Diabetic Complications Consortium

Application Title: Changes in energy generating pathways as a cause of diabetic bladder dysfunction.

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1. Project Accomplishments:

2.

The main accomplishment of this project has been to document the progressive changes in bladder metabolism that are associated with diabetes. Some of this work has been presented at scientific meetings, has been published or will result in several papers that are in preparation and will be published over the course of the next year.

An additionally outcome of these studies was that evidence was generated supporting the presence of "hyperglycemic memory" in diabetic bladder disease. This evidence comes from the observation that after three months of diabetes, insulin treatment for one month results in the normalization of most, but not all, metabolic pathways. This observation was the basis of an R01 application submitted to the NIH/NIDDK entitled "The Role of Metabolism in Bladder Hyperglycemic Memory"

Specific Aims:

Specific Aim 1: Determine if changes in the bladder metabolome occurs with hyperglycemia, but prior to the exhibition of bladder pathophysiology.

Our preliminary data demonstrated that after one month of Type I diabetes in the streptozotocin-(STZ)-induced male rat model there are changes in the metabolism of the bladder detrusor and urothelium. Because this is the earliest time point at which the bladder exhibits significant pathophysiology (it is physiologically compensated and over-active) we hypothesized that the changes in metabolism are a direct cause of the pathology rather than a response to pathology.

To substantiate this hypothesis we performed a similar study at a one week time point. We confirmed that one-week after STZ-treatment animals have established hyperglycemia but no significant change in bladder physiology. We isolated mucosal and detrusor layer from the one-week diabetic group and age-matched control groups for metabolomics analysis. We are presently fully analyzing the data set and anticipate submitting a paper based on the findings early in 2018. At present are major finding are as follows:

Overview: There were between 422 and 572 detected biochemicals in this dataset, with detrusor tissue giving slightly more compounds than urothelial tissue(519 versus 422). Principal Component Analysis (PCA) can be useful in providing a high-level overview of a dataset. Here, there is clustering and separation of control and diabetic samples in both rat detrusor muscle and

urothelium. This supports that even at one week, the hyperglycemic conditions lead to a distinct metabolic profile in the two groups. Analysis of the specific biochemical differences between the groups follows.

Consequences of hyperglycemic conditions in the diabetes model: At one week post-streptozotocin treatment to induce diabetes in rats, there is a significant rise in glucose levels in both tissues, as well as a decrease in 1,5-AG, both expected results from a diabetes model (Slide 7). Glycolytic intermediates (3-phosphoglycerate is shown) become elevated, consistent with an increased throughput to make use of the higher glucose levels. There is also shunting of excess glucose into the pentose associated pathways, fructose/mannose/galactose pathways, and nucleotide sugar pathways. This is possibly a mechanism of accommodating the excess glucose. The effect is stronger in the detrusor muscle than in the urothelium. Of note, the carbohydrate changes in detrusor tissue are uniformly elevated in diabetic samples, while the urothelium tissue instead shows lower levels of some pentoses, glycogen related metabolites, and amino sugars (i.e. N-acetylneuraminate). This difference might suggest that muscle tissue is better able to deal with elevated glucose levels, possibly up regulating activity of the alternate pathways while the urothelium is already beginning to show mis-regulation of carbohydrate processing pathways. These differences may also represent tissue specific changes to elevated glucose. There is also elevation of glycolysis intermediates and pentose metabolism in the high glucose condition for human urothelial cells, consistent with what is observed in the rat tissues.

TCA cycle alterations in rat tissue: Both detrusor and urothelium show elevations in TCA cycle and oxidative phosphorylation intermediates in the diabetic group. The changes are more apparent in the urothelium, with citrate, aconitate, alpha ketoglutarate, and succinate being significantly elevated. Detrusor muscle shows only trending increases (fumarate and malate), and a significant elevation in homocitrate. homocitrate can be formed when alpha-ketoglutarate and acetyl-CoA or propionyl-CoA are in excess. Increases in TCA intermediates can reflect changes to inflow or outflow, though it can be difficult to draw definitive conclusions as to carbon flow through the cycle. Here, a possible cause is elevated input from the increased glycolytic use noted above. Another possible source is from anapleurotic input of branched chain amino acids (BCAA; see below). The slight differences in TCA cycle alterations between urothelium and detrusor muscle may indicate that urothelium is using the TCA cycle to a more significant extent for energy generation. Another possibility would be that citrate is being siphoned away for lipid biosynthesis, perhaps causing a fatty bladder condition that could interfere with normal distension during filling. There is not a strong signature of elevated mono- or diacylglycerols that might suggest elevated lipid biosynthesis, however.

Elevated ketone body markers and lipid β-oxidation: In tissues, the rate of β-oxidation is regulated in part by controlling mitochondrial uptake of fatty acids by the carnitine shuttle. Initially, fatty acids are activated by long-chain acyl-CoA synthetase to form acyl-CoA. The acyl group of the fatty acid is then transferred to the hydroxyl group of carnitine to form acylcarnitine (e.g. palmitoylcarnitine, stearoylcarnitine) in the outer membrane of the mitochondria. Acylcarnitine can then enter the mitochondrial matrix via carnitine-acylcarnitine translocase and exchange carnitine for acyl-CoA on the inner membrane. Thus, carnitine shuttling removes fatty acyl-CoA from the cytosol and generates fatty acyl CoA in the mitochondrial matrix that can then be utilized for β-oxidation and ATP generation. Here, there are multiple acylcarnitines

significantly elevated in the detrusor muscle, and relatively few in the urothelium. This could suggest either the muscle tissue is utilizing lipid β-oxidation to a more significant extent, or that there is an oxidative dysfunction which is commonly seen in diabetes. Excessive β-oxidation can produce ketone bodies, such as acetoacetate and 3-hydroxybutyrate (BHBA). There is a large increase in BHBA in detrusor muscle, consistent with elevated β-oxidation. The increase in BHBA in the urothelium, rather than signal tissue specific β-oxidation, may be the result of overall higher levels in the animals due to the rapid onset of the diabetic state. Ketones are predominantly produced in the kidney and liver, and it should be noted that it can be difficult to disambiguate changes in levels in peripheral tissues from them. In general both tissues have high levels of ketones, and it is possible that the urothelium is also being exposed from high urine levels.

Branched chain amino acid markers of hyperglycemia: The branched-chain amino acids (BCAAs) valine, isoleucine, and leucine are important constituents of proteins but are also readily degraded into carbon skeletons that may enter anabolic pathways (i.e., gluconeogenesis or fatty acid synthesis) or pathways of energy generation (i.e., the TCA cycle). BCAAs are first converted to their α-keto acid derivatives in the cytosol through the activity of branched-chain aminotransferase (BCAT), and then further broken down in the mitochondrial matrix via the branched-chain α-keto acid dehydrogenase (BCKD) complex. It is accepted that elevations in BCAA levels is a biomarker in metabolic disease and diabetes. Here, in detrusor muscle there are elevated levels of multiple BCAA metabolites, including the three products of BCAT, 4-methyl-2-oxopentanoate, 3-methyl-oxovalerate, and 3-methyl-2-oxobutyrate. One possibility is that in detrusor muscle, catabolism of the BCAAs is elevated, possibly caused by mitochondrial lipid oxidation dysfunction (as also suggested above with elevated ketones). The changes are largely absent in rat urothelium, consistent with subtly different changes occurring in the two tissue types (similar to glycolysis and the TCA cycle discussed above).

Bile acid changes in detrusor tissue: Bile acids emulsify dietary fats, eliminate cholesterol, and clear hepatic catabolites. They are synthesized in the liver by cytochrome P450-mediated oxidation of cholesterol, stored in the gall bladder, and released into the small intestines. Primary bile acids like cholate, chenodeoxycholate, and glycocholate are conjugated to either taurine or glycine to decrease toxicity and increase solubility. The majority of bile acids released to the intestinal tract are recycled to the liver in the process termed enterohepatic recirculation. Bile acids in the gut are subject to modification by the gut microbiota, which creates the secondary bile acids like deoxycholate. Here, the increased levels of bile acids in the rat detrusor may suggest an underlying liver dysfunction that is causing higher serum levels and subsequent uptake in muscle tissue. This is not surprising as streptozotocin treatment also causes liver damage. In this respect, it is noted that all changes in the dataset could be from a combination of disease effects and liver toxicity. Only cholate was detected in the urothelium, and it was also significantly higher in the diabetic group compared to control.

Specific Aim 2: Determine if there are progressive changes in the bladder metabolome caused by diabetes as bladder physiology progresses from the compensated to decompensated.

The goal of this specific aim was to determine if there were progressive changes in bladder metabolism with diabetes as bladder physiology progresses from the compensated to decompensated state. In addition we added to this aim a study to determine if one-month insulin treatment was able to fully restore normal bladder physiology/ metabolism. We isolated mucosal and detrusor layer from the three-month diabetic group, three-month diabetic group treated with insulin for one-month, and age-matched control groups for metabolomics analysis. We are presently fully analyzing the data set and anticipate submitting a paper based on the findings early in 2018. At present are major finding are as follows:

In (as yet) unpublished studies we investigated the ability of insulin treatment to reverse changes in metabolism. Metabolomic analysis was performed on the bladder (urothelium and detrusor) of 4-month STZ-diabetic rats (N=5) and 3-month STZ diabetic rats treated for 1-month with insulin (N=5). The metabolomes of these two groups were compared to non-diabetic age matched controls (N=5). Analysis was as described above and results depicted in Fig. 1 highlight the metabolites/pathways that were reversible/irreversible with insulin treatment. Although

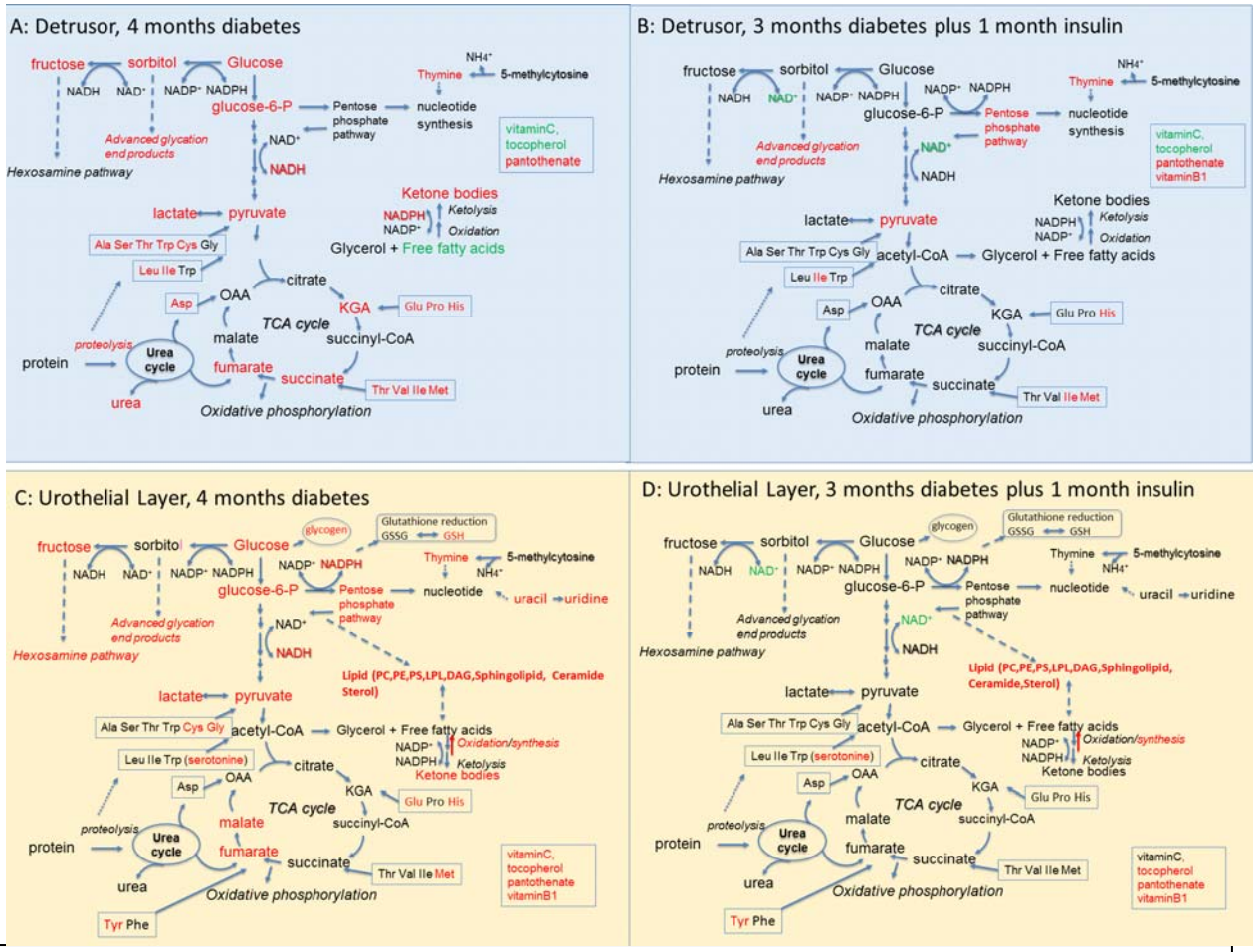

Fig 1. Metabolomic analysis was performed on the bladder (urothelium and detrusor) of 4-month STZ-diabetic rats (N=5) and 3-month STZ diabetic rats treated for 1-month with insulin (N=5). Metabolomes of these groups were compared to non-diabetic age matched controls (AMC) (N=5). Analysis was as previously described (26). **Red color** means significantly higher metabolite level than AMC; **Green color** means significantly decreased metabolite level compared to AMC**; Black color** indicates not a significant difference, or, partial restoration of metabolite levels to those in AMC.

insulin did appear to reverse the majority of the effects of diabetes on the energy generating

pathways in both detrusor and urothelium, the levels of several metabolites were not significantly ($P < 0.05$) changed in expression following insulin treatment (22 metabolites in the urothelium and 31 in the detrusor). In the urothelium lipid membrane components did not respond well to insulin treatment. This potentially could affect signaling pathways between the urothelium and detrusor. In the detrusor, several metabolites involved in amino acid metabolism were not normalized by insulin treatment, which could impact several pathways involved in bladder physiology.

3. Publications:

Wang, Y., Deng G.G. and Davies K.P. "Urothelial MaxiK-activity regulates mucosal and detrusor metabolism". Submitted to Plos One

Presentations at meetings:

Wang, Y., Deng G.G. and Davies K.P. @ Society for Pelvic Research. "Metabolomics provides novel insights into the effect of diabetes on bladder detrusor and urothelial metabolism". (2016) Transl. Androl. Urol .5(Suppl 2); PMC5143264

Wang, Y., Deng G.G. and Davies K.P. @ International Continence Society "Metabolomic and epigenomic studies provide evidence for "hyperglycemic memory" in diabetic bladder disease" (2016) https://www.ics.org/2017/abstract/609

Wang, Y., Deng G.G. and Davies K.P. @ American Urological Society "Metabolomics reveals differential changes in the energy generating pathways of detrusor and urothelium caused by diabetes" (2016) The Journal of Urology, Vol. 195, Issue 4, e414