Diabetes-altered gene expression in rat skeletal muscle corrected by oral administration of vanadyl sulfate

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Willsky, Gail R., Lai-Har Chi, Yulan Liang, Daniel P. Gaile, Zihua Hu, and Debbie C. Crans. Diabetes-altered gene expression in rat skeletal muscle corrected by oral administration of vanadyl sulfate. Physiol Genomics 26: 192–201, 2006. First published May 9, 2006; doi:10.1152/physiolgenomics.00196.2005.—Treatment with vanadium, a representative of a class of antidiabetic compounds, alleviates diabetic hyperglycemia and hyperlipidemia. Oral administration of vanadium compounds in animal models and humans does not cause clinical symptoms of hypoglycemia, a common problem for diabetic patients with insulin treatment. Gene expression, using Affymetrix arrays, was examined in muscle from streptozotocin-induced diabetic and normal rats in the presence or absence of oral vanadyl sulfate treatment. This treatment affected normal rats differently from diabetic rats, as demonstrated by two-way ANOVA of the full array data. Diabetes altered the expression of 133 genes, and the expression of 30% of these genes dysregulated in diabetes was normalized by vanadyl sulfate treatment. For those genes, the ratio of expression in normal animals to the expression in diabetic animals showed a strong negative correlation with the ratio of expression in diabetic animals to the expression in diabetic animals treated with vanadyl sulfate (P = −0.85). The genes identified belong to six major metabolic functional groups: lipid metabolism, oxidative stress, muscle structure, protein, and carbohydrate metabolism. The identification of oxidative stress genes, coupled with the known oxidative chemistry of vanadium, implicates reactive oxygen species in the action of this class of compounds. These results imply that early transition metals or compounds formed from their complexes represent a unique class of antidiabetic agents. Specific lowering of diabetic hyperglycemia has been obtained by treatment with compounds (33), the detailed mechanism of its antidiabetic action remains unclear. Inhibition of phosphotyrosine phosphatase (PTP)-1B is one mechanism by which V compounds may alleviate diabetic symptoms (16); however, alternative mechanisms could also be important contributors. Oral administration of V alleviates symptoms of diabetes in humans (9, 14, 15, 23) and rodents (25, 35) without causing any clinical symptoms of hypoglycemia, although blood glucose levels are slightly lowered in normal animals by V treatment. This differential effect on normal (N) and diabetic (D) animals is seen with many other metabolic processes. For example, diabetic hyperglycemia is significantly lowered by vanadyl sulfate (VS) treatment, whereas serum lipid levels in N animals are not substantially reduced by VS treatment (35). Given the importance of tight control of blood glucose levels in the diabetic patient, this class of compounds is a promising alternative for the treatment of diabetes.

Various strategies are being explored to lower the toxicity of VS compounds, including the coating of orally administered pills (20) and continuing investigation of the effect of different ligands (21). Many investigators have measured the activity of various enzymes and metabolite levels in muscle from D rats and D rats treated with V compounds. However, few reports address the effects of V compound treatment on specific gene expression (12, 35). Microarray technology allows investigation of global gene expression changes caused by complex diseases such as diabetes. The purpose of these experiments was to find out whether the physiological changes seen in diabetic animals after chronic treatment with VS were mirrored in global gene expression changes, as seen with insulin and other antidiabetic drugs. Obtaining information on gene expression regarding the insulin-enhancing activity of V is timely, as diabetic patients are being advised to maintain low blood glucose levels to avoid diabetic complications.

The role of oxidative stress in the etiology of diabetes and progression of diabetic complications has been increasingly recognized. The unifying mechanism for the pathobiology of diabetic complications has been proposed to be hyperglycemia-induced production of superoxide in the mitochondria (10). Diabetes has been shown to increase the production of reactive oxygen species (ROS) and mitochondrial antioxidant defense systems in rats with streptozotocin (STZ)-induced diabetes (40). The oxidant stress caused by glucose-induced free radical production is implicated in the development of insulin resistance in both type 1 (48) and type 2 diabetes (41). The presence of antioxidants such as glutathione (GSH) has been shown to protect against the development of diabetes and diabetic complications (51, 56). Because low levels of ROS are both critical in normal metabolism and involved in insulin signaling, it is difficult to correlate ROS levels with the pathophysiology of diabetes (45). This apparent paradox of ROS function in insulin action is currently being widely investigated (24).

In this study, we examined the effect of VS treatment on gene expression and found that VS-normalized diabetes altered gene expression and found that VS-normalized diabetes altered gene
expression for some of the genes involved in oxidative stress metabolism.

The insulin-enhancing activity of V compounds is likely to be related to both the direct inhibition of PTP-1B (12) and interaction of V compounds with cellular redox chemistry and ROS (11). PTP-1B, a current potential drug target in diabetes research, is important in the development of insulin resistance, resistance to obesity, and activity of the lipoprotein system (39). Inhibition of PTP-1B is proposed to be controlled by redox regulation (46). The insulin-stimulated formation of \( \text{H}_2\text{O}_2 \) modulated by NAD(P)H oxidase homolog Nox 4 is believed to play an integral role in insulin signal transduction, perhaps in part by affecting the transcription of PTP-1B (34). This study is the first report using DNA microarray technology to examine the effects of V, a representative of the antidiabetic early transition metals, on global gene expression in a diabetic animal model. Oral VS treatment is shown to have differential effects on global gene expression in N and D rats. Lipid metabolism and oxidative stress pathways highlight the specific areas of metabolism that respond to the insulin-enhancing effects of V identified here. We demonstrate that VS treatment normalizes gene expression in 30% of the genes identified with altered gene expression in muscle from Wistar rats with STZ-induced diabetes. In addition, VS treatment is shown to regulate metabolic processes via gene expression changes in different genes than those altered by diabetes, where both genes are part of the same metabolic pathway. The gene expression studies reported here will be useful in the development of potential therapeutic agents for diabetes.

**MATERIALS AND METHODS**

**Animal Protocol**

The procedures for STZ induction of diabetes in male Wistar rats and subsequent animal care have been previously described (54) and followed a protocol approved by the University at Buffalo Institutional Animal Care and Use Committee for this study. VS was present in the drinking water for 28 days, with the concentration of VS varying from 3 to 10 mM to assure that animals were ingesting similar amounts of V per day per kilogram. Dose ingested was calculated (mmol V kg\(^{-1}\) day\(^{-1}\)). Blood glucose levels were determined using an Accu-Check monitor. Animals were killed by decapitation, and the biceps femoris muscle of the rear leg of one animal was separately hybridized to one gene chip. Labeled cRNA was prepared and hybridized to the Affymetrix U34A rat chip, and the chip was scanned at the Gene Expression Core Facility at Roswell Park Cancer Institute (Buffalo, NY). Affymetrix Microarray Suite (MAS) 5.0 and Data Mining Tool 3.0 were used to prepare the data set for statistical analysis. The array data can be found in the National Center for Biotechnology Information (NCBI)’s Gene Expression Omnibus (GEO) site at this accession number: GSE3068. Probe sets not called Present by MAS (5.0) in any sample in this experiment and the Affymetrix internal control probe sets were deleted, which left 5,170 of the 8,799 probe sets on the chip for data analysis. False-positive rates are a problem in the analysis of microarray data (5). A \( t \)-test for signal intensity was performed for data set comparisons of interest [D compared with N (D:N), D/VS:D, D/VS:N, and N/VS:N], and a \( P \) value was found meeting the test for a 5% false-positive rate. Two-way ANOVA comparing treatment effect and array effects was carried out using SAS 8.0 after a log transformation of the data set to stabilize the variances.

To select individual probe sets, pairwise comparisons of each set were done using Affymetrix MAS 5.0. For each comparison, a 5 \( \times \) 5 matrix was generated, and only those probe sets with increased or decreased expression in \( > 20 \) of the 25 comparisons were selected. Genes were annotated using EMBL Nucleotide Sequence Database (28) and GenBank (6). Metabolic function and pathways were identified using Gene Ontology (2).

The heatmap was generated using the \( R \) statistical computing environment (http://www.r-project.org/) (43). The hierarchical clustering heatmap was created using the log\(_2\) fold change in expression calculated for all samples with a modified version of the \( R \) function heatmap.

**RESULTS**

**Rationale for Diabetic Model Used for Microarray Analysis**

The diabetic model chosen for these experiments was the Wistar rat with STZ-induced diabetes. This rodent model is the most widely used model to study the antidiabetic effects of V compounds. The purpose of these experiments was to demonstrate that the physiological changes seen in D animals after chronic treatment with VS were mirrored in global gene expression changes. Four weeks were chosen for the time period to assure that altered diabetic physiology was established. Vanadate correction of compromised cardiac function in rats with STZ-induced diabetes usually has been studied at 4 wk (26), because time is needed for diabetes-altered cardiac physiology to be observed. Vanadate correction of alterations in kidney weight and sorbitol content have been reported in this model at 4 wk (32); however, an even longer time is needed to see pathological changes in kidney tissue sections. A month of diabetes is also needed to see fully alterations of metabolic

RT-PCR

Total RNA was reverse transcribed and used to generate cDNA with Invitrogen SuperScript First-Strand Synthesis System kit (Carlsbad, CA). Primers for selected genes were designed using Primer3 developed at Whitehead Institute and Howard Hughes Medical Institute (Cambridge, MA) and synthesized by Sigma-Genosys (The Woodlands, TX). Real-time PCR was performed using cDNA, gene-specific primers, SYBR Green PCR Core Reagent kit (obtained from Applied Biosystems; Foster City, CA), and the iCycler IQ Real Time PCR detection system from Bio-Rad (Hercules, CA).

**DNA Microarray Analysis**

Gene expression data were obtained from four groups of rats: N, D, D treated with VS (D/VS), and N treated with VS (N/VS). In each group of five animals, RNA obtained from the biceps femoris muscle of the rear leg of one animal was separately hybridized to one gene chip. Labeled cRNA was prepared and hybridized to the Affymetrix U34A rat chip, and the chip was scanned at the Gene Expression Core Facility at Roswell Park Cancer Institute (Buffalo, NY). Affymetrix Microarray Suite (MAS) 5.0 and Data Mining Tool 3.0 were used to prepare the data set for statistical analysis. The array data can be found in the National Center for Biotechnology Information (NCBI)’s Gene Expression Omnibus (GEO) site at this accession number: GSE3068. Probe sets not called Present by MAS (5.0) in any sample in this experiment and the Affymetrix internal control probe sets were deleted, which left 5,170 of the 8,799 probe sets on the chip for data analysis. False-positive rates are a problem in the analysis of microarray data (5). A \( t \)-test for signal intensity was performed for data set comparisons of interest [D compared with N (D:N), D/VS:D, D/VS:N, and N/VS:N], and a \( P \) value was found meeting the test for a 5% false-positive rate. Two-way ANOVA comparing treatment effect and array effects was carried out using SAS 8.0 after a log transformation of the data set to stabilize the variances.

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markers. Serum lipid levels are shown for 2 and 4 wk for D and D/VS rats in Fig. 1. Although cholesterol and triglyceride levels show only a modest increase from 2 to 4 wk in the D rat, the serum levels of free fatty acids show a very statistically significant rise ($P < 0.005$) in the D rat after 4 wk compared with 2 wk. The lipid levels observed for the D/VS rats are similar to those seen in normal animals for all time points. Muscle tissue was chosen for this analysis because of the predominance of insulin-dependent metabolism in this tissue.

Normalization of Diabetic Hyperglycemia and Hyperlipidemia by Oral Administration of VS

The rats used for this experiment were divided into four groups, with five rats per group: N, D, N/VS, and D/VS. Oral VS treatment lowers diabetic hyperglycemia in 40–70% of Wistar outbred rats (12). For these gene expression studies, D/VS rats with significantly lowered blood glucose values for all time points tested were selected to maximize homogeneity of response. The blood glucose levels of the D rats were significantly different from that of the N, N/VS, and D/VS rats ($P < 0.01$) at all times tested (Fig. 2). N/VS showed reduced blood glucose levels compared with those seen in rats. The final serum lipid levels are shown in Table 1. D/VS rats had significantly ($P < 0.001$) lower amounts of all the diabetes-elevated lipids; however, N/VS rats has insignificantly lower serum lipid levels compared with the N rats. The N rats drank 217 ± 0.44 ml/day, N/VS rats drank 20 ± 1.2 ml/day, ingesting 0.44 ± 0.02 mmol V·kg$^{-1}$·day$^{-1}$ during the last 2 wk. Diabetic rats drank 257 ± 6.4 ml/day. D/VS rats drank 38 ± 1.4 ml/day, ingesting 0.49 ± 0.04 mmol V·kg$^{-1}$·day$^{-1}$ during the last 2 wk. These results demonstrate that diabetic hyperglycemia and hyperlipidemia were lowered by oral administration of VS to D rats. In contrast, ingestion of similar amounts of VS by N rats did not substantially lower blood glucose or serum lipid levels.

Effect of Oral VS Treatment on Gene Expression in Both N and D Rats

Analysis of the full array data set. Significance was demonstrated for effect of array and treatment using a two-way ANOVA of the global array data for all probe sets in the four groups, with $P < 0.0001$. VS treatment of D rats restored gene expression values toward that of the N rats, with all groups significantly different from each other except for the D/VS and the N groups. With the use of treatment and disease as separate categorical variables, a two-factor ANOVA showed a high interaction term for the two parameters, with $P < 0.001$. This result implies that global gene expression in N and D rats is different after VS treatment. The comparison of the effect of treatment within the disease groups was highly significant, with $P < 0.0001$, and the comparison of the effect of disease within the treatment groups was also very significant, with $P = 0.013$. Thus there are significant differences within groups for the two parameters (treatment and disease).

Selection and analysis of transcripts with differential gene expression. Comparisons of gene expression array data for D:N, D/VS:D, D/VS:N, and N/VS:N were carried out to select differentially expressed transcripts. The $5 \times 5$ matrix method was selected because it is robust with respect to outlying expression values and, unlike a k-fold cutoff rule, would allow for genes with significant but small changes. In total, 371 different probe sets representing 243 separate, annotated genes were identified.

Table 1. Effect of oral administration of VS

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Triglyceride, mg/dl</th>
<th>Cholesterol, mg/dl</th>
<th>Free Fatty Acid, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>5</td>
<td>175 ± 52*</td>
<td>102 ± 5*</td>
<td>0.27 ± 0.02*</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>2,335 ± 126</td>
<td>271 ± 53</td>
<td>1.21 ± 0.14</td>
</tr>
<tr>
<td>D/VS</td>
<td>5</td>
<td>173 ± 13*</td>
<td>79 ± 6*</td>
<td>0.30 ± 0.03*</td>
</tr>
<tr>
<td>N/VS</td>
<td>5</td>
<td>105 ± 22*</td>
<td>78 ± 6*</td>
<td>0.32 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Effect of oral administration of vanadyl sulfate (VS) on serum cholesterol, triglyceride, and free fatty acid levels in rats with streptozotocin (STZ)-induced diabetes used for DNA microarray experiments. N, normal; D, diabetic; *$P \leq 0.001$ compared with D animals.
Analysis of the identified transcripts demonstrated that VS treatment had different effects on N and D animals, supporting the results obtained using the global gene expression data. There were 39 probe sets identified in N/VS:N, and 178 probe sets identified in D/VS:D. In addition to the different numbers of probe sets identified when N or D animals were treated with VS, only seven probe sets were found in both sets.

Transcripts with diabetes-altered gene expression, for which expression in D rats was corrected by VS treatment, were selected. In D/VS:D, 178 probe sets were identified. The intersection of this set with the set of 213 probe sets selected in D:N contained 62 probe sets. A heatmap using hierarchical clustering and data from all arrays was built using these 62 probe sets (Fig. 3). Expression data from all probe sets were normalized to the median sample from the N group. Note that the expression levels in the N group are predominantly either black (representing the median) or dark red or dark green, showing similar slight levels of up- or downregulation. Two major probe set clusters visualized primarily in bright red and green are seen when comparing the expression pattern from the D rats with that of the median value among the N rats: green is upregulated and red is downregulated by diabetes. The overall expression pattern of the D/VS group resembles the N or the N/VS group rather than the D group. For each of the 62 probe sets showing opposite expression in D:N and D/VS:D, $\log_2$ changes in median expression levels were calculated and plotted against one another (Fig. 4). In this individual animal array data set, the expression levels of the differentially expressed probe sets selected in D:VS showed a strong negative correlation to the expression levels of the differentially expressed probe sets selected in D:N ($P = -0.85$). These data demonstrate that V treatment restored diabetic gene expression in muscle to normal levels, but not beyond, assuming that both data sets had the same false-negative rate.

**Annotated genes identified in D:N and D/VS:D.** Among the 62 probe sets showing opposite expression in D:N and D/VS:D, there were a minimum of 38 genes both upregulated by diabetes and downregulated by VS treatment of D rats and two genes with the opposite response. The genes selected in both comparisons are shown in Table 2 and are divided into functional groups with the corresponding mean fold change values given. The variability of the expression levels given in Table 2 can be visualized in the heatmap (Fig. 3) that shows expression of the 62 probe sets for each individual array. Approximately 30% (40/133) of the genes selected in D:N (see Table S4, Supplemental Materials; the online version of this article contains supplemental data) were corrected in D/VS rats. Approximately 33% (40/121) of the genes selected in D/VS:D (see Table S5, Supplemental Materials) showed diabetes-altered gene expression.

**Verification of the Affymetrix gene expression data by quantitative RT-PCR.** Quantitative RT-PCR was done for 10 genes, representing different functional groups identified in this study from the N, D, and D/VS groups. Comparison between the RT-PCR data and the array data are shown in Fig. 5. Data for six animals using RT-PCR (3 animals used in the microarray analysis and 3 animals receiving the same VS treatment that were not part of the microarray experiment) were included. The averages for the mean fold change in expression for the Affymetrix data were determined using the five arrays in each group. Note that the direction of change for all comparisons of the Affymetrix data and the quantitative RT-PCR was the same.

**Functional grouping of genes selected in both D:N and D/VS:D.** Overrepresentation of particular functional groups of genes shown in Table 2 was analyzed based on a modified gene function categorization. The significance of the categories was estimated by a Fisher’s exact test (Table 3). For comparison, the same functional groupings for genes selected in D:N and D/VS:D from Tables S4 and S5 (Supplemental Materials) are included, showing that all of these functional groupings are significantly overrepresented for the selected genes. Lipid metabolism, oxidative stress, and signal transduction categories of genes are very significantly overrepresented ($P < \ldots$.}
0.00004) in the intersection of these two data sets shown in Table 2. In these categories, VS treatment of D animals appears to restore normal function by reversing diabetes-altered changes in gene expression. Treatment of D rats with VS appears to restore diabetes-altered carbohydrate and energy metabolism by affecting the expression of genes other than those altered by diabetes. Supporting this hypothesis, the number of genes selected in both comparisons in the category of carbohydrate and energy metabolism in Table 3 is only three, which is not significant (P = 0.15).

**DISCUSSION**

**Implications of the Effects of VS Treatment on Rats with STZ-Induced Diabetes**

The physiological changes seen in diabetic animals after chronic V treatment are accompanied by global gene expression changes, in a manner similar to other antidiabetic treatments. VS treatment affected gene expression in N and D rats differently in these experiments. The effects of insulin, a normal growth hormone, are not strongly moderated by meta-
bolic state. These data strongly imply the existence of both insulin-independent and insulin-enhancing mechanisms for V action. Lipid metabolism and oxidative stress represent major metabolic pathways, where diabetes changed gene expression and VS treatment normalized the expression of many of the altered genes. The identification of lipid metabolism pathways supports and adds to the observation that V treatment alleviates diabetic hyperlipidemia, as shown here in Table 1 and reported by others (12). Differential changes in gene expression in oxidative stress pathways imply that redox properties of V characterized in vitro are also important in vivo. VS treatment counters part of the diabetes-induced inflammatory response in our studies. These damage-reducing properties of V treatment may help to explain V-mediated reduction of diabetes complications (26). Diabetes decreased the expression of genes involved in carbohydrate and energy metabolism through effects on known pathways such as glycolysis, trichloroacetic acid cycle, and oxidative phosphorylation in this study. VS treatment of D rats also moderated metabolism in this study by increasing the expression of other key transcripts in these pathways. The results described here involve the effects of long-term V treatment on gene expression in muscle. Some of these changes may be the consequence of effects in other organs, and further experiments must be done to address this point.

VS treatment normalized overall diabetes-induced changes in gene expression in parallel with lowering blood glucose and serum lipid levels. The overall effects of V on gene expression reported here are likely to involve more than V inhibition of PTP-1B in vivo. However, the overall mechanism of action of V is likely to include some aspects distinct from those of insulin. The results of this study support the role of V as a

Table 3. Overrepresented functional categories selected in the D:N and D/VS:D comparisons*

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Genes in Rat Genome</th>
<th>D:N (from Table 4)</th>
<th>D/VS:D (from Table 5)</th>
<th>Selected in Both D:N and D/VS:D Sets (from Table 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of genes</td>
<td>No. of genes</td>
<td>No. of genes</td>
<td>No. of genes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total unique genes</td>
<td>2,210</td>
<td>140</td>
<td>NA</td>
<td>131</td>
</tr>
<tr>
<td>Lipid metabolism</td>
<td>74</td>
<td>12</td>
<td>0.00417</td>
<td>10</td>
</tr>
<tr>
<td>Oxidative stress</td>
<td>31</td>
<td>12</td>
<td>&lt;0.00001</td>
<td>11</td>
</tr>
<tr>
<td>Cell and muscle structure</td>
<td>60</td>
<td>12</td>
<td>0.00086</td>
<td>15</td>
</tr>
<tr>
<td>Protein biosynthesis and breakdown</td>
<td>117</td>
<td>16</td>
<td>0.00450</td>
<td>13</td>
</tr>
<tr>
<td>Signal transduction</td>
<td>130</td>
<td>32</td>
<td>&lt;0.000001</td>
<td>17</td>
</tr>
<tr>
<td>Carbohydrate and energy metabolism</td>
<td>77</td>
<td>19</td>
<td>0.0000017</td>
<td>22</td>
</tr>
</tbody>
</table>

*The 5,170 probe sets, including 2,210 unique annotated genes, were expressed in at least one condition and were included in the analysis. Entries in bold represent categories with significant overrepresentation in both N:D and D/VS:D NA, not available. P value calculated from Fisher’s exact test.
general transcriptional modulator, either directly or through the formation of other metabolites, a function not usually associated with this metal.

V is now recognized to be insulin enhancing, as it requires the presence of insulin for full activity as an antidiabetic therapy (12). The most widely utilized animal model to study V treatment is the STZ-induced diabetes model, generally classified as a type 1 diabetes model due to the cytotoxic action of STZ on β-cells in the pancreas. Type 1 diabetes is caused by a >90% reduction in the number of pancreatic β-cells. In STZ-induced diabetes, a 50% reduction in plasma insulin levels with remaining pancreatic insulin content of <5% of control have been reported (42). Insulin supplementation is not required for maintaining animals with STZ-induced diabetes. Chromium, whose mechanism of insulin enhancement is better understood than that of V, does not work in alleviating the symptoms of diabetes in the STZ diabetic rat model, but it is effective in the Zucker Fatty rat type 2 diabetic model (13).

The fact that V is effective in alleviating diabetic symptoms in the STZ type 1 model of diabetes implies that V must have both insulin-dependent and insulin-independent mechanisms. Other data support this multiple mechanism hypothesis of V action. In N animals, V treatment reduces plasma insulin levels in serum (12, 26). This observation has been hypothesized to result from partial substitution of V action for insulin action in V-treated animals. In the BB rat model (another type 1 model for diabetes), V treatment does not eliminate the need for insulin but does lower the amount of insulin needed (4). The pancreas of D animals has <0.1% of normal insulin content in the BB rat model (19), and D animals require insulin supplementation for survival. V treatment is effective in animal models of type 2 diabetes such as the Zucker (fa/fa) rat, Zucker Diabetic Fatty rat, (db/db) mouse, and (ob/ob) mouse (12). In addition, positive effects of V therapy have been seen in human patients with both type 1 and type 2 diabetes, as reviewed in Ref. 23. In the STZ-induced type 1 diabetes animal model, there are sufficient insulin stores remaining for V to be an active therapeutic agent. In the more stringent type 1 diabetes models, such as the BB rat, V is not able to substitute for insulin as the sole antidiabetic agent. Studies with V in multiple model systems of diabetes and D patients support the hypothesis that V has both an insulin-enhancing and an insulin-independent mode of action.

Many of the groups of genes altered by diabetes and corrected by VS treatment (Table 2) were found altered by STZ-induced diabetes in mouse (55) and rat (29) muscle. In mouse skeletal muscle, using Affymetrix arrays after 4 wk of diabetes, lipid metabolism, transport, and signal transduction genes were identified with diabetes-induced changes in gene expression (55). The diabetes-altered gene expressions for fatty acid transport protein, carnitine acetyltransferase, hormone-sensitive lipase, and a fatty acid-binding protein, which were identified in this rat study, were also identified in that mouse study (55). Approximately 50% of the genes identified in the mouse experiment as altered in diabetes were corrected by insulin treatment. In our experiment, 30% of the diabetes-induced changes in muscle gene expression selected in D:N were normalized by VS treatment of rats with STZ-induced diabetes. Interestingly, expressions of genes for oxidative stress, lipid metabolism, and ribonuclear proteins were also identified as altered by diabetes in skeletal muscle from rats after 2 wk of STZ-induced diabetes using cDNA arrays (29).

**Effects of VS on Diabetes-Altered Gene Expression by Functional Grouping**

The complete list of genes with VS correction of diabetes-altered gene expression is shown in Table 2.

**Lipid metabolism.** Lipid mobilization and breakdown is one of the biological pathways identified here in which VS treatment normalized many diabetes-induced changes in gene expression. The 11 genes from lipid metabolism identified in D:N treatment are shown in Fig. 6 and belong to different pathways of lipid metabolism. The expression of a majority of these genes (70%) was normalized in D/VS rats. These results complement the data that VS treatment corrects diabetic hyperlipidemia, shown in Table 1. Both glucose and lipid metabolism have been implicated in the pathophysiology of diabetes (47). In diabetes, there is increased demand for fatty acids for energy needs in muscle. Changes in serum nonesterified fatty acids represent an important link between obesity and insulin resistance in type 2 diabetes and have been implicated in the regulation of glucose production (8). Our results showing that VS treatment of diabetic rats normalized diabetes-induced changes in expression of lipid metabolism genes demonstrate that V treatment can improve a major area of diabetic dysfunction in humans.

**Oxidative stress.** Genes involved in oxidative stress that were both upregulated in D:N and downregulated in D/VS:D are shown in Table 2. VS treatment corrected 54% of the oxidative stress genes identified in D:N (Table S4, Supplemental Materials). Many subunits of glutathione-S-transferase, which transfers glutathione to different substrates, have been identified. Also, genes in cellular oxidative stress pathways encoding metallothionein, ferritin, and transferrin were identified with this regulation pattern. The interaction of V com-

![Fig. 6. Schematic representation of diabetes-altered genes in lipid metabolism. Only 3 of the 11 genes selected in the D:N comparison (shown in italics) were not normalized by VS treatment. Expression levels are listed in Table 2.](http://physiolgenomics.physiology.org/)

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pounds with processes involved in oxidative stress such as GSH metabolism, redox regulation, and superoxide production have been previously studied. Vanadate was reduced to vanadyl in yeast cells, presumably by cellular GSH (53). The levels of the antioxidant enzymes catalase, superoxide dismutase, and GSH peroxidase were increased in diabetic rat liver after oral administration of vanadate (22). The gene expression results reported here support the hypothesis that reduction of oxidative stress is central to the insulin-enhancing activity of V.

The expression of 5-aminolevulinate synthase was downregulated by diabetes and upregulated by VS treatment (Table 2). This enzyme, the rate-limiting step in heme biosynthesis, may also be involved in oxidative stress metabolism. Bilirubin, a heme breakdown product, has been hypothesized to protect from oxidative stress (3). Increased heme production in D/VS rats could protect from oxidative stress by increasing cellular bilirubin levels. Iron is frequently bound to heme. Iron overload causes loss of insulin sensitivity, and excess tissue iron promotes free radical-induced injury (18). Our results suggest that downregulation of heme biosynthesis or other genes in D/VS animals may protect from excess iron.

Muscle structure, protein synthesis and breakdown, and complement system. The transcript for myosin heavy chain protein exhibited an 11-fold increase in D:N, countered by a 17-fold decrease in expression in D/VS:D. Muscle structural genes were expected to be identified in a gene expression study in muscle. Coordinated changes in expression of genes encoding ribosomal and complement system proteins found in this study may reflect changing metabolic processes and damage caused by diabetes that are reversed by VS treatment. An inhibitor of metalloproteinase exhibited a fourfold increased expression in D:N coupled with fourfold reduction in transcription in D/VS:D. Decreased metalloproteinase activity in the vascular system has been proposed to lead to increased collagen deposition and pathological remodeling in diabetes (38). These results are consistent with VS treatment protecting D rats from damage caused by decreased metalloproteinase activity. Inflammatory mechanisms have been proposed to mediate insulin resistance (24). Elevated serum NEFA induced inflammation and impaired vascular reactivity in N humans (52). It is interesting to speculate that the differences in gene expression reported here occur because the D/VS rats do not develop the elevated NEFA associated with diabetes (Table 1).

Signal transduction. VS correction of diabetes-altered gene expression was seen for several signal transduction genes. Some of them are strongly associated with the insulin signaling system. The Rap protein is a diabetes-related ras gene (7). Phosphorylated heat and acid stable protein regulated by diabetes and upregulated by VS treatment in D rats is reflected by upregulation of another probe set representing malate dehydrogenase.

Oxidative phosphorylation is also regulated by differential expression of several transcripts. Expressions of the cytochrome oxidase subunit-5b, the F1F0-ATPase subunit c, and iron sulfur protein were all lowered in D:N. In contrast, the expression of the transcripts for mitochondrial uncoupling factor 3 expressed predominantly in muscle (44) was lowered, and cytochrome oxidase subunits-7a and -8h were increased in D/VS:D in this study. Creatine kinase was the only energy metabolism transcript both decreased in D:N and increased in D/VS:D (Table 2).

The major type of metabolic correction observed for carbohydrate and energy metabolism in our study would not be seen in analysis of gene array data only using changes in expression of the same genes. These examples demonstrate the importance of looking at changes in gene expression for complete pathways in addition to differential gene expression of specific genes.

In summary, the characterization of VS partial correction of diabetes-induced gene expression changes provided important clues to processes involved in the therapeutic action of V compounds. These studies demonstrate that global gene expression changes accompany the changes in diabetic physiology caused by oral treatment with VS. In addition, this report identifies new areas of study for development of better therapeutic agents for diabetes.

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REFERENCES

11. Vanadyl sulfate corrects diabetes-altered gene expression

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Drake PG and Posner BI.


15. Benjamini Y and Hockberg Y.


17. Benjamini Y and Hockberg Y.


39. Rusnak F and Reiter T. Sensing electrons: protein phosphatase reductase and Barford D.


