Alterations in Intervertebral Disc Matrix Homeostasis in the UCD-T2DM Rat Model of Type 2 Diabetes

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Introduction: Low back pain (LBP) is among the leading causes of disability and health care expenditures in adults worldwide. Moreover, the frequency and severity of LBP are higher in adults with type 2 diabetes (T2D)[1], who also report less favorable treatment outcomes [2]. Since LBP is often related to intervertebral disc degeneration, identifying the mechanisms by which T2D affects disc health is an important step towards improving the management of LBP in diabetes. One potential mechanism that could link T2D with poor disc health relates to the accumulation advanced glycation end-products (AGEs). AGEs are formed through non-enzymatic reactions between glucose and proteins. Interactions between AGEs and one of their receptors (RAGE) can have a number of pathologic effects, including elevated oxidative stress and inflammatory factor expression [3,4]. In T2D, hyperglycemia could increase the formation AGEs in the disc. Thus, using a rat model of T2D that isolates hyperglycemia as the only remaining independent variable between groups, we sought to test the hypothesis that T2D compromises disc matrix homeostasis by increasing oxidative stress and AGE/RAGE-mediated interactions.

Methods: Animals and tissues: Coccygeal discs were harvested from 6-month-old lean Sprague Dawley rats (“control”), non-diabetic obese Sprague Dawley rats (“obese”), and diabetic obese UCD-T2DM rats (n = 6 rats/group). The UCD-T2DM rats are a cross between Sprague Dawley rats that display polygenic obesity and insulin resistance and Zucker diabetic fatty-lean rats that have an intact leptin receptor and pancreatic beta cell insufficiency [5]. Non-fasted blood glucose was monitored every 2 weeks with a glucose meter, and the age of diabetes onset was defined as the age at which hyperglycemia (blood glucose concentration >200 mg/dl on two consecutive measurements) was first detected. Blood was collected for measurement of circulating glucose, insulin, and HbA1c after an overnight fast at the time of sacrifice. AGE accumulation: ELISA was used to measure the concentration of pentosidine in the disc. Pentosidine is a well-characterized AGE and inter-molecular cross-link that is a sensitive marker of non-enzymatic glycation. Samples of annulus (C9-C10) and nucleus (C10-C11) were pulverized in a freezer mill and assayed for pentosidine by sandwich ELISA (MyBiosource). Pentosidine measurements were normalized to collagen content, which was calculated from the amount of hydroxyproline [6]. Matrix homeostasis: qPCR was used to characterize matrix homeostasis. Samples of annulus and nucleus (C8-C9) were homogenized in Trizol (Sigma), and total RNA was collected using phenol chloroform and purified with RNeasy Kits (Qiagen). RNA samples were reverse transcribed using iScript (Bio-Rad), and the resulting cDNA was pre-amplified and analyzed via qPCR for the following genes: receptor for AGEs (RAGE), vascular endothelial growth factor alpha (VEGFA), hypoxia-inducible factor 1-alpha (HIF1A),
insulin-like growth factor 1 (IGF1), insulin receptor (INSR), glucose transporter 1 (SLC2A1), caveolin 1 (CAV1), tumor necrosis factor alpha (TNFA), twisted gastrulation protein homolog 1 (TWSG1), tissue inhibitor of metalloproteinase 1 (TIMP1), collagen 2 alpha 1 (COL2A1), collagen 1 alpha 1 (COL1A1), and aggrecan (ACAN). The ΔΔCt method was used to calculate relative expression values compared to beta-actin (ACTB), and expression values were reported as fold change over control. Statistics: Results (mean ± SEM) were compared using ANOVA with Tukey post-hoc tests.

Results: Blood glucose measurements indicated that the diabetic rats were diabetic for 69 ± 7 diabetic days. As expected, diabetic and obese rats had significantly higher body weights than the age-matched lean controls (Table 1). Diabetes, but not obesity, significantly increased AGE concentrations in the disc (Figure 1). In the annulus, pentosidine concentration was elevated by 29% (p < 0.01); in the nucleus, by 104% (p < 0.05). Diabetes also shifted disc cell metabolism and matrix homeostasis in a manner that was consistent with increased AGES and oxidative stress (Figure 2). Diabetes tended to associate with elevated expression of hypoxia-sensitive genes, including VEGFA (p < 0.10) and HIF1A (p < 0.10). In the nucleus pulposus, RAGE expression was up-regulated nine-fold in the diabetic rats (additional samples needed to reach significance), which was consistent with their tendency of having lower expression levels of TIMP1 (p < 0.10) and COL2A1 (p < 0.05), and of having higher levels of the cytokine TWSG1 (p < 0.05). In the annulus fibrosus, notable findings included elevated expression of the senescence indicator CAV1 (p < 0.05) and of TWSG1 (p < 0.05). Relative to the lean controls, diabetic rats also had higher insulin receptor expression in the nucleus (INSR, p < 0.05) and annulus (p < 0.10); IGF1 expression tended to be higher in the annulus (p < 0.10). There was no difference in ACAN expression, and reliable amplification data were not obtained for TNFA.

Table 1: Body weight, glucose, insulin and HbA1c data (mean ± SEM) for 6-month-old lean Sprague Dawley (SD), obese SD, and diabetic obese UCD-T2DM rats.

<table>
<thead>
<tr>
<th></th>
<th>Lean SD (n = 5 rats)</th>
<th>Obese SD (n = 6 rats)</th>
<th>UCD-T2DM (n = 6 rats)</th>
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<tbody>
<tr>
<td>Diabetes duration (days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>426.0 ± 12.1</td>
<td>693.7 ± 29.0a</td>
<td>563.5 ± 18.9c,d</td>
</tr>
<tr>
<td>Non-fasting glucose (mg/dl)</td>
<td>112.2 ± 3.4</td>
<td>112.2 ± 4.7</td>
<td>546.2 ± 18.5a,b</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>91.8 ± 3.2</td>
<td>98.7 ± 2.8</td>
<td>246.4 ± 52.6c,d</td>
</tr>
<tr>
<td>Fasting insulin (mg/ml)</td>
<td>0.6 ± 0.07</td>
<td>1.4 ± 0.2a</td>
<td>1.4 ± 0.3a</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.5 ± 0.09</td>
<td>4.3 ± 0.06</td>
<td>11.8 ± 1.28ab</td>
</tr>
</tbody>
</table>

ANOVA w/ Tukey test: a p < 0.0001 vs. lean; b p < 0.0001 vs. obese; c p < 0.05 vs. lean; d p < 0.05 vs. obese; e p < 0.10 vs. lean.
Figure 1: Diabetes but not obesity significantly increased the concentration of pentosidine in the annulus fibrosus and nucleus pulposus. Bars show mean ± SEM for \( n = 6 \) rats/group.

\(^a\) \( p < 0.01 \) vs. control

\(^b\) \( p < 0.05 \) vs. obese
Discussion: Hyperglycemia in T2D increased AGE accumulation in the disc. Although intradiscal AGE accumulation has physical and biomechanical consequences -- AGEs lower the hydrophilic charge of the proteoglycans [7] and stiffen the disc matrix [8] -- our findings here show that there are important biological consequences too. For example, in the nucleus pulposus, elevated expression of hypoxia-inducible genes (VEGFA and HIF1A) and catabolic markers (reduced COL2A1 and TIMP1 expression) in the diabetic rats indicated shifts in matrix homeostasis that were consistent with greater oxidative stress and interactions between AGEs and RAGE. It was previously reported that T2D could also compromise disc health by causing endplate sclerosis [9]. Therefore, taken together, the collective evidence indicates that endplate sclerosis, increased oxidative stress, and AGE/RAGE-mediated interactions are consequences of T2D that are linked to detrimental changes in the composition, homeostasis and biomechanical function of the disc.

Figure 2: Diabetes tended to up-regulate hypoxia-sensitive factors (HIF1A, VEGF) and also increased expression of the senescence indicator CAV1 and the BMP antagonist TWSG1. Relative to obesity, diabetes decreased expression of the glucose receptor-encoding gene SLC2A1, the metalloproteinase inhibitor TIMP1, and COL1 and COL2. Mean ± SEM for n = 5-6 rats/group. Fold changes relative to control: 
- $a$ $p < 0.05$ vs. control; 
- $b$ $p < 0.05$ vs. obese
- $c$ $p < 0.10$ vs. control; 
- $d$ $p < 0.10$ vs. obese
**Significance:** Enhanced care for patients with discogenic low back pain must take into account not only the obvious pathology but also the relevant co-morbidities that affect disc health. Using a rat model of polygenic obese type 2 diabetes, we show that hyperglycemia in type 2 diabetes elevates the expression of hypoxia-inducible genes and catabolic markers, and that these alterations in matrix homeostasis are consistent with increased oxidative stress and AGE/RAGE-mediated interactions.

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