: Intact podocytes foot process



Abnormal: Flattened podocytes

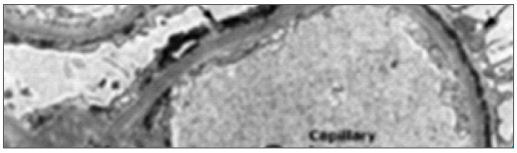


Image adapted from D'Agati et al: N Engl J Med 2011; 365:2398-2411

Figure 2.

Effects of Podocyte Lipid Accumulation

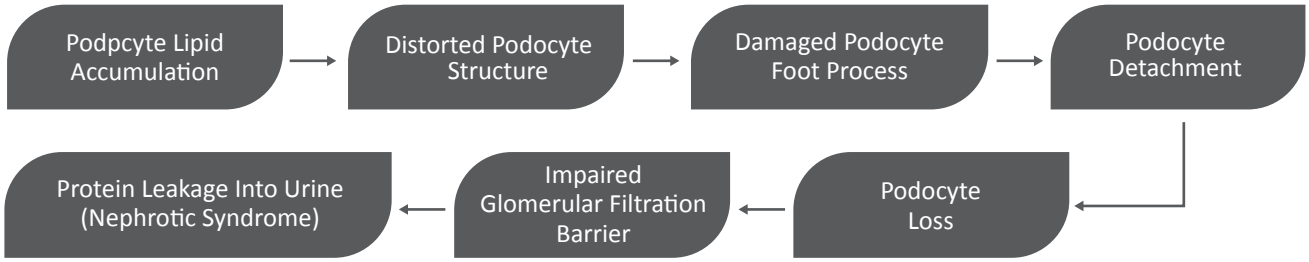


Figure 3.

Recent research suggests that it is the elevated cholesterol within the kidney, rather than that in the blood that contributes to the pathogenesis and progression of kidney diseases[2-6]. Renal lipid accumulation has been demonstrated in *in vitro* podocyte studies, in human biopsy data, and in animal models of various kidney diseases as summarized below.

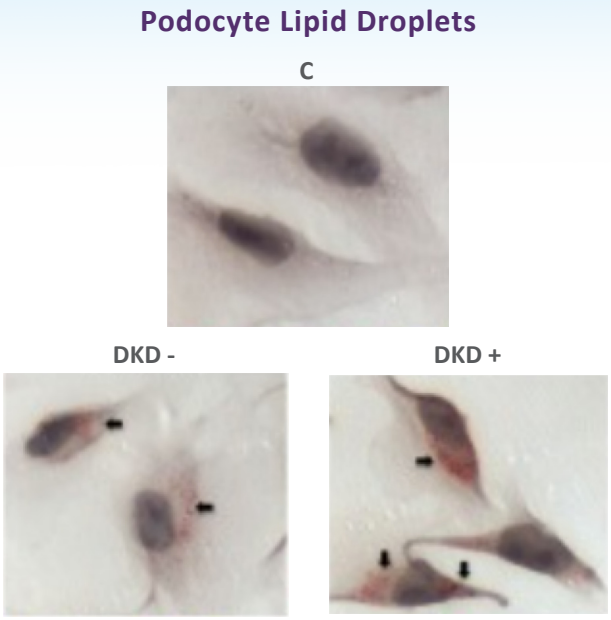
HUMAN PODOCYTES EXPOSED TO SERUM FROM PATIENTS WITH TYPE 1 DIABETIC KIDNEY DISEASE DEMONSTRATED CHOLESTEROL ACCUMULATION RESULTING FROM DECREASED EFFLUX

To determine if dysregulation of cellular lipid homeostasis is associated with podocyte injury in diabetic kidney disease, Mercher-Gomez[7] evaluated immortalized normal human podocytes which were serum and insulin starved and exposed to 4% patient sera for 24 hours from the following groups:

- 10 healthy controls (C)
- 10 patients with type 1 diabetes with no proteinuria, normal albuminuria and creatinine (DKD-)
- 10 patients with type 1 diabetes with proteinuria, albuminuria and increased creatinine (DKD+)

RESULTS

Major lipid droplet accumulation was demonstrated in podocytes exposed to serum from patients with both DKD- and DKD+, with a significantly higher percentage of cells affected than controls (C).



Oil Red O Staining

Figure 4.

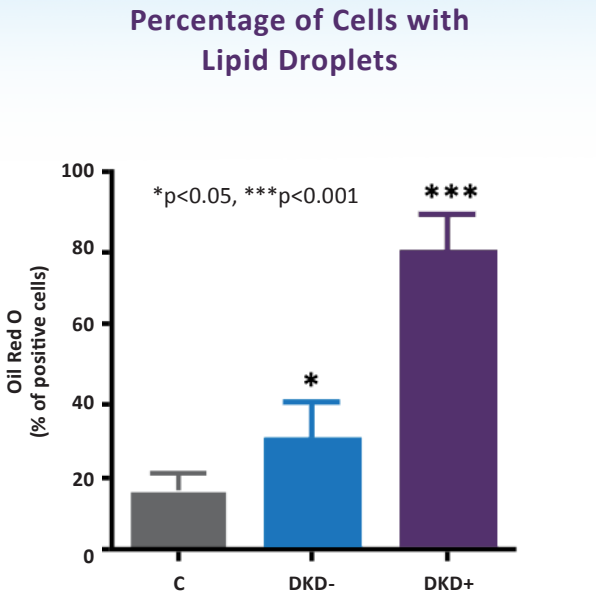


Figure 5.

Podocyte total cholesterol and esterified cholesterol were significantly higher in podocytes exposed to serum from patients with DKD+ compared to those exposed to serum from patients with DKD- or controls (C).

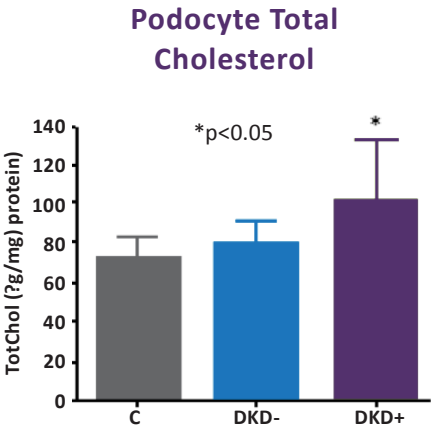


Figure 6.

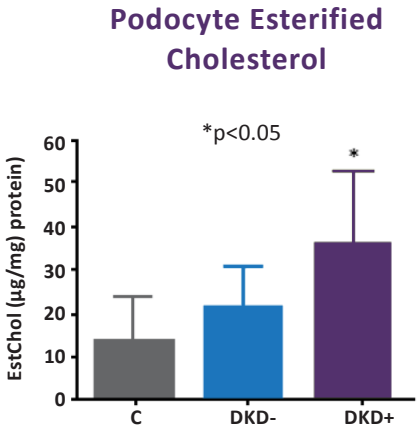


Figure 7.

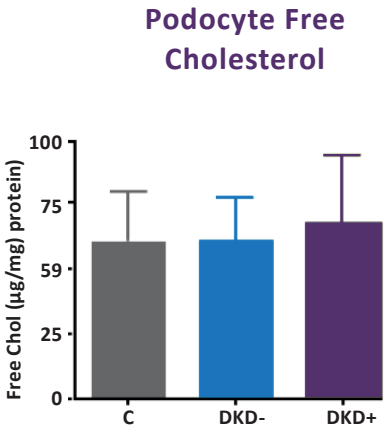


Figure 8.

Data indicate that the increased podocyte lipids were associated with decreased cholesterol efflux (Figure 9). Cholesterol efflux was significantly reduced in podocytes exposed to serum from patients with DKD- and DKD+ compared to controls (C), and ABCA1 mRNA expression, associated with lipid efflux, was significantly downregulated in podocytes exposed to serum from patients with DKD+ (Figure 10).

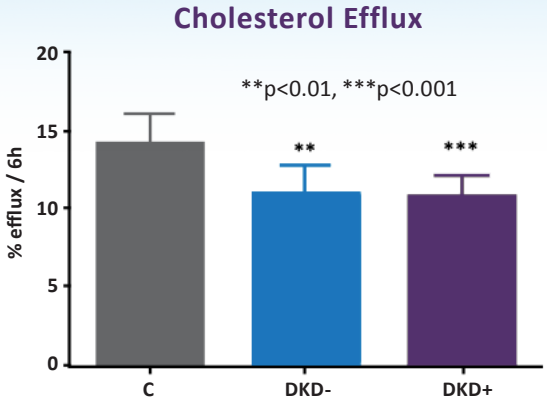


Figure 9.

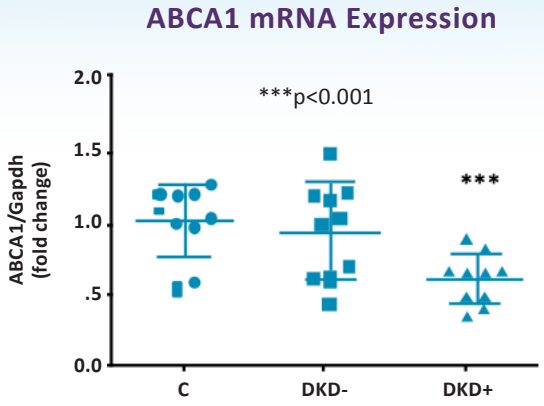


Figure 10.

LDL receptor and HMG-CoA reductase, associated with lipid influx, were unchanged across all three groups (Figures 11 and 12).

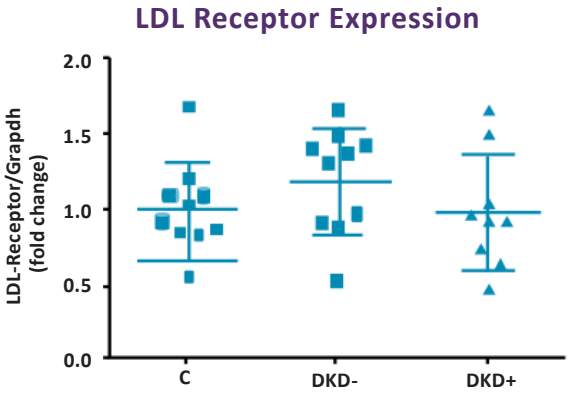


Figure 11.

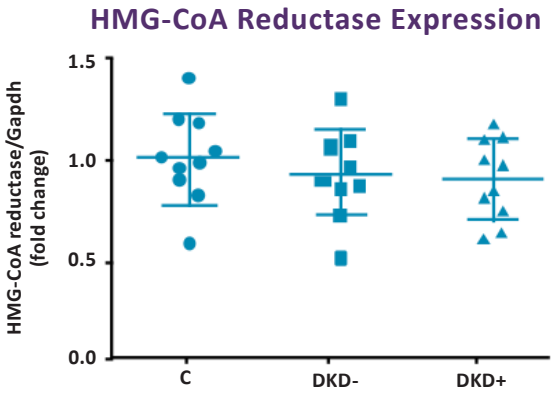


Figure 12.

BIOPSIES FROM PATIENTS WITH TYPE 2 DIABETIC KIDNEY DISEASE DEMONSTRATED KIDNEY DAMAGE ASSOCIATED WITH ACCUMULATION OF LIPIDS RESULTING FROM INCREASED CHOLESTEROL INFLUX AND DECREASED CHOLESTEROL EFFLUX

Herman-Edelstein[2] investigated lipid accumulation in human diabetic nephropathy by evaluating leftover portions of diagnostic kidney biopsies from patients with type 2 diabetic nephropathy (n=34) and normal kidneys (n=12) from the pathological archives of the Department of Pathology at Rabin Medical Center. Clinical information about the diabetic patients and controls was collected from the patients' files. Phenotype analysis represented all clinical and pathological diversity of DN from early CKD (stage 1) to advanced CKD (stage 4–5).

RESULTS

Electron microscopy demonstrated extensive accumulation of intracellular lipid droplets (LDs) in patients with diabetic nephropathy, along with podocyte foot process effacement, widening of glomerular basement membrane, and mesangial expansion. Lipid droplets were found even in early diabetic nephropathy when foot processes were still preserved (Figure 13).

Kidney Damage Associated With Lipid Accumulation

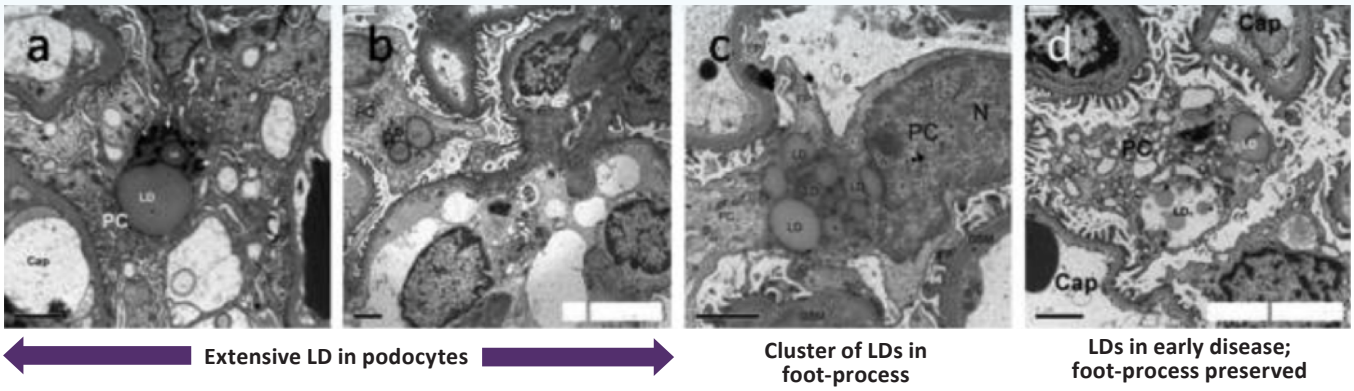


Figure 13.

Marked neutral lipid accumulation was demonstrated with Oil Red O staining in both glomeruli and tubulointerstitium in diabetic kidneys, and there was evidence of cholesterol accumulation based on filipin staining.

Lipid and Cholesterol Accumulation in Diabetic Kidneys

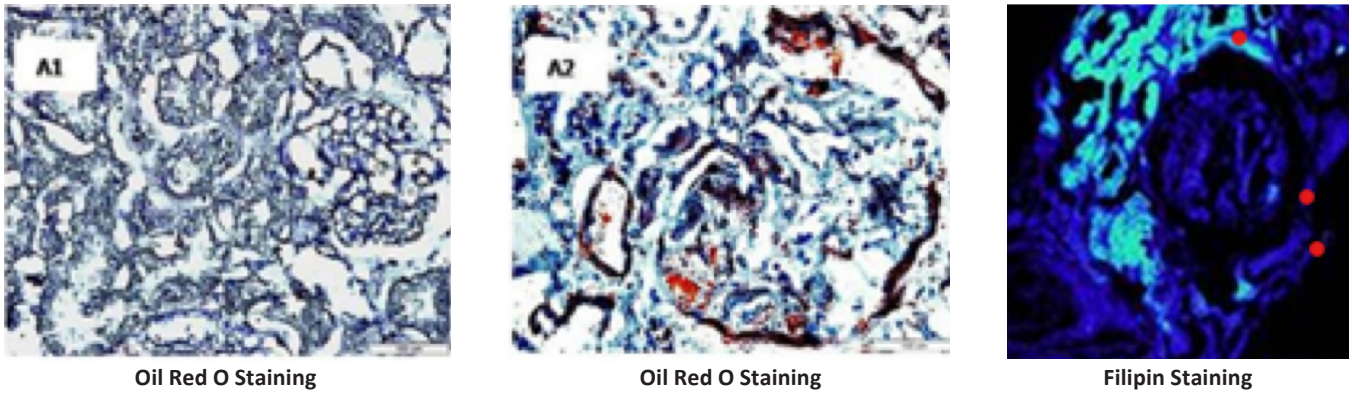


Figure 14.

Data suggest that increased cholesterol uptake and decreased cholesterol efflux led to cholesterol accumulation in the diabetic kidney, based on gene expression involved in cholesterol metabolism (Figures 15 and 16).

Cholesterol Influx Gene Expression

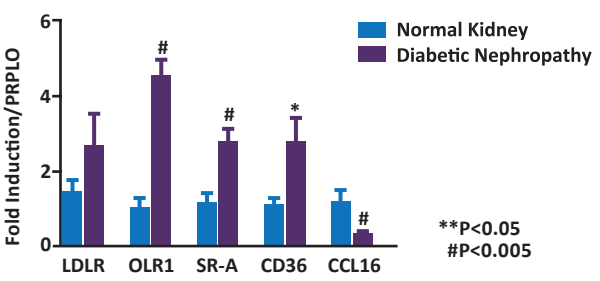


Figure 15.

Cholesterol Efflux Gene Expression

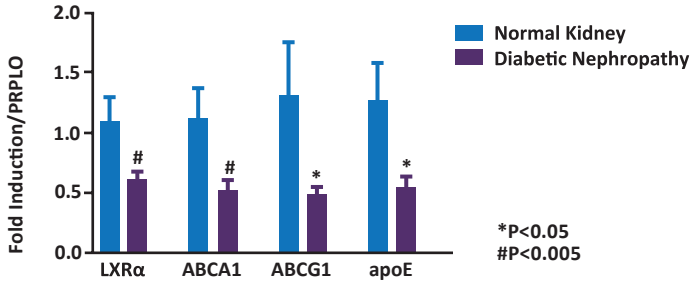


Figure 16.

DIABETIC MOUSE MODEL DEMONSTRATES LIPID ACCUMULATION IN THE KIDNEY, WITH ASSOCIATED KIDNEY DAMAGE

To assess the potential role of renal lipid accumulation in the pathogenesis of diabetic nephropathy, Proctor[8] evaluated male Ins2^{Akita} mice on the C57BL/6 background, a genetic model of type 1 diabetes. C57BL/6 mice served as controls.

RESULTS

There was significant accumulation of neutral lipid deposits in the glomeruli of diabetic mice when compared to controls, based on oil red O staining (Figure 17). Biochemical analysis indicated that the increased neutral lipids corresponded to significant increases in triglyceride and cholesterol content (Figures 18 and 19).

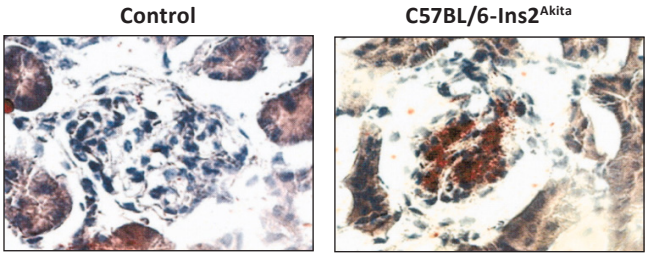


Figure 17.

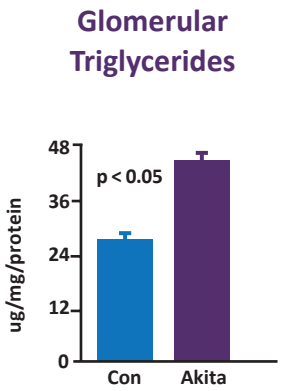


Figure 18.

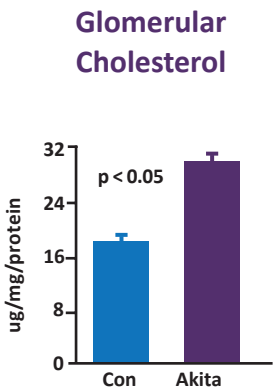


Figure 19.

As shown in Figure 20, the lipid accumulation was associated with kidney damage (mesangial expansion, podocyte injury and glomerulosclerosis), and proteinuria (albumin/creatine of 1.8 versus 0.3 in controls; P<0.01).

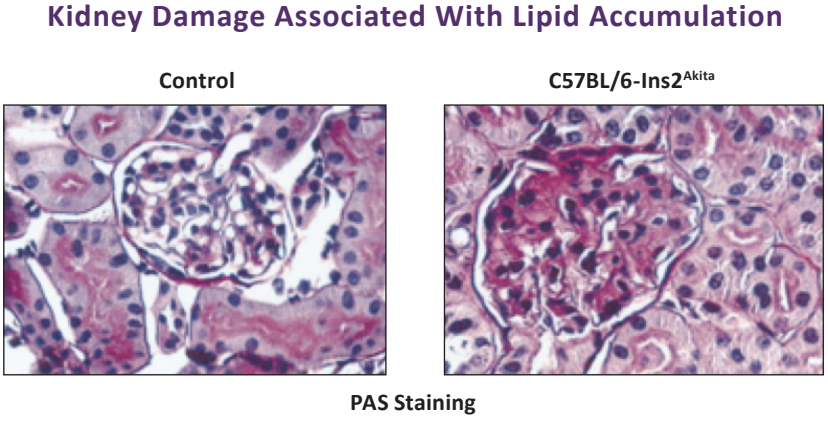


Figure 20.

Lipid accumulation was attributed to increased fatty acid and cholesterol synthesis, and decreased cholesterol efflux (Figure 21).

Nuclear receptor and lipid metabolism enzyme activity, protein, and mRNA expression in wild-type and Akita mice

	WILD TYPE	AKITA	*P* VALUE
FATTY ACID SYNTHESIS			
SREBP-1 Protein	3.0 ± 0.2	5.4 ± 0.2	<0.01
SCD-1 mRNA	1.8 ± 0.2	3.6 ± 0.3	<0.05
SCD-1 Protein	7.2 ± 0.1	11.6 ± 0.3	<0.05
SCD-1 Activity	2.5 ± 0.1	4.2 ± 0.3	<0.05
FXR mRNA	9.1 ± 0.2	2.0 ± 0.4	<0.01
SHP mRNA	3.9 ± 0.2	2.1 ± 0.4	<0.05
CHOLESTEROL SYNTHESIS AND EFFLLUX			
SREBP-2 Protein	1.6 ± 0.2	3.4 ± 0.3	<0.01
ABCA1 mRNA	14.8 ± 1.0	5.8 ± 0.9	<0.01
LXR-α	12.3 ± 0.8	3.1 ± 0.7	<0.01
LXR-β	12.9 ± 0.9	2.1 ± 0.8	<0.01

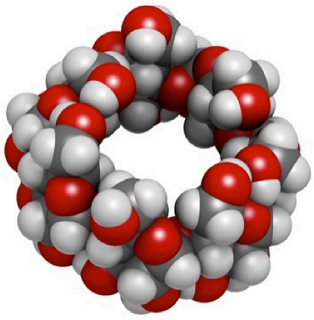
Data are means ± SE (n = 6 mice in each group)

Figure 21.

2-Hydroxypropyl-Beta-Cyclodextrin (2-HPβCD) as a Mediator of Cholesterol Efflux

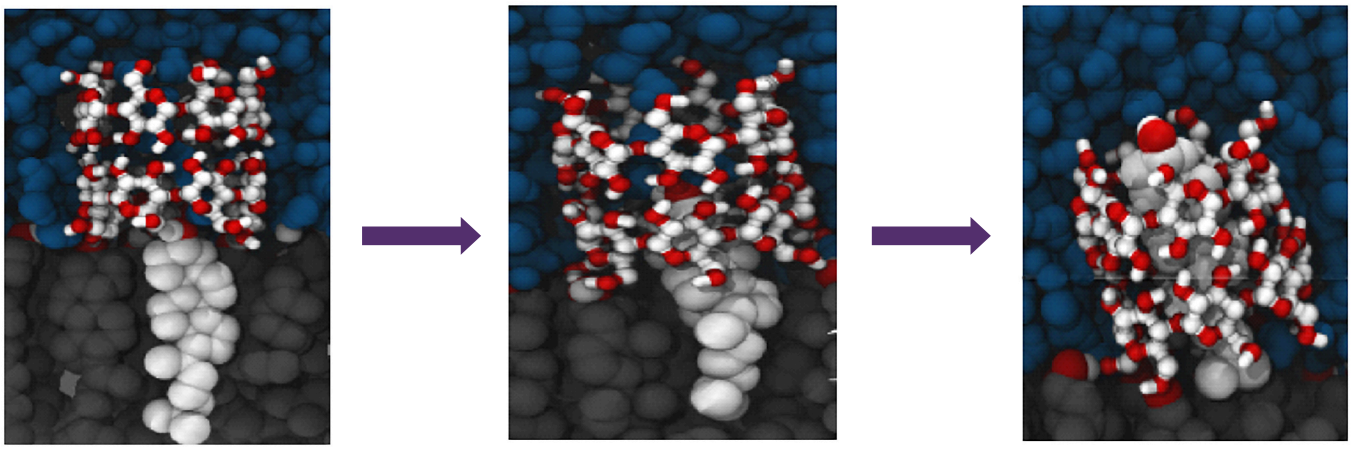
Beta-cyclodextrins (βCD) are oligosaccharides consisting of a cone-shaped 3D-ring of seven glucose subunits. The cone shaped ring forms a hydrophobic cavity, whereas the outer part of the cone is hydrophilic. 2-HPβCD is therefore water soluble, and it can incorporate hydrophobic molecules, such as cholesterol, into its central hydrophobic cavity.

Space filling model of β-Cyclodextrin



Lopez demonstrated that βCD mediates cholesterol efflux by interacting with hydrophilic membrane components and passively extracting lipid molecules by forming non-covalent inclusion complexes with them (Figure 22).[9]

βCD-mediated Cholesterol Efflux



Lopez CA, de Vries AH, Marrink SJ (2011) Molecular Mechanism of Cyclodextrin Mediated Cholesterol Extraction. PLoS Comput Biol 7(3): e1002020.

Figure 22.

It is believed that 2-HPβCD also promotes active cholesterol removal from cells. Once bound to the cell membrane, 2-HPβCD increases turnover of intracellular cholesterol pools, leading to increased intracellular cholesterol metabolism and active cholesterol efflux triggered by upregulation of cholesterol efflux transporters, ABCA1 and ABCG.[10]

Role of 2-Hydroxypropyl-β-Cyclodextrin as a Potential Treatment Option for Kidney Disease

There is increasing evidence from an *in vitro* podocyte study and data from animal models representing three different kidney diseases that 2-HPβCD promotes cholesterol removal from podocytes, protecting the kidney's filtration system from damage and reducing proteinuria (protein spillage into the urine). These types of outcomes are key to delaying or preventing progression of kidney disease.

METHYL-β-CYCLODEXTRIN (MβCD) PROTECTED AGAINST LIPID ACCUMULATION AND DAMAGE TO HUMAN PODOCYTES EXPOSED TO SERUM FROM PATIENTS WITH TYPE 1 DIABETIC KIDNEY DISEASE

To determine if MβCD can effectively sequester intracellular cholesterol and protect podocytes from cholesterol-dependent damage in DKD, Mercher-Gomez[7] evaluated immortalized normal human podocytes, which were serum and insulin starved and pretreated for 1 hour with 5 mmol/L of MβCD prior to exposure to 4% patient sera for 24 hours from the following groups:

- 10 healthy controls (C)
- 10 patients with type 1 diabetes with no proteinuria, normal albuminuria and creatinine (DKD-)
- 10 patients with type 1 diabetes with proteinuria, albuminuria and increased creatinine (DKD+)

RESULTS

MβCD prevented elevations in podocyte total cholesterol and esterified cholesterol in the DKD+ group compared with the untreated DKD+ group (Figures 23 and 24). This was associated with prevention of podocyte damage (actin cytoskeleton remodeling and cell blebbing) seen in the untreated DKD+ group (Figure 25).

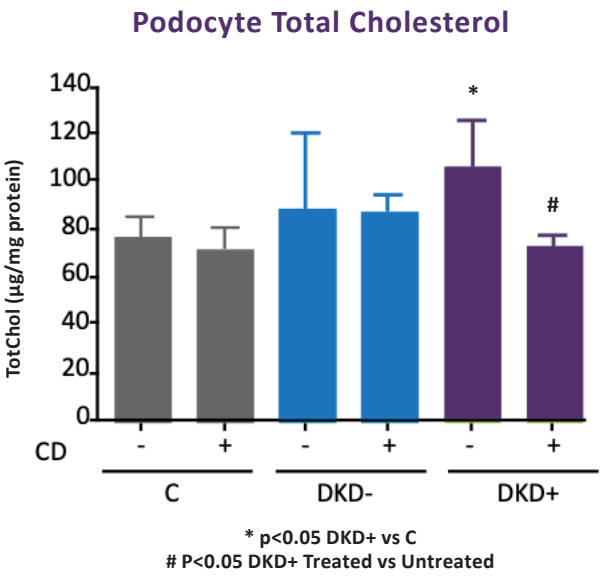


Figure 23.

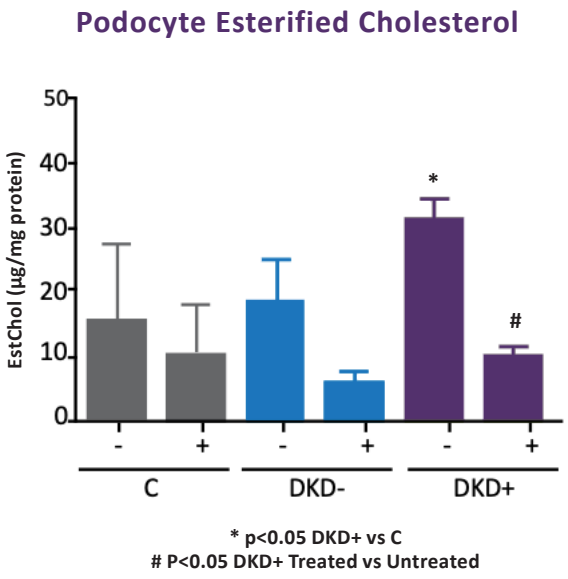
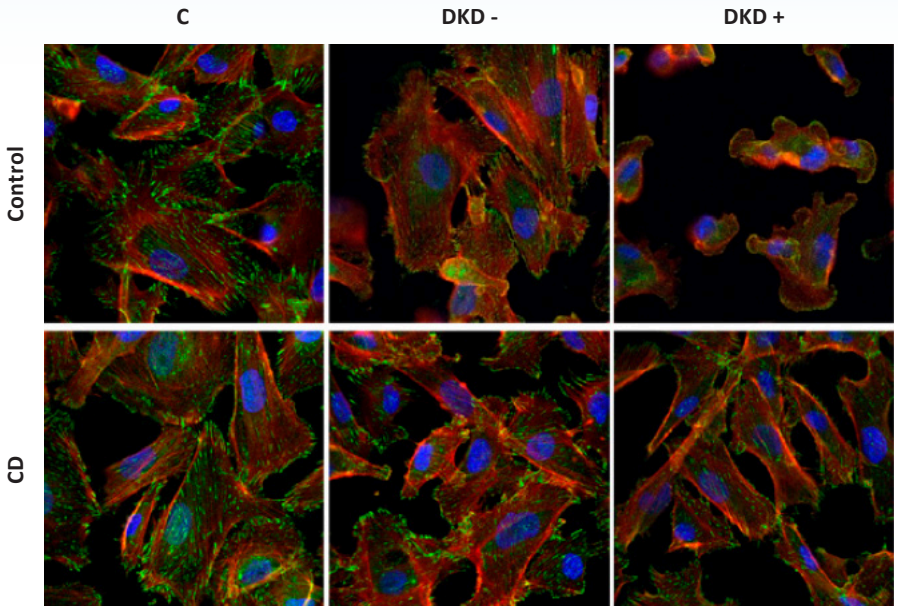


Figure 24.

Podocyte Damage Associated With Elevated Cholesterol



Filipin Staining (Orange); Phosphorylated Caveolin Staining (Green)

Figure 25.

2-HPβCD REDUCED INTRACELLULAR RENAL CHOLESTEROL, PROTECTED AGAINST RENAL INJURY, AND RESTORED FUNCTION IN A DIABETIC MOUSE MODEL

To determine if 2-HPβCD can effectively sequester intracellular cholesterol and protect podocytes from cholesterol-dependent damage in DKD, Mercher-Gomez[7] treated 4-week old BTBR ob/ob homozygous (homo) mice, a diabetic model of progressive kidney disease, with three weekly subcutaneous injections of 2-HPβCD at 4,000 mg/kg for 5 months. Heterozygous (Het) mice served as controls.

RESULTS

2-HPβCD significantly reduced total cholesterol in the kidney cortex compared with untreated diabetic mice (Figure 26). This was associated with a significant reduction in renal damage (mesangial expansion), Figure 27.

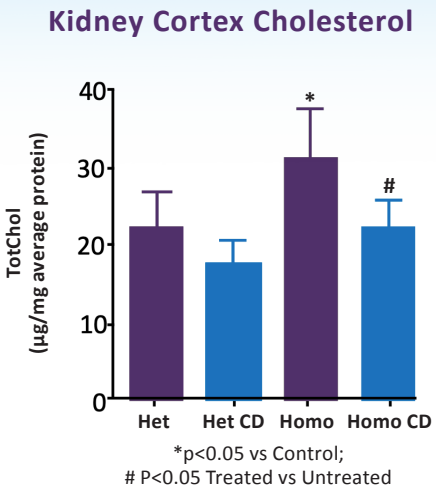


Figure 26.

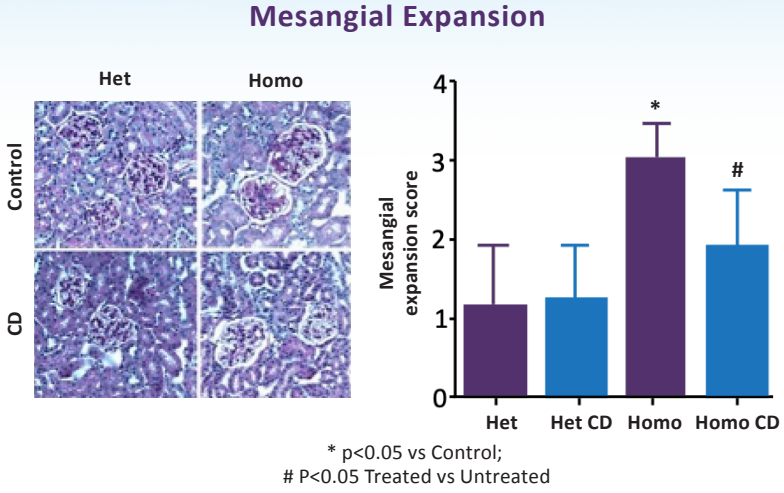


Figure 27.

2-HPβCD - treated diabetic mice demonstrated reduced proteinuria (albumin/creatinine) compared to untreated diabetic mice starting at two months following treatment, with statistically significant reduced levels from three months to end of study (Figure 28).

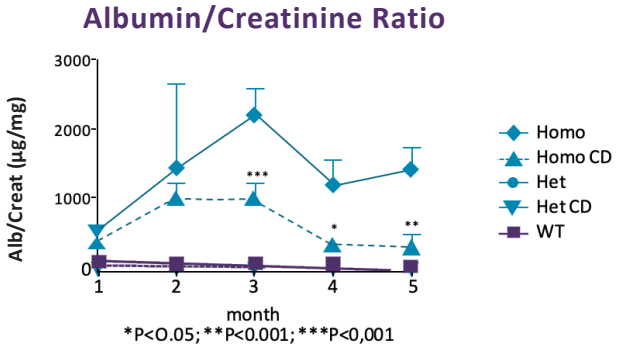


Figure 28.

2-HPβCD REDUCED INTRACELLULAR RENAL CHOLESTEROL, PROTECTED AGAINST RENAL INJURY, AND RESTORED FUNCTION IN FSGS MOUSE MODELS

To evaluate whether 2-HPβCD has a protective effect in non-metabolic glomerular diseases similar to that demonstrated in diabetic animal models, studies were conducted in two FSGS mouse models, an experimental NFAT FSGS model and a more established Adriamycin-induced FSGS model, which is characterized by a milder, less progressive form of nephropathy than the NFAT model.[11]

NFAT MODEL

To examine the role of altered podocyte cholesterol homeostasis in NFAT-mediated podocyte injury and the effects of treatment with 2-HPβCD, Pedigo[4] administered 2-HPβCD subcutaneously at 4,000 mg/kg to 6-week-old Nfatc1^{nuc} mice 24 hours prior to induction with doxycycline, and then every other day for 4 days. Single transgenic mice served as a control (ST).

RESULTS

2-HPβCD significantly reduced cholesterol in the renal cortex of mice with doxycycline-induced FSGS (DT) compared to untreated DT mice (Figure 29). This was associated with a significant reduction in proteinuria (albumin/creatine ratio) as shown in Figure 30.

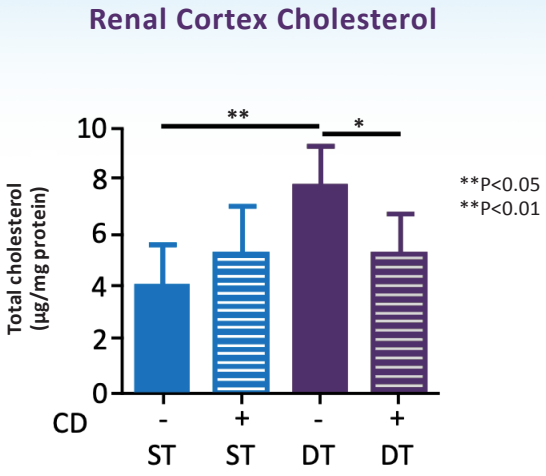


Figure 29.

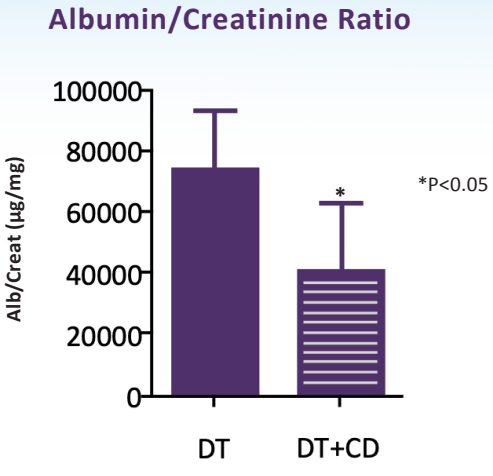


Figure 30.

ADRIAMYCIN-INDUCED MODEL

In the second FSGS model, Mitrofanova[11] injected 5-week-old BALB/c mice with one dose of Adriamycin (11 mg/kg). Twenty-four hours later, 2-HPβCD was administered at 40 mg/kg via subcutaneous osmotic pump for 10 weeks. Non-induced mice served as a control.

RESULTS

2-HPβCD significantly reduced mesangial expansion (commonly associated with lipid deposition) in ADR-induced mice compared to untreated ADR-induced mice (Figure 31).

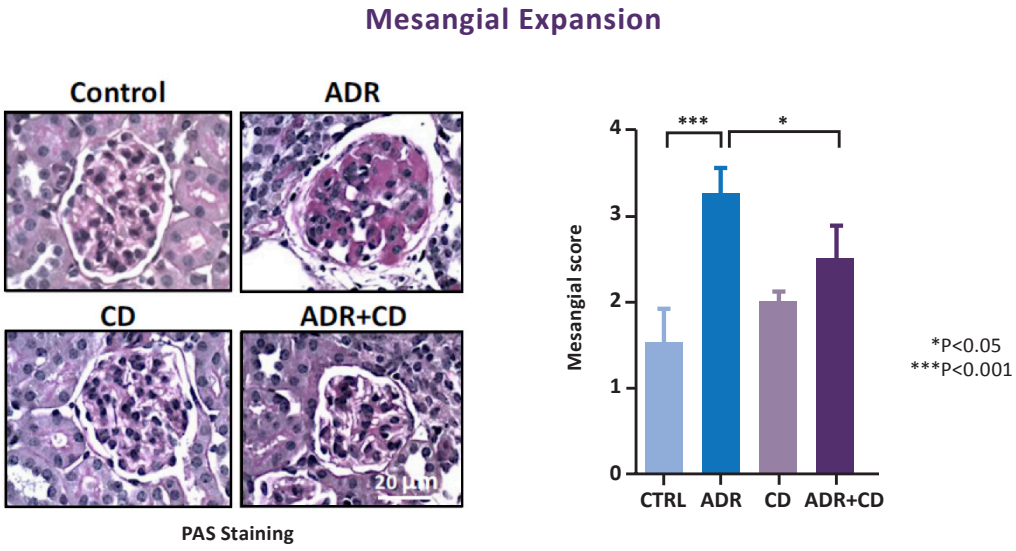


Figure 31.

This was associated with a significant reduction in proteinuria (albumin/creatine) and BUN in 2-HPβCD-treated ADR-induced mice compared to untreated ADR-induced mice (Figures 32 and 33).

Albumin/Creatinine Ratio

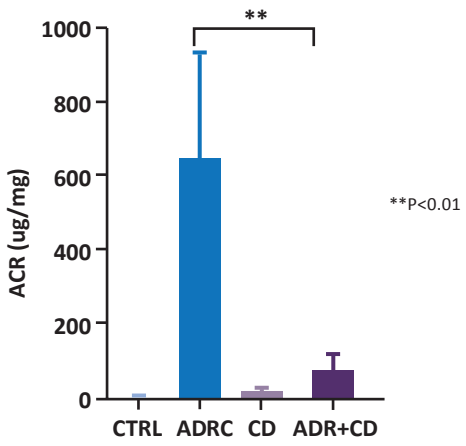


Figure 32.

Blood Urea Nitrogen

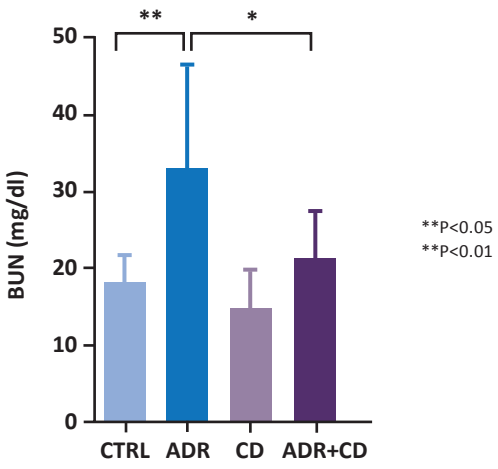


Figure 33.

2-HPβCD REDUCED INTRACELLULAR RENAL CHOLESTEROL, PROTECTED AGAINST RENAL INJURY, AND RESTORED FUNCTION IN AN ALPORT SYNDROME MOUSE MODEL

To evaluate whether 2-HPβCD has a protective effect in a second type of non-metabolic glomerular disease, Alport Syndrome, Mitrofanova[11] injected four-week-old female Col4a3 knockout (Col4a3−/−) mice with 2-HPβCD at 4000 mg/kg subcutaneously 3-times per week for 4 weeks. Wild type Col4a3 (Col43+/+) served as controls.

RESULTS

2-HPβCD significantly reduced renal neutral lipid and cholesterol ester accumulation in Alport Syndrome mice when compared to untreated Alport Syndrome mice (Figure 34).

Renal Neutral Lipids and Cholesterol

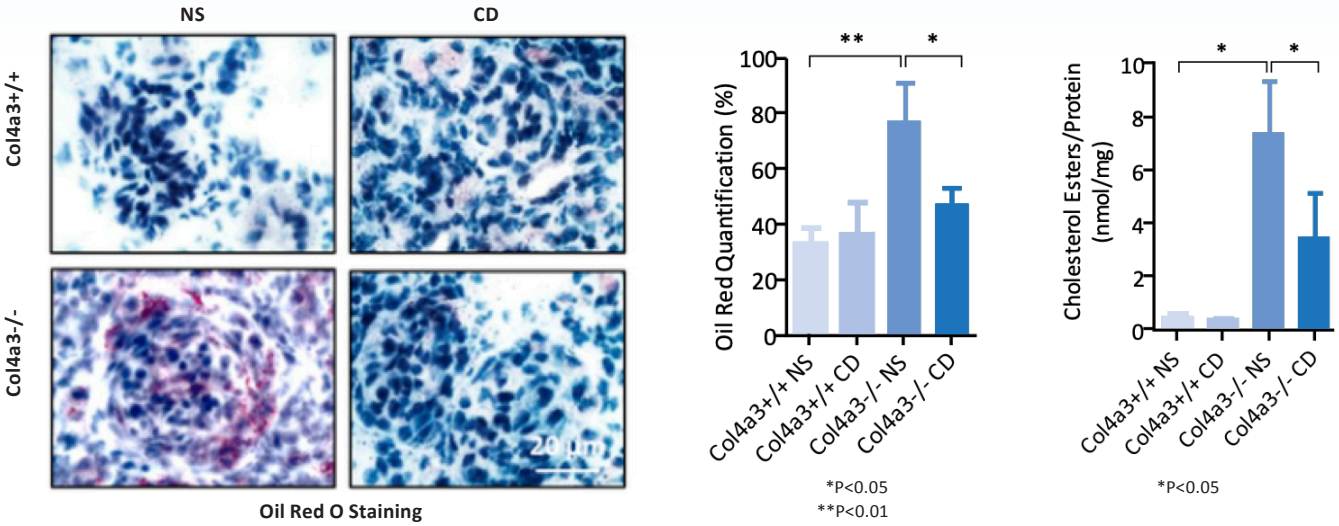


Figure 34.

2-HPβCD administered to Alport Syndrome mice prevented renal cholesterol crystal accumulation seen in untreated Alport Syndrome mice (Figure 35).

Renal Cholesterol Crystals

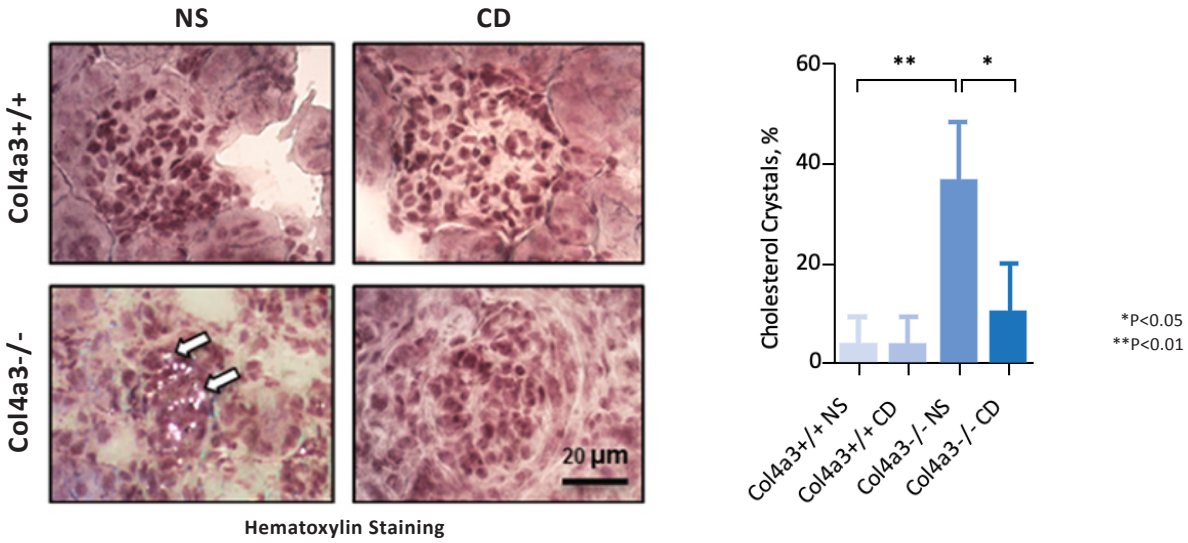


Figure 35.

Decreased intracellular lipids in 2-HPβCD-treated Alport Syndrome mice were associated with a significant reduction in renal damage when compared to untreated Alport Syndrome mice. This included a reduction in mesangial expansion, fibrosis, and foot process effacement (Figures 36 - 38).

Mesangial Expansion

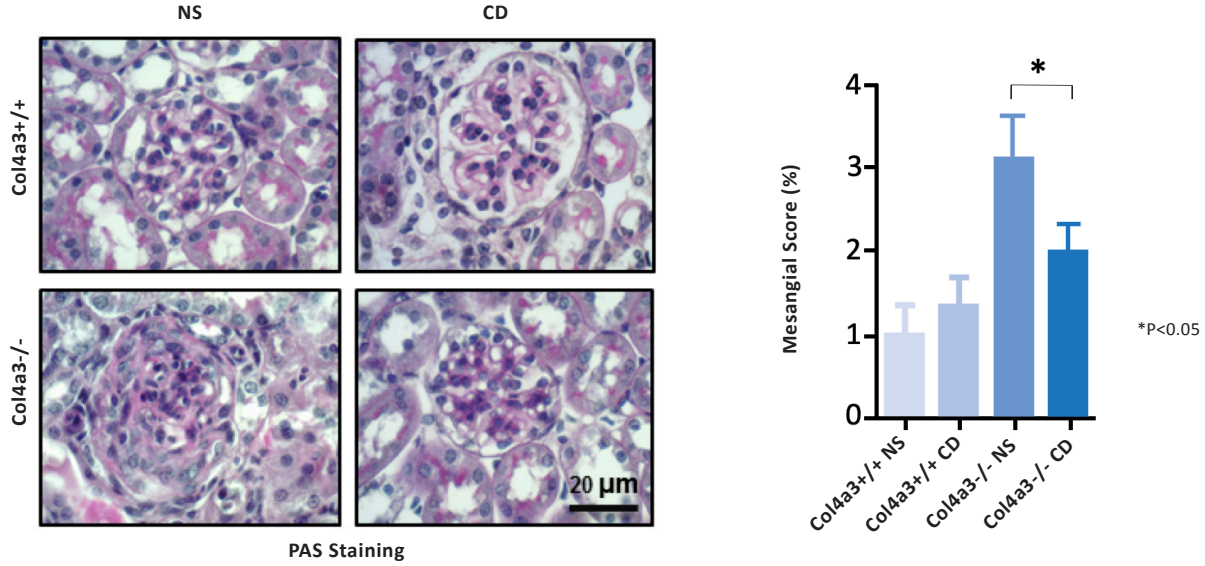


Figure 36.

Fibrosis

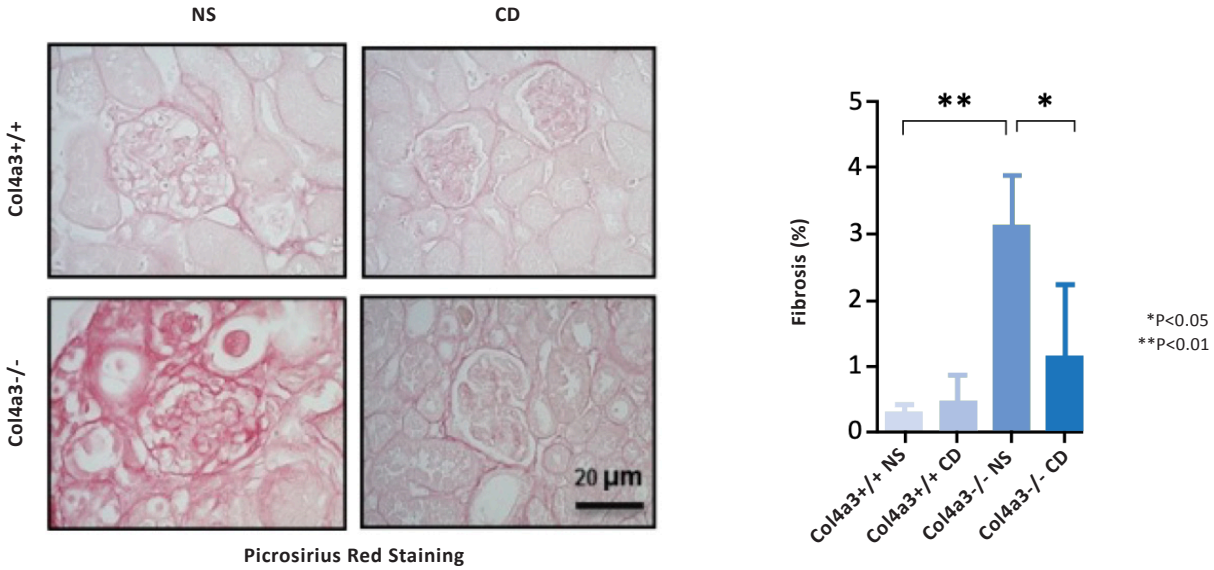


Figure 37.

Foot Process Structure

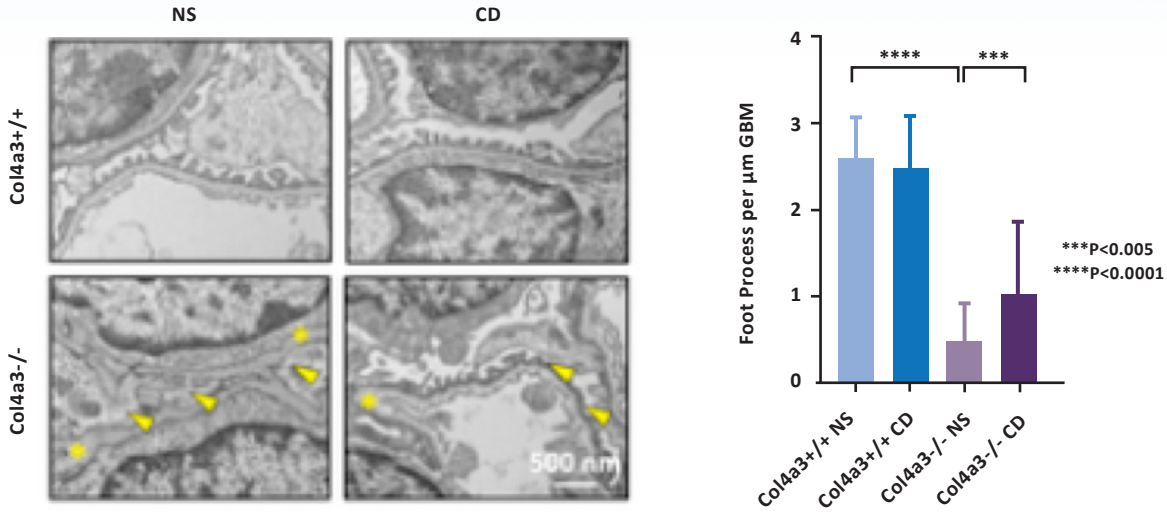


Figure 38.

Renal function was maintained in Alport Syndrome mice treated with 2-HPβCD, as evidenced by reduced proteinuria (albumin/creatinine), BUN, and serum creatinine when compared to untreated Alport Syndrome mice.

Albumin/Creatinine Ratio

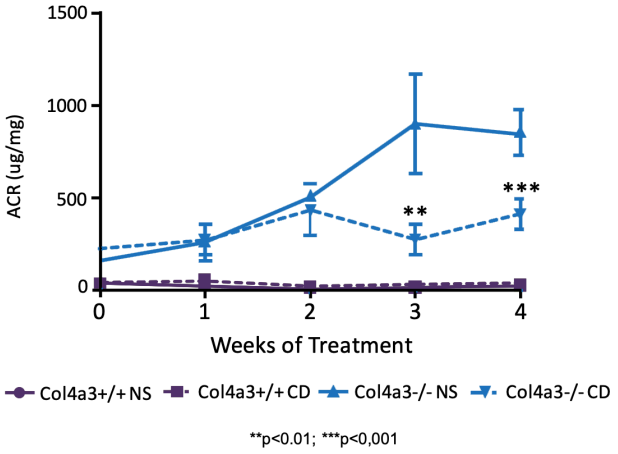


Figure 39.

Blood Urea Nitrogen

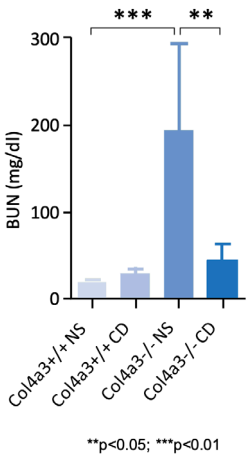


Figure 40.

Serum Creatinine

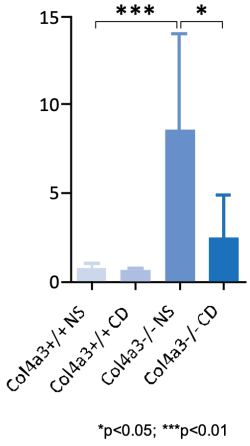


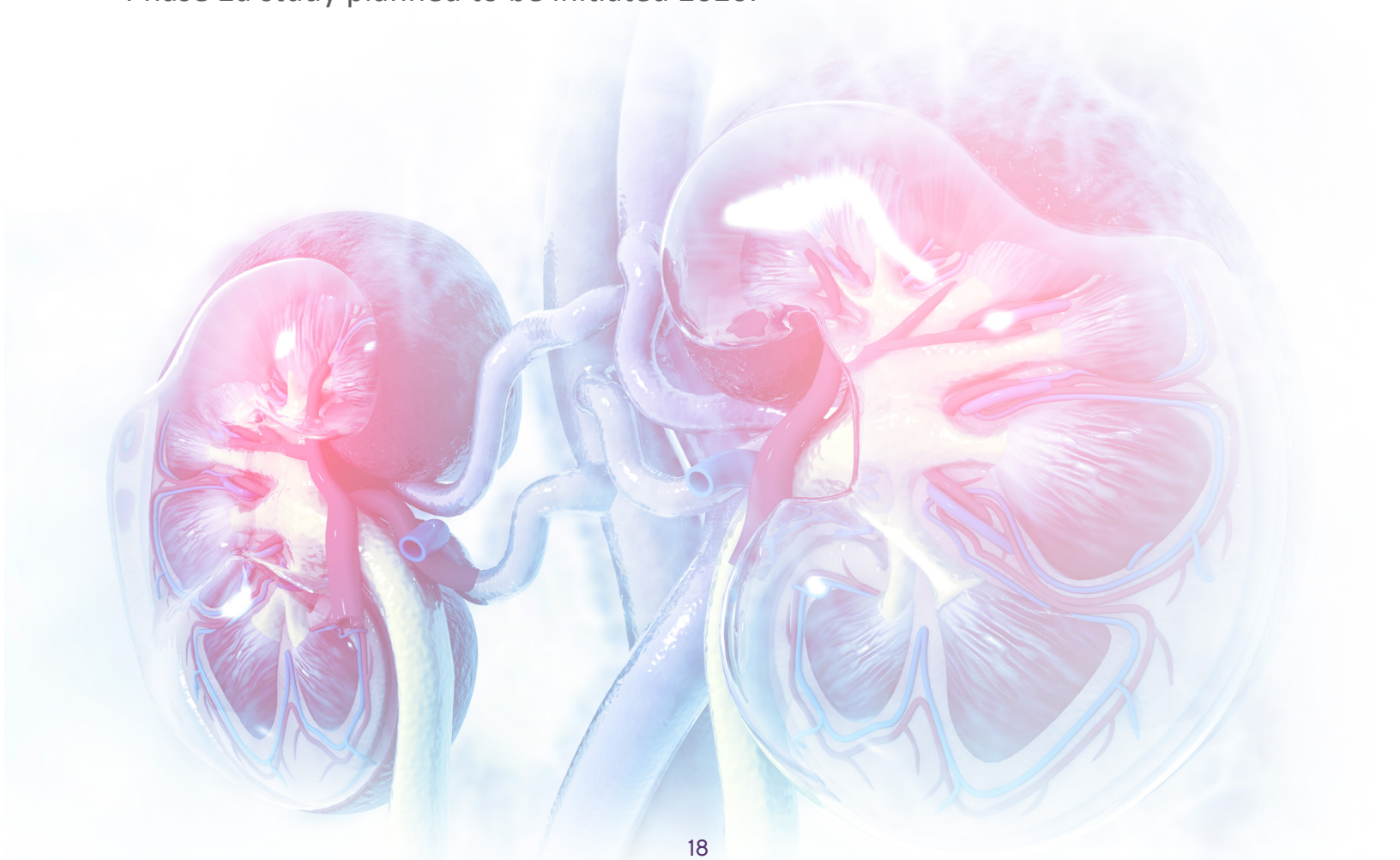
Figure 41.

Conclusion

There is significant data, as summarized in this White Paper, to support that glomerular/podocyte lipid homeostasis is dysregulated in certain kidney diseases, resulting in lipid accumulation leading to glomerular damage and kidney dysfunction.

The pathogenic nature of lipid accumulation in kidney disease is further supported by data demonstrating that reducing intracellular renal lipids with 2-HPβCD, prevents glomerular damage and kidney dysfunction, suggesting that 2-HPβCD has potential to become a disease-modifying treatment option. Results have been consistent across *in vitro* and *in vivo* studies in animal models representing three different types of kidney disease (diabetic nephropathy, FSGS, and Alport Syndrome).

ZyVersa is looking forward to obtaining data in patients with FSGS from a Phase 2a study planned to be initiated 2020.



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2200 N. Commerce Parkway, Suite 208
Weston, FL 33326

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