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Re: Final Report for Cost-Containment Project

Dear Panel Members,

I appreciate the opportunity to receive funding in the past year for a cost-containment proposal! The support from the Missouri Kidney Program has enabled our lab to generate a tremendous amount of impactful data and we have been very productive with the funding.

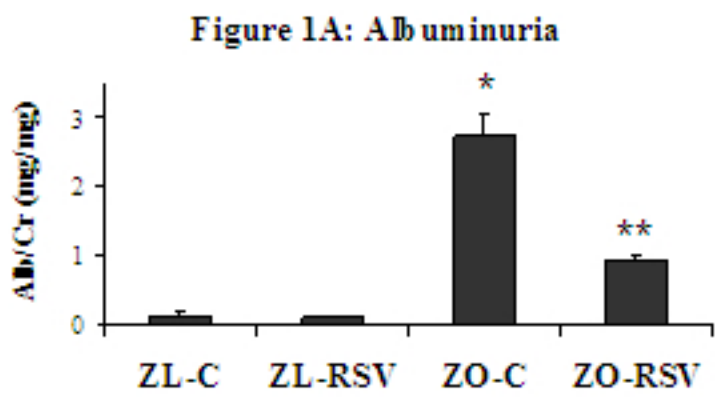
Our investigation centered on the role of HMG-CoA reductase inhibitors (statins) in reduction of oxidative stress and inflammation as it relates to diabetic glomerulosclerosis. In response to the initial critiques regarding the ability to translate our project to cost-containment, part of my project also included work with the NKF-Kidney Early Evaluation Program. In the response to reviewers I had outline that I would generate data from the KEEP for a future MoKP proposal in regards to the KEEP as part of the current funding cycle. Both aspects of the project as outlined have been successful to date.

Since there was a lapse in time from proposal to actual time of funding (e.g. 2 years), I had to resubmit my animal safety protocols. To adapt, I received approval from the MoKP to utilize other animal models of diabetes for this investigation that I already had approved by our institution. Thereby we utilized the Zucker obese (ZO) and the transgenic Ren2 rats for the proposal.

This investigation addressed the impact of statins on the relationship between insulin resistance and hyperinsulinemia, oxidative stress, and functional and structural changes of the glomerular filtration barrier. Filtration barrier integrity was evaluated in an animal model of insulin resistance, the ZO rat (Table 1), and results from our study demonstrate enhanced renal tissue oxidative stress as a causative factor in albuminuria and filtration barrier injury (Fig 2 and 3). In the ZO rat, obesity, insulin resistance and the compensatory hyperinsulinemia develop at an early age consistent with the pre-diabetic state as seen in those with the cardiometabolic syndrome. Thus, the age of ZO rats at initiation of statin treatment likely reflects an early stage of insulin resistance/hyperinsulinemia. The metabolic abnormalities (i.e. insulin resistance/hyperinsulinemia) at 6 weeks parallel proteinuria and early glomerular injury from human studies. However, to our knowledge our work is the first to uncover statin intervention to investigate the relationship of insulin resistance and hyperinsulinemia to oxidative stress-mediated filtration barrier injury. The results of the present work suggests for the first time, a role for hyperinsulinemia and oxidative stress in the pathogenesis of podocyte/filtration barrier injury and albuminuria.

Experimental Parameters				
	ZL-C	ZL-RSV	ZO-C	ZO-RSV
Systolic Blood Pressure (mm Hg)				
Initial	138 ± 7.9	150.7 ± 3.7	141.8 ± 4.3	134 ± 3.4
Final	144.3 ± 10.4	141.3 ± 7.9	151.6 ± 7.9	148.7 ± 12.1
Total Body Weight (gm)				
Initial	130.3 ± 2.7	131.5 ± 4.1	162.8 ± 8.7 *	167.83 ± 9.3 *
Final	231.8 ± 14.0	239.5 ± 6.9	351.80 ± 18.7 *	360.3 ± 22.7 *
Serum Glucose (mg/dl)	152.3 ± 22.0	142.6 ± 4.6	240.9 ± 17.08*	278.4 ± 7.2*
Serum Insulin (pmol/L)	1.9 ± 0.6	1.4 ± 0.5	8.4 ± 1.6*	6.1 ± 0.8*
HOMA-IR index	0.12 ± 0.05	0.45 ± 0.17	0.73 ± 0.16*	0.61 ± 0.07
Beta-N-acetylglucosaminidase				
(beta-NAG) (mgmgof Cr)	0.157 ± 0.01	0.178 ± 0.03	0.016 ± 0.01 *	0.022 ± 0.01 *

(HOMA-IR), Homeostasis Model Assessment-Insulin Resistance index. *, $p<0.05$ when compared to Zucker Lean control ZL-C



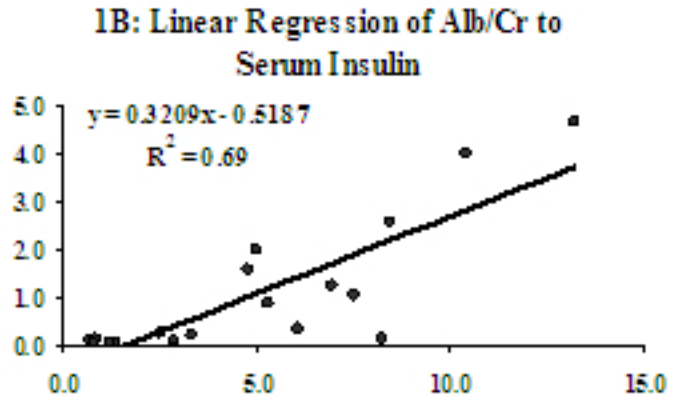


Figure 1: A) Depicts albuminuria measures in Zucker rats. **B)** Depicts linear regression analysis of albuminuria to serum insulin. *, $p<0.05$ when Zucker Obese controls (ZO-C) is compared to Zucker Lean controls (ZL-C), **, $p<0.05$ when rosuvastatin treated ZO rats (ZO-RSV) are compared to ZO-C/.

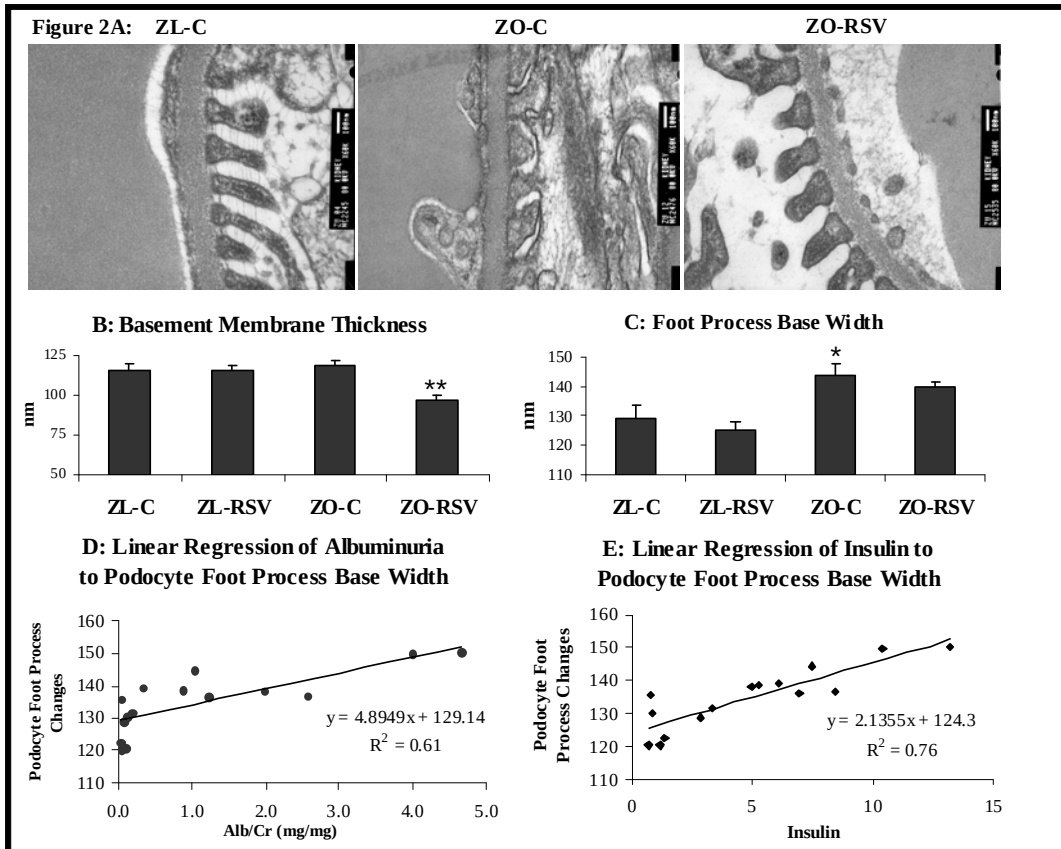


Figure 2: Glomerular Filtration Barrier. A) Depicts Transmission Electron Miscroscopy images of the gomerular filtration barrier. **B)** Basement membrane measures and **C)** Podocyte foot-process base width. **D)** Depicts linear regression of albuminuria to podocyte foot-process base width **E)** Depicts linear regression analysis of insulin to podocyte foot process base width. *, p<0.05 when Zucker Obese controls (ZO-C) is compared to Zucker Lean controls (ZL-C); **, p<0.05 when rosuvastatin treated ZO rats (ZO-RSV) are compared to ZO-C.

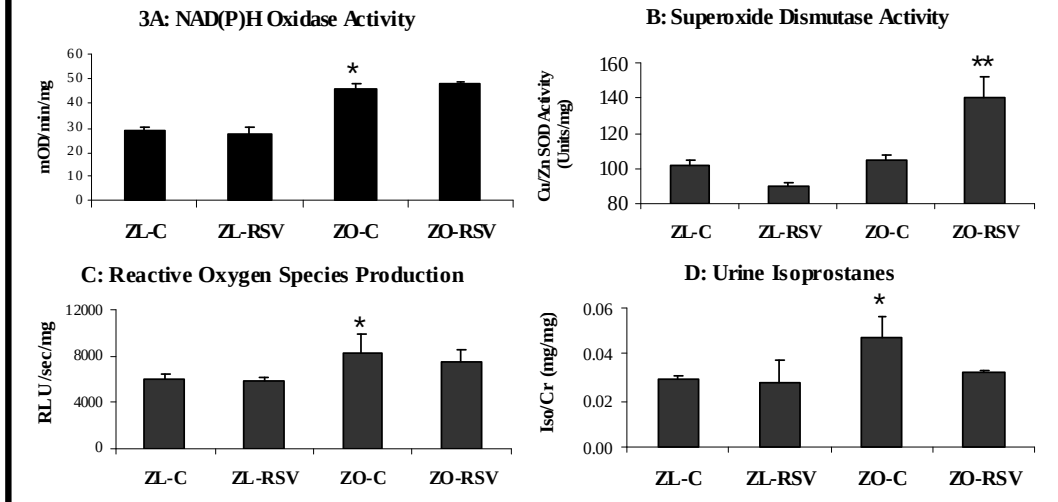


Figure 3: Oxidative Stress Measures. A) NADPH oxidase activity. **B)** Copper/Zinc Superoxide Dismutase activity. **C)** Reactive oxygen species formation. **D)** Urine isoprostane measurement in the urine. *, p<0.05 when Zucker Obese controls (ZO-C) is compared to Zucker Lean controls (ZL-C); **, p<0.05 when rosuvastatin treated ZO rats (ZO-RSV) are compared to ZO-C.

When we utilized the Ren2 model, we unraveled the hierarchal importance that statins have on reductions in oxidative stress for improving proteinuria (e.g. albuminuria). Reductions in systolic blood pressure are a prominent confounder in understanding the role of various interventions on proteinuria. Fig 4 depicts that statins reduce proteinuria without significantly reducing systolic blood pressure.

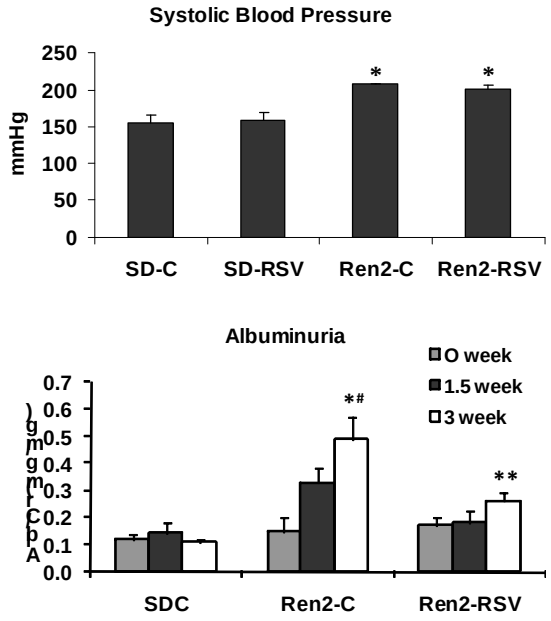


Figure 4: Systolic Blood Pressure and Albuminuria in Transgenic Ren2 rats. (A) SBP in Ren2 rats at the end of treatment period. (B) Albuminuria in the Ren2 rat as measured at the beginning of treatment (6 weeks of age or 0 week of treatment), middle of treatment period (7-8 weeks of age or 1.5 weeks of treatment), and end of treatment period (9 weeks of age or 3 weeks of treatment). *, $p < 0.05$ when compared to age-matched Sprague-Dawley controls (SD-C) (n=6); #, $p < 0.05$ when Ren2-C (n=6) 3 week is compared to 0 week; **, $p < 0.05$ when rosuvastatin treated Ren2 rats (Ren2-RSV) (n=4) are compared to age-matched SD-C.

Similar to the Zucker rat, we observed changes in the glomerular filtration barrier as the etiology of the proteiuria and that statins improved proteinuria via improvements in loss of podocytes (Fig 5). Collectively, data from the current investigation indicate statin therapy has reno-protective effects as demonstrated by reductions in albuminuria, oxidative stress, and improvements in filtration barrier indices; basement membrane thickening and podocyte foot process effacement. Findings from this study support that statins decrease NADPH oxidase-related ROS species generation through inhibition of cytosolic small molecular weight G proteins such as Rac and the membrane subunits of NADPH oxidase

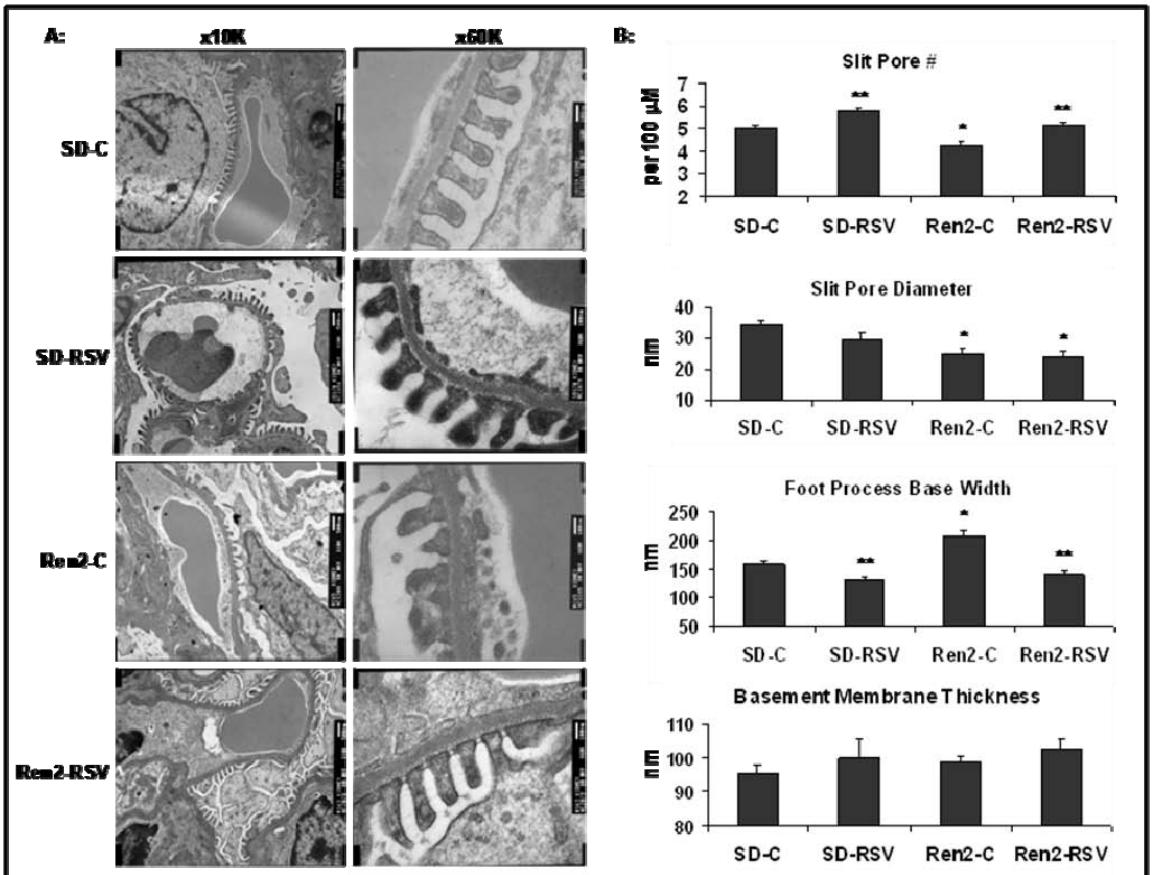


Figure 5: Statin improves indices Podocyte Foot Process Effacement on Transmission Electron Microscopy (TEM). (A) Representative TEM images at x10K (left panel) and x60K (right panel). (B) Indices for glomerular filtration barrier integrity. *, $p < 0.05$ when Ren2 controls (Ren2-C) (n=6) are compared to age-matched Sprague-Dawley controls (SD-C) (n=6); **, $p < 0.05$ when statin treated Ren2 rats (Ren2-RSV) (n=4) or SD (SD-RSV) (n=4) are compared to age-matched controls.

We have also generated data within the NKF-KEEP program to support a proposal for the next round of funding for the Cost Containment Program. In summary, all manuscripts directly and indirectly attributable to MoKP funding, now and in the future, will be attributed and forwarded to the appropriate personnel.

Once again, we appreciate the opportunity to work with you and look forward to continued success in the future!

Sincerely,

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