Tissue-specific transcriptome responses in rats with early streptozotocin-induced diabetes

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Tissue-specific transcriptome responses in rats with early streptozotocin-induced diabetes. Physiol Genomics 21: 222–229, 2005. First published February 15, 2005; doi:10.1152/physiolgenomics.00231.2004.—The understanding of common and tissue-specific molecular alterations in diabetes, particularly at early stages, is limited and fragmental. In the present study, we systematically compared transcriptome responses in four important diabetic target tissues in rats with 2 wk of streptozotocin (STZ)-induced diabetes. At this stage of diabetes, the skeletal muscle exhibited the highest transcriptome sensitivity to the STZ treatment with nearly 17% of the transcriptome being altered (false discovery rate, 1.6%) compared with ~3% in the cardiac left ventricle, renal cortex, and retina. Similarity in transcriptome response among tissues was low, with the highest similarity being 2.2% between skeletal muscle and the left ventricle. Several biological processes or cellular components, such as lipid metabolism in the left ventricle and collagen in the renal cortex, were significantly overrepresented in the responsive genes than in the entire array. Particularly interesting cases of common or tissue-specific regulation included decorin and CD36, which were upregulated in several tissues, and serum/glucocorticoid-regulated kinase and four and a half LIM domains 2, which were upregulated only in the renal cortex. Further biochemical analyses indicated that the thiol and oxidative stress pathway was altered in a tissue-specific manner at several levels including transcript abundance, content of reduced thiols, and lipid peroxidation, providing an example of the potential biological relevance of tissue-specific transcript regulation. These results provided a transcriptome-wide view of the molecular alterations across several key tissues in early diabetes. It appears that both common pathways and, perhaps more importantly, tissue-specific mechanisms are involved in the adaptation to diabetes or the initiation of diabetic complications.

diabetic complications; microarray; gene expression; decorin; CD36

Diabetes is the leading cause of end-stage renal disease, adult blindness, and nontraumatic lower extremity amputation in the United States. Diabetes is also a major risk factor of cardiovascular disease. While other abnormalities present in diabetic patients may participate in causing certain complications, hyperglycemia is believed to be the most important common factor. Controlling hyperglycemia has been shown to be effective in preventing or slowing the progression of many diabetic complications (33, 35). This suggests the presence of common molecular pathways underlying the development of diabetic complications in different organ systems.

However, it is also evident that the development of diabetic complications in different organ systems follows distinct courses. It is known that hyperglycemia could damage cells through the stimulation of several interrelated pathways involving, for example, oxidative stress, advanced glycation end-products, the polyol pathway, protein kinase C, the hexosamine pathway, and transforming growth factor (TGF)-β (3). It is not yet fully understood how these molecular pathways interplay to cause diabetic complications. Particularly, current knowledge regarding the relative importance of each of these and potentially other mechanisms in diabetic complications in different tissues and organ systems is limited and fragmental. It is important to understand both common pathways and tissue-specific mechanisms mediating diabetic complications because they could have significant, but distinct, implications for the diagnosis and treatment of these important abnormalities.

High-throughput transcriptome analysis using techniques such as DNA microarray is a powerful approach for extending fragmental knowledge of complex biological processes into integrative global views (17, 18). It has also proven useful for generating novel hypotheses regarding regulatory pathways or disease mechanisms. The DNA microarray approach has been applied to the study of diabetes. Examples of tissues examined include β-cells (5, 36), immune cells or organs (7, 38), adipose tissue (25), the retina (11), skeletal muscle (23, 30, 40), and the kidney or kidney cells (24, 31, 37). Many of these studies have provided novel insights into the pathophysiology of diabetes and identified previously unsuspected mechanisms deserving further investigation.

What is lacking, however, is a systematic comparison of transcriptome responses to diabetes across different target organ systems. Previous studies in individual tissues do not allow a meta-analysis across tissues to be performed because the models and designs used varied widely. In the present study, we examined, in parallel, the transcriptome profiles in several key target tissues of diabetes, including the renal cortex, cardiac left ventricle, skeletal muscle, and retina, in streptozotocin (STZ)-treated rats, a widely used model of Type 1 diabetes. This systematic approach, together with a set of extensively characterized microarray protocols and newly developed analytical algorithms, allowed us to obtain important insights into the tissue-specific transcriptome sensitivity to diabetes and the extent of tissue resemblance in transcriptome responses. Further characterization of the thiol and oxidative stress pathway provided a proof of concept for the biological relevance of tissue-specific transcriptome responses in diabetes.

RESEARCH DESIGN AND METHODS

STZ model of diabetes. The animal protocol was approved by the Animal Care and Use Committee of the Medical College of Wisconsin. Rats were treated with STZ to produce Type 1 diabetes as we have described previously (24). Male Sprague-Dawley rats (Harlan; Indianapolis, IN) weighing ~250 g were given a single intraperitoneal injection of STZ (Sigma; St. Louis, MO) at 55 mg/kg body wt.
dissolved in 50 mM citrate buffer (pH 5). Control rats (n = 8) of similar age and body weight were injected with vehicle buffer in comparable amounts. Body weight and blood glucose levels were measured before injection and then 2 days and 2 wk after the injection. STZ rats were considered diabetic (n = 8) if their blood glucose levels were >250 mg/dl 2 days after the STZ injection. Blood glucose levels were >400 mg/dl at 2 wk after the STZ injection. At this time, control and STZ rats were anesthetized, and tissue samples from the right renal cortex, free wall of the cardiac left ventricle, psoas muscle, and retinas were harvested. Each tissue sample was divided into two pieces and snap frozen for later preparation of total RNA and tissue homogenate.

_Construction and hybridization of cDNA microarrays._ Rat cDNA microarrays were constructed and hybridized as described previously (8, 9, 21, 22, 24). This microarray contained 17,664 elements of rat cDNA probes and 768 elements of control solutions printed on a glass slide. Total RNA samples from control and STZ rats were reverse transcribed and labeled with Cy3 or Cy5 fluorescent dyes. Differentially labeled cDNA samples were combined and hybridized with a microarray. Cy3 and Cy5 fluorescent intensities of each array element were quantified, and the ratio between them provided an index of the mRNA expression level of the gene represented by each element in the STZ rat relative to the control rat.

**Microarray experimental design.** The two-color hybridization method allowed us to directly compare control and STZ samples on one array. However, it was shown in a previous analysis (16) that bias introduced by different fluorescent dyes, for example, Cy3 and Cy5, was the most important source of technical variation in this type of microarray data. Therefore, each hybridization was repeated using a second microarray with Cy3 and Cy5 switched between the control and STZ samples. This is referred to as dye switching. To preserve limited materials such as the retinal tissue and reduce cost, one control and one STZ RNA pool per tissue was created by combining equal aliquots of RNA from each of eight control rats or eight STZ rats. The RNA pools were then used for cDNA labeling and microarray hybridization. The remaining individual RNA samples were used in real-time PCR analysis to validate microarray results and to obtain an estimate of the variability of gene expression between individual rats. 

**Microarray data analysis._** Microarray data were categorized, filtered, adjusted, log transformed, and normalized to generate “qualified data” as described previously (21, 22, 24). For each cDNA element that passed quality filters, a mean log2 (ratio), R, and an index of consistency between initial and dye switching hybridizations, C, were calculated as follows: R = (R1 + R2)/2 and C = R/s, where R1 and R2 are normalized log2 (ratios) obtained from the initial and dye switching hybridizations, respectively, and s is the standard deviation calculated based on R1 and R2.

A reference distribution approach described previously (22) was used to determine the criteria of differential expression and to obtain an estimate of the false discovery rate (FDR). To construct a reference distribution, two aliquots of the renal cortical RNA pool were labeled with Cy3 and Cy5 and hybridized to a microarray. This hybridization was repeated once with sham dye switching. Data from these reference hybridizations were similarly processed to generate R and C values for each cDNA element. Certain R and C values were then selected as tentative thresholds of differential expression. The number of cDNA elements with R and C values above these thresholds (D) was counted in hybridizations for each tissue as well as the reference hybridizations. FDR in each tissue examined was calculated as D/Nc, where Nc is the total number of cDNA elements that passed quality filters in both initial and dye switching hybridizations for that tissue. The resemblance of transcriptome responses between any two tissues examined was calculated as (c – c’)/(maximum (D1, D2), where c is the number of cDNA elements that were considered upregulated or downregulated in both tissues, c’ is the number of cDNA elements that were considered upregulated in one tissue but downregulated in the other, and D1 and D2 are numbers of cDNA elements differentially expressed in tissue 1 and tissue 2, respectively. The range of possible resemblance values is between −100% and 100%, with −100% and 100% corresponding to completely opposite and identical transcriptome responses, respectively.

**Bioinformatic analysis.** Gene affiliations with known biological processes or cellular components defined in Gene Ontology (http://www.geneontology.org) were retrieved based on GenBank accession numbers using DAVID (6, http://david.niaid.nih.gov) with the level 3 definitions and a minimum of 3 entries/category. Chromosomal locations were retrieved from the Rat Genome Database based on gene symbols (http://rgd.mcw.edu). The proportion of each category (a biological process, cell component, or chromosome) in all cDNA elements represented on the array (E; for enrichment) or in the differentially expressed cDNA elements in each tissue (Ee) was calculated. Ee was adjusted for the overall enrichment for classifiable cDNA elements in the differentially expressed elements as follows: Ee = E/(N/Nc) where N is the number of differentially expressed, classifiable cDNA elements in a particular tissue, Nc is the total number of differentially expressed cDNA elements in that tissue, N is the number of classifiable cDNA elements on the entire array, and Nc is the total number of cDNA elements on the entire array. A χ²-test was performed to examine the statistical significance of any difference between E and Ee. The differentially expressed cDNA elements were considered specifically enriched for a category if the P value of the χ²-test for that category was <0.05.

**Real-time PCR.** Quantitative real-time PCR analysis of selected genes was performed in individual control and STZ samples either involved (n = 8 for each group) or not involved (n = 4–6 in the microarray analysis). The SYBR green method was used in the first pass of analysis as described previously (24). The measurement was repeated using the dual-labeled probe method (4, 10) if results from the SYBR green analysis indicated problems of nonspecificity. Probes were designed using Primer Express 2.0 (Applied Biosystems; Foster City, CA) and labeled with a reporter dye at the 5’ end and a quencher dye at the 3’ end. The RT-PCR reaction mixture of the dual-labeled dye method contained 1× TaqMan One-Step RT-PCR Master Mix (Applied Biosystems), 0.25 U/μl Multiscribe reverse transcriptase, 0.4 U/μl RNase inhibitor, 400 nM forward and reverse primers, 200 nM dual-labeled probe, and 10 ng total RNA, in a volume of 10 μl. Each reaction was performed in triplicate in clear 384-well plates at 48°C for 30 min and 95°C for 10 min and then at 95°C for 15 s and 60°C for 1 min for 40 cycles, using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The fluorescent intensity of the reporter dye at each cycle of PCR reflected the accumulated amount of PCR products. Ct numbers (the number of PCR cycles at which reporter intensity reached a detection threshold that was set with the exponential phase of PCR) were used to calculate the relative expression levels of genes of interest, which was normalized to endogenous cellular 18S rRNA measured in parallel. mRNA levels of each gene were expressed as per 10⁶ 18S rRNA molecules. Such expression allowed comparisons of abundance not only between treatment conditions but also between tissues. The comparison between genes should be interpreted with caution because differences in kinetic characteristics might exist between genes. Primer and probe sequences are shown in supplemental Table 1 (see http://physiolgenomics.physiology.org/cgi/content/full/00231.2004/DC1).

**Lipid peroxidation assay._** Thioarbituric acid-reactive substances were quantified as an index of the amount of peroxidated lipids (19, 20, 24).

**Cellular reduced thiol assay.** Ellman’s reagent was used to quantify the level of cellular reduced thiols as described previously (24).
Serum ketone bodies and lipids. Serum levels of β-hydroxybutyrate, triglycerides, and free fatty acids were measured using a Hitachi Analyzer.

Statistical analysis. Microarray data were analyzed as described above. For other data, Student’s t-test was used. P < 0.05 was considered significant. Data are shown as means ± SE unless otherwise indicated.

RESULTS

Characteristics of the STZ model. Over the 2-wk period after the STZ treatment, rats with STZ-induced diabetes (n = 8) had a weight gain of 9 ± 6 g, whereas control rats (n = 8) gained 73 ± 4 g (P < 0.05 vs. STZ rats). Right kidney-to-body weight ratios at the time of tissue collection were significantly higher in STZ (0.0051 ± 0.0001) than in control (0.0037 ± 0.0001, n = 8, P < 0.05) rats. Right kidney weight tended to be higher in STZ rats (1.29 ± 0.03 g) than in control rats (1.19 ± 0.05 g). Another group of control or STZ rats were prepared similarly and used for additional biochemical and real-time PCR analysis. At 2 wk after the STZ treatment, the STZ rats (n = 4), compared with the control rats (n = 6), had significantly higher blood glucose (470 ± 27 vs. 94 ± 4 mg/dl, P < 0.05), serum β-hydroxybutyrate (14.9 ± 3.3 vs. 3.5 ± 0.4 mg/dl, P < 0.05), right kidney weight-to-body weight ratio (0.0057 ± 0.0001 vs. 0.0038 ± 0.0002, P < 0.05), and less body weight gain (9 ± 4 vs. 55 ± 2 g, P < 0.05) and tended to have higher serum triglycerides (102 ± 26 vs. 52 ± 7 mg/dl) and free fatty acids (0.42 ± 0.05 vs. 0.34 ± 0.03 meq/l).

Differentially expressed genes. The threshold of differential expression was determined to be “R > 0.816 and C > 1, or 0.816 > R > 0.490 and C > 2”, where R is the mean log2 ratio and C is the consistency index (see RESEARCH DESIGN AND METHODS); 0.816 and 0.490 corresponded to five and three times, respectively, of the standard deviation of all log2 ratios obtained from the reference hybridizations. The number of differentially expressed cDNA elements identified in each tissue in response to the 2-wk STZ treatment is shown in Fig. 1A. The FDR was 5.7% for the renal cortex, 8.3% for the left ventricle, 1.6% for skeletal muscle, and 18.8% for the retina. A complete list of differentially expressed cDNA elements, together with their mean log2 ratios, is shown in supplemental Table 2 (see http://physiolgenomics.physiology.org/cgi/content/full/00231.2004/DC1).

The overall reliability of the microarray results was assessed through real-time PCR analysis of 11 genes in 64 individual samples involved in the microarray analysis (n = 8) and 3 additional genes in 40 individual samples not involved in the microarray analysis (n = 4–6). These genes covered a wide range of R and C values and represented diverse, interesting biological pathways that will be discussed later. As shown in Tables 1 and 2, 14 of 19 cases, or 74%, of differential expression identified by microarray were validated to be statistically significant by real-time PCR. If directionally consistent changes were accepted as “validated,” 18 of 19 cases, or 95%, of differential expression identified by microarray would be considered validated by real-time PCR. One of sixteen cases of nondifferential expression according to microarray was found to be different by real-time PCR. There were two cases that did not have qualified microarray data points but were found differentially expressed according to real-time PCR. The correlation coefficient between all log2 ratios generated by microarray and by real-time PCR was 0.80 (n = 35, P < 0.001). This level of consistency between microarray and a second technique was comparable with several previous reports that showed both validated and unvalidated results (12, 21, 22, 27).

Transcriptome sensitivity to the STZ treatment. Skeletal muscle exhibited the highest transcriptome sensitivity to the STZ treatment. Of a total of 7,693 cDNA elements that passed quality filters, 16.8% were identified as differentially expressed. The number of cDNA elements that passed quality filters in the renal cortex, left ventricle, and retina was 10,414, 6,735, and 4,221, respectively. Approximately 3% of them responded to the STZ treatment in each of the three tissues (Fig. 1B). The calculated transcriptome sensitivity would be even higher in skeletal muscle and lower in the retina if the threshold of differential expression had been adjusted for each tissue to obtain a medium level of the FDR, for example, 7%, in all tissues.

Similarities and differences in transcriptome response. The largest similarity of transcriptome response was found between
the left ventricle and skeletal muscle (Fig. 2). However, only 2.2% of similarity was found between even these two tissues. Lower levels of similarity were found between the cortex and skeletal muscle, the cortex and left ventricle, and the left ventricle and retina. No net similarity was found between skeletal muscle and the retina or between the cortex and retina.

To examine the potential biological relevance of the observed tissue specificity in the transcriptome response, cDNA elements were classified according to their affiliations with biological processes as defined in Gene Ontology. Of all cDNA elements represented on the array, 12.1% were classifiable with 81 biological processes. The percentages in the differentially expressed elements in the cortex, left ventricle, skeletal muscle, and retina that were classifiable with 31 biological processes. Twenty-one biological processes with at least five cDNA elements being differentially expressed in at least one tissue are shown in Table 3. Compared with all cDNA elements on the array, differentially expressed cDNA elements in the cortex were specifically enriched for genes related to cell adhesion (Fig. 3A). Those in the left ventricle and skeletal muscle were specifically enriched for organic acid metabolism. The left ventricle was also enriched for lipid metabolism.

On the array, 9.4% of all cDNA elements were known to affiliate with 23 cellular components. The corresponding percentages in the differentially expressed elements in the cortex, left ventricle, skeletal muscle, and retina were 14.2%, 14.7%, 13.4%, and 8.0%, respectively. Compared with all cDNA elements.

Table 2. Expression of thioli- and oxidant-related genes in rats with STZ-induced diabetes

<table>
<thead>
<tr>
<th>TISSUE-SPECIFIC TRANSCRIPTOME RESPONSES IN DIABETES</th>
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<tbody>
<tr>
<td>Decodonin</td>
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<tr>
<td>Cortex</td>
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<tr>
<td>Array</td>
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<tr>
<td>Real-time PCR control</td>
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<tr>
<td>Real-time PCR STZ</td>
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<td>LV</td>
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<tr>
<td>Array log2 (STZ/control)</td>
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<tr>
<td>Real-time PCR control</td>
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<tr>
<td>Real-time PCR STZ</td>
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<tr>
<td>SKM</td>
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<tr>
<td>Array log2 (STZ/control)</td>
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<tr>
<td>Real-time PCR control</td>
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<tr>
<td>Real-time PCR STZ</td>
</tr>
<tr>
<td>Retina</td>
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<tr>
<td>Array log2 (STZ/control)</td>
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<tr>
<td>Real-time PCR control</td>
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</tbody>
</table>

Values are means ± SE; real-time PCR analysis of four and a half LIM domains 2 (FHL-2) and AI113079 [an expressed sequence tag (EST) similar to human endothelial Per-ARNT-Sim (PAS) domain protein 1] was performed in samples not involved in the microarray experiment (n = 4–6), whereas the rest was done in samples involved in the microarray experiment (n = 8). Real-time PCR results were normalized to 18S rRNA. Missing values indicate that either those genes were not represented on the microarray, or the fluorescent intensity of those cDNA elements in microarray did not pass quality filters. See RESEARCH DESIGN AND METHODS for more explanation. GPX, glutathione peroxidase; SOD, superoxide dismutase; eNOS, endothelial nitric oxide synthase. *Considered differential expression based on microarray results; †P < 0.05 vs. control.
elements on the array, differentially expressed elements in the cortex were specifically enriched for genes associated with collagen (Fig. 3B). Those in skeletal muscle were enriched for genes associated with the ribonucleoprotein complex and cytoplasm.

On the array, 16.6% of all cDNA elements had known chromosomal locations (only the ones with gene symbols were analyzed). The corresponding percentage in the differentially expressed elements was ~30% in all four tissues. No specific enrichments for any chromosomes were found in the differentially expressed genes.

Representative genes exhibiting common or tissue-specific responses. Decorin and CD36 exhibited differential expression common to several tissues (Table 1). Decorin is a proteoglycan that interacts closely with TGF-β (2, 39) and is possibly involved in the regulation of extracellular matrix deposition seen in diabetic complications. As shown in Table 1, decorin was significantly upregulated in the renal cortex, left ventricle, and skeletal muscle in STZ-treated rats. CD36 is a fatty acid transporter importantly involved in metabolic syndrome (1). It was significantly upregulated by the STZ treatment in the left ventricle and skeletal muscle, and although it appeared upregulated in the renal cortex, real-time PCR did not confirm it.

Serum/glucocorticoid-regulated kinase (SGK), sorbitol dehydrogenase (SDH), and four and a half LIM domains 2 (FHL-2) are examples of genes regulated in a tissue-specific manner (Table 1). SGK has a wide range of biological activities, including the regulation of sodium reabsorption in the distal nephron in the kidney, a process possibly affected by diabetes. SGK was specifically and substantially upregulated in the renal cortex, real-time PCR did not confirm it. FHL-2 is an adaptor protein mediating a variety of protein interactions important for metabolism and cell signaling (15, 32). FHL-2 was specifically upregulated in the renal cortex, the functional significance of which would be an interesting subject for future studies.

The thiol and oxidative stress pathway. The biological relevance of the tissue specific transcriptome response was further investigated by studying the thiol and oxidative stress pathway. As shown in Table 2, several genes related to the thiol and oxidative stress pathway were regulated in a tissue-specific manner. Glutathione peroxidase 1 (GPX1) was modestly upregulated in the renal cortex. Endothelial nitric oxide synthase (eNOS) was downregulated in the left ventricle. In skeletal muscle, GPX1 was substantially downregulated, whereas catalase and superoxide dismutase 3 (SOD3) were upregulated. Thioredoxin 2 appeared upregulated in the retina but was not statistically confirmed by real-time PCR.

Similarly, lipid peroxidation, an index of oxidative stress, and cellular contents of reduced thiols were altered in a tissue-specific manner. Lipid peroxidation was not changed in the renal cortex or left ventricle but was decreased in skeletal muscle (Fig. 4A). Levels of cellular reduced thiols, however, were increased in the renal cortex and left ventricle but remained unchanged in skeletal muscle (Fig. 4B). The retina was not analyzed due to limited tissues available.

DISCUSSION

The present study provided the first systematic comparison of gene expression profiles across several key target tissues in diabetes. The results suggested a high degree of tissue specificity in the molecular mechanism underlying early responses to diabetes. The differential expression of a number of particularly interesting genes or pathways was validated and demonstrated to have potential functional significance.

The high transcriptome sensitivity seen in skeletal muscle was likely because this tissue heavily relies on insulin to maintain its energy metabolism and other cellular activities.

Table 3. Biological processes that were likely affected in rats with STZ-induced diabetes

<table>
<thead>
<tr>
<th>GO Biological Processes</th>
<th>Cortex</th>
<th>LV</th>
<th>SKM</th>
<th>Retina</th>
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<tbody>
<tr>
<td>Alcohol metabolism</td>
<td></td>
<td></td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Amine metabolism</td>
<td></td>
<td></td>
<td>10</td>
<td></td>
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<tr>
<td>Amino acid and derivative metabolism</td>
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<tr>
<td>Biosynthesis</td>
<td>6</td>
<td>5</td>
<td>31</td>
<td></td>
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<tr>
<td>Carbohydrate metabolism</td>
<td></td>
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<td>13</td>
<td></td>
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<tr>
<td>Catabolism</td>
<td>12</td>
<td>27</td>
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<td></td>
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<tr>
<td>Cell adhesion</td>
<td></td>
<td>8</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Cell death</td>
<td></td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell growth and/or maintenance</td>
<td>21</td>
<td>11</td>
<td>59</td>
<td></td>
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<tr>
<td>Coenzyme and prosthetic group metabolism</td>
<td>5</td>
<td></td>
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<tr>
<td>Electron transport</td>
<td></td>
<td>8</td>
<td></td>
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<tr>
<td>Energy pathways</td>
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<td>11</td>
<td></td>
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<tr>
<td>Lipid metabolism</td>
<td>9</td>
<td>11</td>
<td>20</td>
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<tr>
<td>Nucleobase, nucleoside, nucleotide, and nucleic acid metabolism</td>
<td>5</td>
<td></td>
<td>28</td>
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<tr>
<td>Organic acid metabolism</td>
<td>11</td>
<td>17</td>
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<tr>
<td>Organogenesis</td>
<td>7</td>
<td>5</td>
<td>11</td>
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<tr>
<td>Phosphorus metabolism</td>
<td></td>
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<td>14</td>
<td></td>
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<tr>
<td>Protein metabolism</td>
<td>15</td>
<td>5</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Response to external stimulus</td>
<td>5</td>
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<tr>
<td>Response to stress</td>
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<td></td>
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<tr>
<td>Signal transduction</td>
<td>9</td>
<td></td>
<td>29</td>
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</table>

Numbers of differentially expressed cDNA elements are shown for each category in each tissue. GO, Gene Ontology. See http://www.geneontology.org for explanations for the biological processes.
The substantial decrease of the insulin level, which is a characteristic of the STZ model of diabetes, would render the skeletal muscle inefficient in the uptake of its main energy source, glucose. Indeed, muscle atrophy is an early change observed in the STZ model of diabetes (28). The term skeletal muscle was used in this paper to refer to the psoas muscle examined in the present study. It is important to note that the response of different types of skeletal muscle to diabetes might differ to some extent.

The very low degree of similarity in the transcriptome response among target tissues cannot be explained with technical variability of the microarray technology because we observed a much higher degree of reproducibility of this technology in several previous studies (21, 22, 24). The tissue specificity suggests that different molecular mechanisms might be involved in the adaptation to diabetes or in mediating the development of diabetic complications in different organ systems. This may be due to the fact that organ systems have distinct characteristics in their utilization of glucose and insulin. It is conceivable, however, that some distinct early changes may lead to pathological alterations common to many tissues, such as fibrosis, in later stages of diabetic complications. In fact, we chose to examine the early time point in the present study to avoid common terminal changes. It would be interesting to determine the temporal convergence of some of these pathways during the progression of diabetes, although that would require extensive time course studies. On the other hand, time points earlier than 2 wk may be complicated by the fluctuation of other hormones as a result of the STZ treatment.

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Fig. 3. Biological processes (A) and cellular components (B) that were specifically overrepresented in the differentially expressed cDNA elements. The enrichments shown were significantly higher in genes differentially expressed compared with genes examined (i.e., the entire array), as explained in RESEARCH DESIGN AND METHODS.

Fig. 4. STZ-induced diabetes had tissue-specific effects on the levels of lipid peroxidation (A) and cellular reduced thiols (B). TBARS, thiobarbituric acid-reactive substance. *P < 0.05 vs. control; n = 8.
because it has been shown that the levels of most hormones are not stabilized until about 1 wk after the STZ treatment (34).

The tissue specificity in the transcriptome response, however, should not be overemphasized. Several interesting genes such as decorin (2, 39) and CD36 (1) exhibited changes common to two or even three tissues examined, suggesting that processes such as the regulation of extracellular matrix and fatty acid transport might be important for many diabetic complications. Moreover, some of the genes regulated in a tissue-specific manner might be associated with the same biological process, as suggested by the process overlap among tissues shown in Table 3.

The overrepresentation of genes related to certain pathways or cellular components in the differentially expressed genes was another significant finding. The enrichments were not due to overrepresentation of those pathways on the array because they were statistically higher compared with the entire array. Some of those categories, such as collagen genes in the renal cortex, are not surprising because they are known to be important for diabetic nephropathy (29). The enrichment of lipid metabolism genes in the left ventricle further emphasizes the importance of the interaction between cardiac glucose and lipid metabolism (26) in causing diabetic complications. Interestingly, there appeared to be an overall enrichment of classifiable cDNA elements in the differentially expressed genes. This seems to support the possibility that genes that are biologically more active are more likely to be studied and classified first.

The thiol and oxidative stress pathway was used as a proof of concept for the biological relevance of tissue-specific transcriptome response. While the definitive role of the differential expression of each member gene in this pathway requires further study to delineate, the data obtained in the present study have provided interesting indications. The upregulation of catalase and SOD3 in skeletal muscle appeared consistent with a decrease of lipid peroxidation in this tissue, which could be further reflected in the downregulation of GPX1. On the other hand, increases of cellular reduced thiols in the cortex and left ventricle and the upregulation of GPX1 in the cortex appear to indicate an attempt of these tissues to counteract oxidative stress likely present due to hyperglycemia. The thiol pathway has been shown in a previous study (24) to be important for counteracting high glucose-induced oxidative stress in cultured renal mesangial cells.

Type 1 diabetes was presumably the main cause of differential gene expression observed in the present study. Although potential non-diabetic effects of STZ cannot be completely ruled out, numerous studies have shown that Type 1 diabetes is the predominant feature of rats treated with STZ at the dose used in the present study. Future studies incorporating insulin treatment may also help to further establish the role of diabetes in the observed transcriptome response. Although hyperglycemia is the landmark of the STZ model, other metabolic changes also occur (see RESULTS) and may contribute to causing differential gene expression. It should be noted, however, that many of those changes, such as dyslipidemia, may also contribute to the development of diabetic complications.

It is difficult to compare the results of the present study with those of earlier studies examining a single tissue, due to different experimental designs (species, diabetic models, time points, etc.). The design of the study by Yechoor et al. (40), which examined skeletal muscle from mice with 4 wk of STZ-induced diabetes, was more similar to the present study than most other studies were. It is interesting that several pathways emphasized by Yechoor et al. were also found altered in the present study. For instance, several cytochrome c- or NADH dehydrogenase-related genes that encode components of the mitochondrial electron transport chain were found by both studies to be downregulated in skeletal muscle. Moreover, glucose transporter 4, a gene well known to be controlled by insulin, was found to be downregulated in both studies.

Pooled RNA samples were used for microarray hybridization in the present study. The advantage of pooling samples is that limited or costly materials could be preserved. The disadvantage is that it would be difficult to perform statistical analysis on a gene-by-gene basis. This disadvantage was partially offset by using a reference hybridization approach to estimate FDRs and by rather extensive real-time PCR analyses (14 genes analyzed in a total of 104 individual samples) that allowed conventional statistical analyses to be performed.

The transcriptome responses found in the present study should facilitate a wide range of future studies to establish common and tissue-specific molecular mechanisms underlying the development of diabetic complications, with the aim of discovering new diagnostic markers or treatments for one or more diabetic complications.

REFERENCES


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