# Diabetic Complications Consortium

**Application Title:** Chronic aldosterone induction of renal fibrosis in Drosophila

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## 1. Project Accomplishments:

Hyperaldosteronism contributes to diabetic kidney fibrosis. We developed a new *Drosophila melanogaster* model of kidney fibrosis where human aldosterone produces proteinuria, nephrocyte (glomerular) filtration defects and accumulation of renal extra-cellular matrix. We demonstrated that ECM accumulation upon fly nephrocytes causes the functional defects in proteinuria and filtration. We localized the source of the injurious ECM: muscles cells of the heart adjacent to the nephrocytes produce and secrete excess collagen (and this as well impairs heart function). This muscle cell secretion parallels the production of ECM by myofibroblasts in mammalian fibrotic disease associated with diabetes and excess aldosterone. Aldosterone mimics the fly's endogenous steroid hormone ecdysone. We demonstrate that aldosterone and ecdysone require dopEcR, a G-protein coupled receptor (GPCR), to induce fibrosis. This finding contrasts to expectations where aldosterone contributes to fibrosis *only* through a cognate nuclear hormone receptor (in the fly: EcR; in mammals: MR). These unexpected observations provide insights for novel regulatory control of human renal fibrosis. We propose dopEcR alternatively represses fibrosis through cAMP/Crtc/CREB signaling while licensing fibrotic signaling of EGFR/ERK/TGF-beta/Smad. We identify candidate mammalian homologs of DopEcR. We now propose using Drosophila to direct research on aldosterone receptive GPCRs in humans and to dissect new elements of signaling for how hyperaldosteronism modulates renal fibrosis in diabetes.

## 2. Specific Aims:

**Aim 1. Characterize renal pathology induced by chronic aldosterone in Drosophila.**
Examine renal
functional pathology by measuring how chronic aldosterone impairs Malpighian tube excretion and nephrocyte filtration.

**Results:** We treated adult Drosophila with aldosterone for two weeks, and for the same duration with endogenously Drosophila steroids (ecdysone (E) and 20 hydroxyecdysone) for the same duration. For each treatment we assessed (and developed the assay) proteinuria, nephrocyte filtration, and ECM accumulation of collagen. For continuous treatment with the steroids we assessed survival upon high salt diet, and upon standard diet.

High salt or high sugar diet does not induce proteinuria in Drosophila (Fig 1A). Proteinuria is induced by chronic treatment with E or aldosterone (Fig 1B). We suspected proteinuria could arise from defects at Malpighian tubules or from nephrocytes. We show nephrocyte pathology is sufficient to cause proteinuria by depleting their slit diaphragm protein gene expression (Fig 1C). Therefore, we focused on nephrocytes for the remainder of our study. It is possible to measure nephrocyte filtration efficiency in an ex vivo assay where fluorescent dextran beads are added to

the tissue culture media. Healthy nephrocytes filter and accumulate these beads, while depleting slit diaphragm proteins limits this filtration. We reproduced these results (Fig 1E) and then tested the impact of aldosterone, E and 20HE upon filtration. Aldosterone and E strongly reduced filtration, indicating chronic exposure to these hormones impairs nephrocyte function (Fig 1D, F). Survival upon a high salt diet (1.5%) is reduced in control flies (by 50%) and even more so in flies treated with aldosterone, E or 20E (Fig 1G). The mechanism for how chronic steroids reduce stress-associate survival is unknown, but this result does not parallel trends for renal pathology (because 20HE and control are affected). The tested hormones do not affect survival when adults are fed a normal diet (Fig 1H).

**Aim 2. Determine if renal pathology induced by chronic aldosterone requires collagen/pericardin and dopEcR.** Use tissue-directed RNAi against pericardin and dopEcR to determine if these genes are required for aldosterone to induce renal pathology. Identify which tissue produces renal collagen/pericardin that accumulates

**Results:** E and aldosterone quickly induce (overnight) mRNA for the collagen-like protein pericardin (fig 2A; this figure element is in production although the data are complete) but not col4a1 (Fig 2B).

Chronic (2 w) exposure to E or aldosterone induces accumulation of Pericardin protein as extracellular matrix around nephrocytes and the fly heart (cardia). We identified the source of Pericardin in the ECM (Fig 2C). Depletion of pericardin mRNA in nephrocytes does not prevent accumulation of Pericardin in ECM. Likewise, reducing pericardin gene expression with nephrocyte specific drivers did not prevent steroid induced proteinuria or filtration defects (Fig 2 F, G, I, J).

Importantly, steroid induced ECM development is inhibited by depletion of pericardin mRNA from heart muscle cells (which are adjacent to the nephrocytes). And blocking pericardin expression from heart muscle cells is sufficient to prevent the steroid induced renal pathology (Fig 2H, K)

Heart muscle cells are the source of pathogenic ECM affecting nephrocyte/renal function. This outcome is important because renal ECM in humans arises from myofibroblasts, and we can now consider the fly myocardiocytes to produce a similar function.

Drosophila steroid signaling is induced by cytoplasmic 20E that interacts with a classic nuclear hormone receptor, EcR. A resulting complex of proteins locates to the nucleus to affect transcription. Ecdysone is converted to 20HE within the target cells. Ecdysone on its own is thought to not directly affect cell function, until recently when a cell membrane associated GPCR was identified to interact with both Ecdysone and dopamine, so called dopEcR.

We proposed that fibrosis might be mediated by dopEcR in response to both aldosterone and E since 20HE itself does not produce fly renal pathology or ECM accumulation. We now have sufficient data to confirm this hypothesis, but cannot yet show this as a figure because we are still assembling all the replicates and are building the images. I can provide a brief description: using RNAi for dopEcR we demonstrate the same outcomes (from pericardin) of Fig 2 C-K. We

show that dopEcR in myocardiocytes is sufficient and necessary for aldosterone and E to induce fibrosis. We conclude these hormones are acting via dopEcR in the heart muscle cells to induce pericardin mRNA and secretion into the ECM of the nephrocyte/heart system. This ECM accumulation produces renal pathology.

We have established a model of renal fibrosis in Drosophila and identified a highly translatable signaling pathway. We can suggest several potential human homologs of dopEcR. We can now propose specific, testable genomic and non-genomic signaling mechanisms of aldosterone through these GPCR, and how they will contribute to renal fibrosis during diabetes-associated hyperaldosteronism**.**

**3. Publications:**

I will submit to *Disease Models and Mechanisms* in 2018.

I will attend the Keystone meeting *Uncomplicating Diabetes* to present the project.

I am working to continue this research; I applied to ADA for Innovative Core Program in 2017 (triaged, will resubmit in 2018, but very skeptical given the current set of reviews).