Transcriptional analysis of the endothelial response to diabetes reveals a role for galectin-3

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Darrow AL, Shohet RV, Maresh JG. Transcriptional analysis of the endothelial response to diabetes reveals a role for galectin-3. Physiol Genomics 43: 1144 –1152, 2011. First published July 26, 2011; doi:10.1152/physiolgenomics.00035.2011.—To characterize the endothelial dysfunction associated with Type II diabetes, we surveyed transcriptional responses in the vascular endothelium of mice receiving a diabetogenic, high-fat diet. Tie2-GFP mice were fed a diet containing 60% fat calories (HFD); controls were littermates fed normal chow. Following 4, 6, and 8 wk, aortic and leg muscle tissues were enzymatically dispersed, and endothelial cells were obtained by fluorescence-activated cell sorting. Relative mRNA abundance in HFD vs. control endothelium was measured with long-oligo microarrays; highly dysregulated genes were confirmed by real-time PCR and protein quantification. HFD mice were hyperglycemic by 2 wk and displayed vascular insulin resistance and decreased glucose tolerance by 5 and 6 wk, respectively. Endothelial transcripts upregulated by HFD included galectin-3 (Lgals3), 5-lipoxigenase-activating protein, and chemokine ligands 8 and 9. Increased LGALS3 protein was detected in muscle endothelium by immunohistology accompanied by elevated LGALS3 in the serum of HFD mice. Our comprehensive analysis of the endothelial transcriptional response in a model of Type II diabetes reveals novel regulation of transcripts with roles in inflammation, insulin sensitivity, oxidative stress, and atherosclerosis. Increased endothelial expression and elevated humoral levels of LGALS3 supports a role for this molecule in the vascular response to diabetes, and its potential as a direct biomarker for the inflammatory state in diabetes.

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TYPE II DIABETES IS ASSOCIATED WITH increased atherosclerosis, retinopathy, skin ulceration, and other vascular-related diseases, all of which may involve damaged or dysfunctional endothelium. A major consequence of diabetes is endothelial exposure to elevated glucose and fatty acids, leading to endothelial nitric oxide synthase uncoupling and subsequent generation of reactive oxygen and nitrogen species (46) as well as the formation of advanced glycation end-products (AGEs) (45). Furthermore, hyperinsulinemia and other hormonal changes can alter endothelial signaling pathways (34). These changes may promote inflammation, impair vasoregulation, disrupt hemostasis, and inhibit reverse cholesterol transport (2, 32). By examining the transcriptional changes that occur in both the micro- and macrovascular endothelium of mice exposed to a dietary model of Type II diabetes, we expect to gain further insight into the underlying mechanisms of the endothelial dysfunction characteristic of diabetes and the metabolic syndrome.

Transcriptional analysis has been used to examine the responses of cultured endothelium exposed to high glucose and insulin (9, 44). However, an in vitro analysis may only partially reflect the complex in vivo state, where numerous hormonal, metabolic, and cellular perturbations combine to influence the endothelial cell. In vivo analysis of aortic endothelium from mice exposed to a Type I, insulin-deficient, model of diabetes revealed dysregulation of transcripts involved in inflammation and insulin sensitivity (24). Here, we study mice rendered diabetic by a high-fat diet (HFD) and comprehensively evaluate arterial as well as capillary endothelial transcriptional responses in a model of Type II, insulin resistant, diabetes. For this purpose, we utilize transgenic mice expressing green fluorescent protein (GFP) under the endothelial-specific Tie2 promotor (Tie2-GFP). By selecting our endothelial cell population based on GFP expression as well as CD31 surface staining, we achieve a high degree of purity of the analyzed cells, assuring that our results represent transcriptional changes specifically in the diabetic endothelium.

METHODS

Animals and diet. Mice homozygous for the Tie2-GFP transgene [Tg(TIE2GFP)2875Sat0, stock no. 003658; Jackson Laboratories, Bar Harbor, ME] were bred for these experiments. Beginning at 8 wk of age, male mice were allowed to feed ad libitum on an HFD containing 60% fat calories (BioServ cat. no. S3282) for a period of 4, 6, or 8 wk. Littermates fed a normal chow diet containing 12% fat calories (LabDiet cat. no. 5001) served as controls. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Hawaii.

Fasting glucose levels and glucose tolerance test. Before the start of the respective diet regimens and after 2, 3, 4, 6, and 8 wk of feeding, glucose levels were determined by glucometry of the tail blood following an overnight fast (OneTouch Ultra; LifeScan). A glucose tolerance test was performed after 6 wk on the diet regimen. Glucose (1 mg/g body wt ip) was administered following an overnight fast. Glucometry of the tail blood was performed prior to glucose injection and every 20 min afterwards for 2 h. The area under the curve was determined using the statistical software in Graphpad.

Determination of serum galectin-3 and insulin levels. We collected 200 μl of blood from the tail vein, allowed it to clot, and centrifuged it at 5,000 rpm for 10 min to separate serum. Quantification of galectin-3 in the serum of mice after 4, 6, and 8 wk on the diet following an overnight fast was performed using the Mouse Galectin-3 DuoSet ELISA Development Kit (R&D Systems cat. no. DY1197). Following an overnight fast, serum insulin levels were measured at 3, 6, and 8 wk with a Merodia Mouse Insulin ELISA kit (cat. no. 10-1149-01). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the fasting glucose and insulin concentrations at 3, 6, and 8 wk on the diet using the following

\[
\text{HOMA-IR} = \frac{(\text{fasting glucose } \text{mg/dl})(\text{fasting insulin } \text{mcU/ml})}{22.5}
\]

where glucose is in mg/dl and insulin is in mcU/ml.
columns (Qiagen) to yield and phycoerythrin staining on a FACSAria (Becton Dickinson) di-
12-0311). We isolated 10,000 endothelial cells positive for both GFP dispersed as previously described (24, 25). Suspensions of collageno-
three animals were each pooled, minced into 1 mm fragments, and chow-fed controls were processed on the same day. In each experiment, pooled cells from three experimental and three control
endothelium from mice exposed to HFD vs. control diet for 4, 6, and

Amino Allyl MessageAmp kit (Ambion) according to the manufac-
10ng, which was then amplified using an

Yeast tRNA (4

spectrophotometer (Thermo Scientific), were combined and frag-

CyDye Post-Labeling Reactive Dye Pack (GE Healthcare) according

Endothelial cell isolation. Following 4, 6, or 8 wk on the diet regimen, animals were killed 15 min after injection. The thoracic aorta was excised, dissected from adherent fat, and snap-frozen in liquid nitrogen. Protein was subsequently extracted from the tissue and phospho-AKT (serine 473) levels were determined by ELISA (R&D Systems, cat. no. DYC887-2) and normalized to total protein concentration.

Endothelial cell isolation. Following 4, 6, or 8 wk on the diet regimen, animals were killed by CO2 asphyxiation. HFD-fed animals and chow-fed controls were processed on the same day. In each experiment, pooled cells from three experimental and three control groups were collected for each tissue. Aortae from the aortic root to the iliac bifurcation were dissected. Leg muscles consisting of the plan-
taris, gastrocnemius, and biceps femoris (which are readily dissected as a single group) were excised. Tissues were placed into ice-cold PBS and freed of adherent fat. The aortic and skeletal muscle tissues from three animals were each minced into 1 mm fragments, and dispersed as previously described (24, 25). Suspensions of collageno-

Measurement of vascular insulin resistance. Following 5 wk on the diet, six control and six high-fat-fed mice received an injection of insulin (0.06 U/g body wt in 300 μl of sterile saline ip); two or three animals of each group received vehicle (normal saline). Mice were killed 15 min after injection. The thoracic aorta was excised, dissected from adherent fat, and snap-frozen in liquid nitrogen. Protein was subsequently extracted from the tissue and phospho-AKT (serine 473) levels were determined by ELISA (R&D Systems, cat. no. DYC887-2) and normalized to total protein concentration.

Table 1. Metabolic characteristics of Tie2-GFP mice receiving high-fat diet (60% fat calories) versus chow diet (12%)

<table>
<thead>
<tr>
<th>Weeks on Diet</th>
<th>Chow</th>
<th>High Fat</th>
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<tbody>
<tr>
<td>Weight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>22.2 ± 0.9</td>
<td>23.8 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>24.1 ± 0.8</td>
<td>30.0 ± 1.6†</td>
</tr>
<tr>
<td>4</td>
<td>26.8 ± 0.8</td>
<td>31.1 ± 1.3†</td>
</tr>
<tr>
<td>6</td>
<td>26.3 ± 0.8</td>
<td>33.8 ± 1.2‡</td>
</tr>
<tr>
<td>8</td>
<td>28.0 ± 0.9</td>
<td>36.5 ± 1.0‡</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>130 ± 20</td>
<td>120 ± 7</td>
</tr>
<tr>
<td>2</td>
<td>148 ± 9</td>
<td>190 ± 10*</td>
</tr>
<tr>
<td>4</td>
<td>116 ± 8</td>
<td>165 ± 9‡</td>
</tr>
<tr>
<td>6</td>
<td>139 ± 9</td>
<td>210 ± 10‡</td>
</tr>
<tr>
<td>8</td>
<td>111 ± 4</td>
<td>182 ± 4*</td>
</tr>
<tr>
<td>Insulin, ng/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.7 ± 0.2</td>
<td>1.3 ± 0.3</td>
</tr>
<tr>
<td>6</td>
<td>0.7 ± 0.2</td>
<td>2.4 ± 0.4†</td>
</tr>
<tr>
<td>8</td>
<td>0.9 ± 0.2</td>
<td>3.3 ± 0.7†</td>
</tr>
</tbody>
</table>

Data are means ± SE. Weight measurements and glucose of the tail blood was performed before the start of the high-fat or chow diet regimen and after 2, 4, 6, and 8 wk of feeding (n = 6–18). Serum insulin levels were quantified by ELISA following 3, 6, and 8 wk on the diet (n = 5–9). *P < 0.05; †P < 0.01; ‡P < 0.001 vs. chow-fed controls.

formula: [fasting blood glucose (mg/dl) × fasting insulin (μIU/ml)] / 405 (1), where 1 mg insulin = 26 IU (0.038 mg/IU) as defined by the 1st International Standard for insulin published by the World Health Organization in 1986.

Microarray analysis. Microarray analyses were performed to de-
Fig. 1. Endocrine responses of Tie2-GFP mice on high-fat diet (HFD). A: homeostasis model assessment of insulin resistance (HOMA-IR) calculated from measured glucose and insulin levels after 3, 6, or 8 wk exposure to HFD vs. control diet (n = 5–9). B: aortic AKT phosphorylation (pAKT) in response to insulin administration (0.06 U/g) vs. vehicle (ip) in Tie2-GFP mice after 5 wk exposure to HFD vs. control diet (n = 3–6). Data shown are means ± SE. *P < 0.05, **P < 0.01.
nucleotides and to span at least one intron. Primer sequences are listed in Supplemental Table S1. cDNA representing 5 ng of total RNA was amplified by PCR performed using SYBR green fluorophore (Roche) in an Applied Biosystems 7900HT fast real-time PCR system. A standard two-phase reaction (95°C 15 s, 60°C 1 min) worked for all amplifications. Dissociation curves run for each reaction verified the presence of a single amplicon peak, and a single, amplified product of the expected size was confirmed by gel electrophoresis. Amplicons were also sequenced by 3730XL DNA Analyzer (ABI), and BLAST was used to verify the alignment of the amplicon sequence with that of the target transcript (Supplemental Table S1).

The expression level for each gene was interpolated from a standard curve of serial dilutions at cycle times where C_T, the threshold intensity, was exceeded. The abundance of Cyclophilin A was assessed in parallel as a loading control to which the genes of interest were normalized. Fold-changes represent the ratio of diabetic to control expression values. Statistical analyses were performed using the one sample t-test function in Analyse-It software for Microsoft Excel.

**Immunofluorescence.** GFP+, CD31+ endothelial cells from the leg muscles of three mice receiving HFD and three control diet for 8 wk were isolated by fluorescence-activated cell sorting (FACS) and sorted into tissue culture medium. Aliquots containing 1,000 cells were deposited onto lysine-coated slides by centrifugation at 450 rpm for 5 min with a Shandon Cytospin (Thermo Scientific). Slides were fixed overnight in 10% formalin, permeabilized with 0.1% Triton X100 and incubated with rat anti-galectin-3 (Santa Cruz Biotechnology, sc-23938) at 1:100 followed by Alexa fluor-568 goat anti-rat (Invitrogen) at 1:800. Images were collected under controlled exposure and gain settings with an Axiohot photometer (Zeiss).

**RESULTS**

**Effect of HFD on metabolic parameters.** As shown in Table 1, controlled exposure of Tie2-GFP mice to HFD resulted in the expected metabolic changes associated with diabetes, including accelerated weight gain as well as hyperglycemia by 2 wk. These responses were accompanied by marked hyperinsulinemia by 3 wk (Table 1) and an increased HOMA-IR index by 3 wk (Fig. 1A). In addition, exposure to HFD for 5 wk resulted in a reduced vascular response to insulin as reflected in attenuation of aortic AKT phosphorylation following an in vivo insulin challenge (Fig. 1B). Furthermore, glucose tolerance was impaired in mice exposed to a HFD vs. control diet (Supplemental Fig. S1).

**Endothelial cell isolation.** FACS yielded at least 10,000 GFP+CD31+ cells from each pooled tissue sample. All GFP+ cells also exhibited phycoerythrin staining (Supplemental Fig. S2A). GFP+CD31+ cell populations repre-

Table 2. Average log2 fold-change determined by microarray analysis of dysregulated transcripts in aortic endothelium following 4, 6, and 8 wk of high-fat diet

<table>
<thead>
<tr>
<th>RefSeq</th>
<th>Name</th>
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<th>6 wk</th>
<th>8 wk</th>
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<td>NM_010705</td>
<td>lectin, galactose binding, soluble 3</td>
<td>3.6</td>
<td>1.5</td>
<td>2.3</td>
</tr>
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<td>NM_010188</td>
<td>Fc receptor, IgG, low affinity III (Fcgri3)</td>
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<td>1.2</td>
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<td>chemokine (C-C motif) ligand 9</td>
<td>2.8</td>
<td>1.4</td>
<td>2.4</td>
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<td>2.0</td>
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<td>4.1</td>
<td>1.5</td>
<td>1.8</td>
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<td>arachidonate 5-lipoxygenase activating protein</td>
<td>1.8</td>
<td>0.7</td>
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<td>NM_009777</td>
<td>complement component 1, q subcomponent, beta polypeptide</td>
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<td>ferritin light chain 1</td>
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<td>NM_010545</td>
<td>CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)</td>
<td>2.5</td>
<td>2.1</td>
<td>0.9</td>
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</tbody>
</table>

Transcripts shown are dysregulated >0.7 log2 fold at all time points in biologically replicate experiments. Boldfaced fold-changes indicate *P* < 0.05.
sented ~2.5 and 1.5% of the total population of cells derived from muscle and aortic tissue, respectively. The ability of the sorted cells to take up fluorescent labeled acetylated-LDL (Dil-AcLDL, Biomedical Technologies) and form tubes on Matrigel Basement Membrane Matrix (BD Biosciences) further confirmed their endothelial identity (Supplemental Fig. S2B) (20, 40).

Tie2-expressing myeloid cells have been reported to account for 2–7% of human blood mononuclear cells (39). Our analysis of peripheral blood mononuclear cells by flow cytometry has shown the percentage of CD11b+GFP+ myeloid cells after 8 wk of HFD to be 0.5 ± 0.5% vs. 0.3 ± 0.4% in controls (n = 3). Furthermore, analysis of the specific inflammatory monocyte population described to express Tie2 revealed that only 0.8% of this CD11b+CD115+ population express Tie2 in blood cell suspensions derived from both HFD- and control-fed animals (0.8 ± 0.5% vs. 0.8 ± 0.3%, n = 3). Most importantly, FACS of muscle tissue suspensions from three independent experiments following 8 wk of diet revealed that only 0.8 ± 0.4% of GFP+ cells derived from HFD animals also displayed the monocyte marker CD11b with similar low percentages observed in control animals (0.3 ± 0.5%).

Microarray results. Microarray hybridization data are available in the Gene Expression Omnibus database under series record GSE14898. Tables 2 (aortic) and 3 (muscle) display fold-change of transcripts with |log2(fold-change)| >0.7 at all time points in aorta or by 6 or 8 wk of feeding in the muscle in biologically replicate, dye-reversed hybridization experi-

Table 3. Average log2 fold-change of dysregulated transcripts determined by microarray analysis in leg muscle endothelium following 4, 6, and 8 wk of high-fat diet

<table>
<thead>
<tr>
<th>RefSeq</th>
<th>Name</th>
<th>4 wk</th>
<th>6 wk</th>
<th>8 wk</th>
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<td>NM_009140</td>
<td>chemokine (C-C motif) ligand 2 (Cxcl2)</td>
<td>1.6</td>
<td>1.5</td>
<td>2.5</td>
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<tr>
<td>NM_011338</td>
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<td>2.4</td>
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<td>0.1</td>
<td>0.3</td>
<td>1.8</td>
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<tr>
<td>NM_010185</td>
<td>Fc receptor, IgE, high affinity I, gamma polypeptide</td>
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<td>0.5</td>
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<td>NM_153795</td>
<td>cDNA sequence BC032204</td>
<td>n.d.</td>
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<tr>
<td>NM_028351</td>
<td>R-spondin 3 homolog (Xenopus laevis)</td>
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<tr>
<td>NM_029295</td>
<td>chemokine-like factor</td>
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<td>0.6</td>
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<tr>
<td>NM_008704</td>
<td>expressed in nonmetastatic cells 1, protein</td>
<td>n.d.</td>
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</tr>
<tr>
<td>NM_009930</td>
<td>Mus musculus collagen, type III, alpha 1 (Col3a1)</td>
<td>1.7</td>
<td>1.9</td>
<td>2.1</td>
</tr>
<tr>
<td>NM_053082</td>
<td>tetraspanin 4</td>
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<tr>
<td>NM_009777</td>
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<td>-0.8</td>
<td>-0.6</td>
</tr>
<tr>
<td>NM_010422</td>
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<tr>
<td>NM_026678</td>
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<tr>
<td>XM_486478</td>
<td>ferritin light chain 2</td>
<td>0.0</td>
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</tr>
</tbody>
</table>

Transcripts with both >0.70 log2 fold-change and P < 0.1 in biologically replicate experiments after 6 or 8 wk of the diet are shown. Boldfaced fold changes indicate P < 0.05.
Table 4. Transcripts commonly dysregulated in aortic and skeletal muscle endothelium in response to a high-fat diet

<table>
<thead>
<tr>
<th>RefSeq</th>
<th>Name</th>
<th>4 wk M</th>
<th>4 wk A</th>
<th>6 wk M</th>
<th>6 wk A</th>
<th>8 wk M</th>
<th>8 wk A</th>
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<td>1.5</td>
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<td>1.1</td>
<td>0.7</td>
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<tr>
<td>NM_013590</td>
<td>Lyz1</td>
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<td>3.1</td>
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</tbody>
</table>

Transcripts shown demonstrate >0.5 log2 fold dysregulation in aortic endothelium after 4 wk of feeding and in skeletal muscle endothelium after 6 wk. A, aortic endothelium; M, muscle endothelium. Boldfaced fold-changes indicate P < 0.05.

In the present study, we performed a controlled, comprehensive survey of the transcriptional responses of the endothelium in vivo to a model of Type II diabetes induced by a HFD in Tie2-GFP mice. Our mouse model recapitulates clinical diabetes, which is characterized by weight gain, hyperglycemia, hyperinsulinaemia, and insulin resistance. It is likely that the endothelial dysfunction observed in diabetic patients is a result of the combinatorial effect of these metabolic and hormonal derangements. Our study was not designed to distinguish endothelial responses to any single diabetic change; rather, our analysis integrates all of the diabetic influences on the endothelium, perhaps allowing our assessment to be more physiologically relevant than in vitro studies looking at isolated components of the diabetic milieu.

Our assessment of the endothelial response in both aorta and skeletal muscle addressed the diversity of endothelial cell populations (10) and may thus reflect distinct endothelial contributions to microvascular and macrovascular disease (3). In the present study, we observed a greater response to diet-induced diabetes in the aortic endothelium compared with that of mice, average levels were two- to threefold higher in the HFD mice after 6 wk (104.7 ± 17.1 vs. 38.9 ± 2.9 ng/ml, P < 0.005) and 8 wk (90.7 ± 12.3 vs. 34.4 ± 3.4 ng/ml, P < 0.001) on the diet (Fig. 4A). Furthermore, serum galectin-3 levels correlated well with HOMA-IR after 8 wk of feeding, exhibiting a Pearson correlation coefficient of 0.92 (P < 0.0001, Fig. 4B).

DISCUSSION

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of skeletal muscle, as evidenced by both the amount and onset of dysregulation: we identified 36 transcripts with \( \geq 1.5 \log_2 \) fold-change in the large vessel endothelium by 4 wk, whereas the capillary endothelium of the muscle had only 16 transcripts dysregulated to this degree, and in general, only after 8 wk of HFD (Table 3). This difference in susceptibility to transcriptional regulation may represent the foundation for the physiological findings of Kim et al. (19), who observed an earlier onset of vascular vs. peripheral insulin resistance in response to an HFD.

Exploration of the enhanced biological pathways by DAVID and IPA emphasizes the commonalities and differences between the macrovascular and microvascular transcriptional response. Highly upregulated pathways in the aortic endothelium include those related to signaling, including chemokines, PLC signaling, and signaling events that precede atherosclerosis (Supplemental Fig. S4). Interestingly, by 8 wk of HFD, the liver X receptor/retinoic X receptor (LXR/RXR) activation pathway was significantly upregulated in the aortic endothelium (Supplemental Fig. S4). These receptors have been shown to modulate the expression of genes involved in lipid metabolism in human endothelial cells in vitro (30). Our findings implicate this pathway in the endothelial transcriptional response to an in vivo model of Type II diabetes, and suggests that LXR/RXR agonists may serve a role in modulating lipid metabolism in the macrovasculature. In the microvasculature of the muscle, we observed upregulation of pathways involved in cytoskeletal rearrangements as well as glycolysis and lipid biosynthesis (Supplemental Fig. S4). Biological functions such as molecular transport and “cellular compromise” are overrepresented, including molecules with roles in oxidative stress and cell damage (Supplemental Fig. S3). Other pathways, such as glycerolipid metabolism, and biological functions such as cell-to-cell signaling and interaction and “small molecule biochemistry”, are shared by the two vascular beds (Supplemental Figs. S3 and S4).

Our comprehensive analysis of the transcriptional response of the endothelium to diet-induced diabetes has identified the dysregulation of genes with recognized roles in atherosclerosis, hyperglycemia, insulin resistance, and inflammation, as well as novel transcripts with previously unknown associations with diabetes. Chemokine (C-C motif) ligand 9 and 8 (Ccl9, Ccl8/Mcp-2) were both found to be dysregulated in aortic and skeletal muscle endothelium. The chemokine CCL9 has recently been suggested as a biomarker of atherosclerosis, and serum protein levels of CCL9 have been closely correlated with gene expression (38). The substantial upregulation of CCL8 may be related to activation of TLR4 by circulating free fatty acids (37) but in any event suggests CCL8 as another potential biomarker of inflammation in diabetes. A catalyst of
leukotriene biosynthesis (7), arachidonate 5-lipoxygenase-activating protein (Alox5ap/FLAP), has been implicated in both coronary restenosis and the development of atherosclerosis (9, 17). Endothelial upregulation of Alox5ap upon HFD suggests roles for this protein in vascular inflammation and insulin insensitivity similar to those of 12/15-lipoxygenase (35).

The upregulation of metallothionein 1 (Mti), a zinc-binding molecule important in maintaining redox homeostasis (4), is consistent with our observed downregulation of early growth response 1 (Egr-1) in the skeletal muscle endothelium; transcription of Egr-1 is regulated by the levels of intracellular zinc (4). Also, ferritin light chain 1 (Ftl1) was upregulated in both muscle and aortae, possibly reflecting the presence of oxidized low-density lipoproteins in diabetic endothelium (16). One other transcript dysregulated in both aortic and skeletal muscle endothelium, lysozyme 1 (Lyz1), is involved in the modulation of AGEs (43) and is a vasodilator (27).

The transcriptional upregulation of another AGE-binding protein, galectin-3/AGE-R3, was also observed in the endothelium of the aorta and the skeletal muscle as shown by microarray and RT-PCR (Table 2, Fig. 2). Galectin-3 is a component of the AGE-receptor complex expressed on the surface of renal cells, including endothelial cells, and is important in the binding and removal of AGEs from the circulation (36). Previous in vitro studies using cultured human umbilical vein endothelial cells have demonstrated increased galectin-3 mRNA and protein levels following exposure to AGEs (36). Galectin-3-deficient mice have been shown to display increased glomerular accumulation of AGEs in a model of Type I diabetes and increased ox-LDL and lipoprotein products when fed an atherogenic diet (14, 33). Therefore, the observed marked upregulation of galectin-3 in the aortic endothelia likely reflects elevated AGEs and modified lipids in the diabetic milieu, and suggests a role for galectin-3 in their binding and uptake by the endothelium.

Galectin-3 has been implicated in the progression of cancer due to its antiapoptotic properties, and recent studies suggest that it may contribute to heart failure, possibly due to activation of inflammation and fibrosis (5, 6, 8, 15, 18). A role for galectin-3 in Type I diabetes has been suggested by studies on the Lgals3−/− mouse, which was found to be more resistant to diabeticogenesis following STZ treatment and displayed a lower expression of inflammatory cytokines and macrophages with a reduced ability to produce TNF-α and nitric oxide (26). Expression of galectin-3 was shown to be upregulated in whole aortic lysates from both db/db mice and mice exposed to an HFD (28), and elevated galectin-3 mRNA and protein have been demonstrated in the atherosclerotic lesions of ApoE−/− mice (31). In our present study, we observed upregulation of galectin-3 expression specifically in the diabetic endothelium, which places it at the appropriate site to recruit monocytes, in concert with the other inflammatory mediators demonstrated. In vitro studies have shown that exogenous galectin-3 acts as a chemotactrant for monocytes and macrophages through a G protein-coupled pathway, and it can also induce the proliferation and migration of vascular smooth muscle cells (31). Our studies suggest that the increased galectin-3 observed in diabetes and cardiovascular pathologies is at least partially originating from endothelial cells and potentially contributing to vascular inflammation.

We also found that serum galectin-3 levels were substantially increased in our HFD model. A recent study has similarly reported an elevation of systemic galectin-3 in obese and Type II diabetic patients compared with normal weight individuals (42). Thus, our murine model is accurately recapitulating the response of diabetic patients and should be useful to study the role of galectin-3 in the vascular complications of Type II diabetes. Future studies stimulated by our findings could include examining the effects of galectin-3 inhibitors, such as modified citrus pectin, and the effects of diabetic treatments on galectin-3 levels and vascular pathology (21, 29). However, it remains a possibility that some element of circulating galectin-3 is contributed by cell types such as monocytes and macrophages, which have also been shown to secrete LGALS3 (31, 41).

Another transcript belonging to the galectin family, galec- tin-1 (Lgals1), was observed to have increased expression in the diabetic skeletal muscle endothelium. LGALS1 may play a role in the adhesion of various cell types to the vascular wall. LGALS1 is expressed on the extracellular surface of endothelial cells and can mediate the adhesion of lymphoma cells to endothelial cells of the liver microvasculature (23). Recently, mass spectroscopic analysis of differentially expressed proteins

![Fig. 4. A: galectin-3 soluble protein levels measured by ELISA in serum from mice receiving HFD vs. control for 3, 6, or 8 wk. B: correlation of soluble LGALS3 with HOMA-IR. Data shown as means ± SE (n = 5–11). **P < 0.01, ***P < 0.001.](http://physiolgenomics.physiology.org/10.220.32.247)
in the plasma of Type II diabetic patients revealed a 4.8-fold increase in galectin-1 protein (22). Galectin-1 also binds the neuropilin-1 receptor on the surface of endothelial cells leading to the increased phosphorylation of the VEGFR-2 co-receptor, which activates the stress-activated protein kinase-1/c-Jun NH2-terminal kinase signaling pathway (11) and could influence angiogenesis in diabetic tissues.

Our analysis of the transcriptional response of the endothelium of both aortic and skeletal muscle tissues to an in vivo model of Type II diabetes revealed novel regulation of several transcripts with roles in inflammation, vasoregulation, redox homeostasis, and modulation of responses to AGEs. While a greater number of differentially regulated transcripts were observed in the macrovasculature compared with the microvasculature, commonly dysregulated transcripts were shared by the two vascular beds, suggesting robust and consistent endothelial markers of diabetes. The dysregulated transcripts identified in this study reflect the various metabolic and hormonal derangements that impinge upon the diabetic endothelium and lead to its damage and dysfunction. These findings suggest potential diagnostic biomarkers of the disease and also implicate gene products and pathways that may be targets for the treatment of vascular pathology in Type II diabetes.

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DISCLOSURES

No conflicts of interest (financial or otherwise) are declared by the author(s).

REFERENCES


