Diabetic Complications Consortium

Application Title: Type II diabetes: phenotype of the diabetic bladder from proteins to organ

Principal Investigator: Robert S. Moreland

1. Project Accomplishments:

During the past approximately one year we have validated the high fat diet/low dose streptozotocin model of Type II diabetes. This model exhibits high blood glucose, increased body weight, bladder hypertrophy, and diabetic bladder dysfunction. Moreover, we have shown that at 3 months post HFD/STZ the bladder exhibits an increased contractility consistent with the compensated state of diabetic bladder dysfunction and by 6 months post HFD/STZ exhibits the decompensated state that leads to a failing bladder. We will continue these studies to increase our experimental values, perform electrical field stimulation, and quantify additional phosphoprotein signaling levels.

2. Specific Aims:

Specific Aim 1: To validate the model and develop the optimal time course for the study of pre-diabetic, compensated, and decompensated states of DBD in the HFD/STZ rat.

We used sprague-dawley rats to induce a high fat diet (HFD)/low dose streptozotocin (STZ) dependent Type II diabetes (T2D). The first aim was to validate that T2D was produced and that the T2D rats exhibited diabetic bladder dysfunction (DBD). Figure 1 shows that elevation in blood glucose following the HFD/STZ injections. Figure 2 shows the significant increase in body weight in the HFD/STZ animal model.

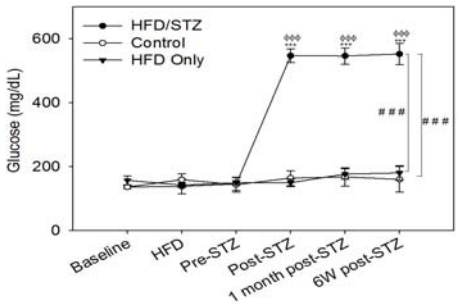


FIGURE 1

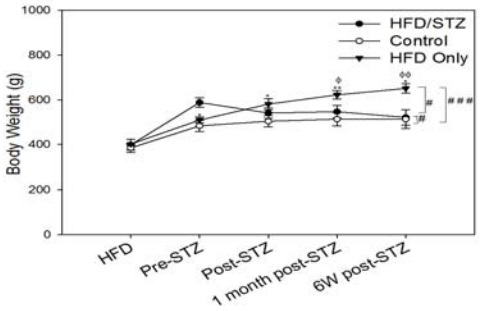


FIGURE 2

In terms DBD, Figure3 shows a significant increase in the volume per void in the HFD/STZ diabetic animals. There was no significant change in the number of voids per unit time.

FIGURE 3

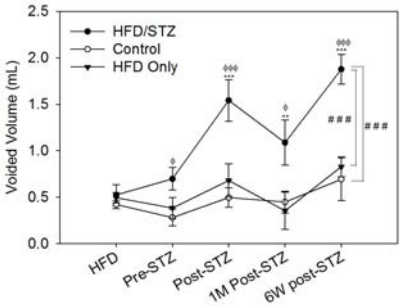


FIGURE 4

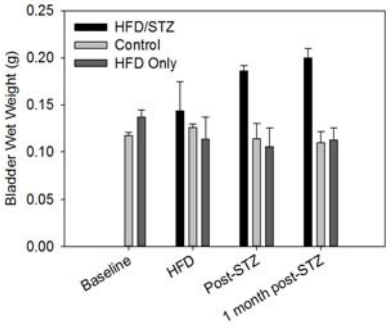


Figure 4 shows the increase in bladder weight following HFD/STZ. We have now carried these studies out to 6 months post-STZ injection. Our results are consistent with the diabetic bladders being in what is termed the compensated state approximately 3 months post HFD/STZ due to bladder hypertrophy and increased contractility (see below) and a decompensated state approximately 6 months post HFD/STZ.

Specific Aim 2: To phenotype intact strips of bladder smooth muscle from control, pre-diabetic, compensated, and decompensated states of DBD in terms of their response to pharmacological agents and electrical field stimulation (EFS).

Three months after the induction of HFD/STZ T2D, contractility to carbachol, KCl, and ATP were significantly increased. The increase in contractility to ATP was the greatest and is shown in Figure 5. This is consistent with the compensated state of DBD. Six months after the induction of HFD/STZ T2D, contractility to ATP was similar to that of controls, consistent with a decompensated state of bladder dysfunction (Figure 6). Responses to carbachol and KCl also returned to control values at the six month time point.

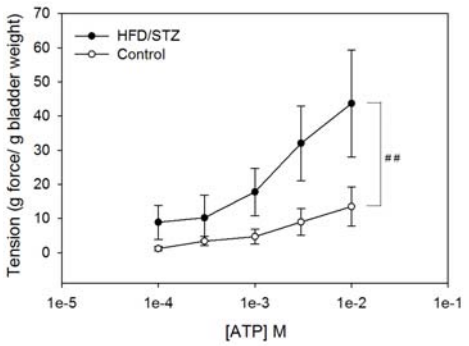


FIGURE 5

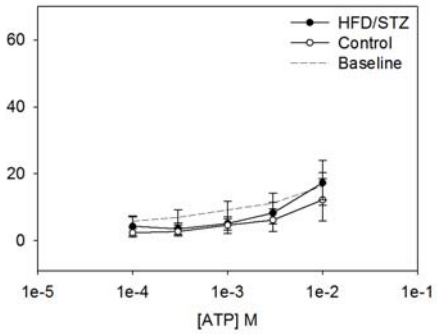


FIGURE 6.

Specific Aim 3: To phenotype skinned strips of bladder smooth muscle from control, pre-diabetic, compensated, and decompensated states of DBD in terms of their Ca^{2+}/force and Ca^{2+}/MLC phosphorylation relationships.

We used Triton X-100 detergent skinned fibers to determine the calcium/force and calcium/myosin light chain (MLC) phosphorylation relationships in bladders from HFD/STZ T2D animals. Figure 7 shows the calcium/force relationship in animals one month post HFD-STZ and Figure 8 shows the calcium/MLC phosphorylation relationship in the same animals.

FIGURE 7

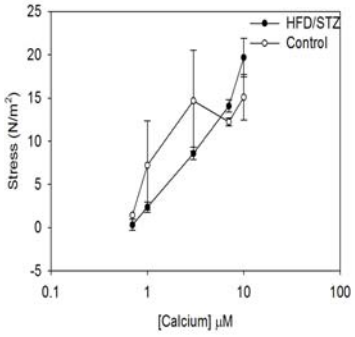
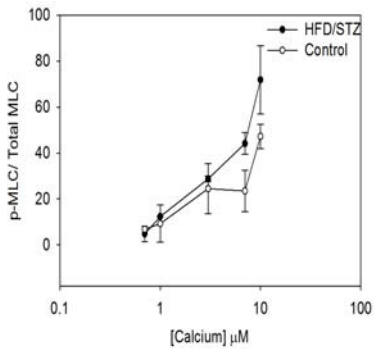


FIGURE 8



Force trended downward (Figure 7) while MLC phosphorylation level trended upwards in the one month T2D animals. These are low "n" values (n=3) and need to be increased. We are currently performing these experiments.

3. Publications:

Klee N, Moreland RS. Cellular and molecular characterization of type II diabetic bladder dysfunction. FASEB J. 28: 865.12, 2014.