Increase in GLUT1 in Smooth Muscle Alters Vascular Contractility and Increases Inflammation in Response to Vascular Injury

Neeta Adhikari, David L. Basi, Marjorie Carlson, Ami Mariash, Zhigang Hong, Ute Lehman, Sureni Mullegama, Edward K. Weir, Jennifer L. Hall

Objective—The goal of this study was to test the contributing role of increasing glucose uptake in vascular smooth muscle cells (VSMCs) in vascular complications and disease.

Methods and Results—A murine genetic model was established in which glucose transporter 1 (GLUT1), the non–insulin-dependent glucose transporter protein, was overexpressed in smooth muscle using the sm22α promoter. Overexpression of GLUT1 in smooth muscle led to significant increases in glucose uptake (n=3, P<0.0001) as measured using radiolabeled 2-deoxyglucose. Fasting blood glucose, insulin, and nonesterified fatty acids were unchanged. Contractility in aortic ring segments was decreased in sm22α-GLUT1 mice (n=10, P<0.04). In response to vascular injury, sm22α-GLUT1 mice exhibited a proinflammatory phenotype, including a significant increase in the percentage of neutrophils in the lesion (n=4, P<0.04) and an increase in monocyte chemoattractant protein-1 (MCP-1) immunofluorescence. Circulating haptoglobin and glutathione/total glutathione were significantly higher in the sm22α-GLUT1 mice postinjury compared with controls (n=4, P<0.05), suggesting increased flux through the pentose phosphate pathway. sm22α-GLUT1 mice exhibited significant medial hypertrophy following injury that was associated with a significant increase in the percentage of VSMCs in the media staining positive for nuclear phosphoSMAD2/3 (n=4, P<0.003).

Conclusion—In summary, these findings suggest that increased glucose uptake in VSMCs impairs vascular contractility and accelerates a proinflammatory, neutrophil-rich lesion in response to injury, as well as medial hypertrophy, which is associated with enhanced transforming growth factor-β activity. (Arterioscler Thromb Vasc Biol. 2011;31:86-94.)

Key Words: Glut1 • hypertrophy • vascular smooth muscle • haptoglobin • phosphoSMAD2/3 • glucose • neutrophil • macrophage

Cardiovascular complications remain the number one cause of death from individuals with diabetes. Epidemiological studies to date have reported conflicting results over the role of glucose as a contributing risk factor to coronary artery disease in individuals with type 1 diabetes.1–9 A recent report found that glycohemoglobin in nondiabetic adults was strongly associated with coronary artery disease, further suggesting that increasing cellular glucose uptake promotes vascular complications and coronary artery disease.10 However, the mechanisms through which glucose increases the risk of coronary artery disease are not well understood. Specifically, the role of increasing glucose uptake in different cell types in contributing to coronary artery disease is not well understood.

We tested the hypothesis that increasing glucose uptake in vascular smooth muscle cells would alter the contractility properties of the vessel. In addition, we tested the hypothesis that in response to vascular injury, increased glucose uptake would exacerbate vascular intima formation.

We used a genetic approach to increase expression of the non–insulin-dependent glucose transporter protein GLUT1 in VSMCs using the sm22α promoter. This led to a significant increase in glucose uptake in smooth muscle without altering circulating levels of glucose, insulin, or nonesterified fatty acids (NEFA). The advantage of this model is the ability to specifically isolate the effects of glucose in the absence of alterations in free fatty acids and insulin specifically in VSMCs in the vessel wall. This model allows us to study how enhanced glucose uptake in VSMCs over the life span of the mouse alters the phenotype of the vasculature. These results represent novel findings, as well as verification of recent work in other new genetic models that are leading to a better

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understanding of how glucose increases the risk of coronary artery disease.

Materials and Methods

Genetic Model

sm22α-GLUT1 mice were generated by using the well-characterized 441-bp region (exon 1 to +41) of the sm22α promoter14 and the full-length human GLUT1 cDNA (a gift from Dr Mike Mueckler, Department of Cell Physiology, Washington University of St. Louis, Mo). The fragment for injection containing the 441-bp sm22α promoter (including exon 1 to +41), human glut1, and SV40 polyA region was removed by digestion with NotI and XhoI, and the fragment was run on a 0.8% gel and excised. The injection concentration was 4 ng/μL. Standard microinjection into C57BL/6 fertilized eggs and implantation into foster females was completed at the Mouse Genetics Laboratory at the University of Minnesota. Mice remained in a C57BL/6 background. Primers for genotyping were (sm22α-forward) 5’-TAAACC-CCTCACCCAGCGGGCGCCC-3’ and (HuGLUT-reverse) 5’-CCGGATTAGAGTATGCTCAACGACGCGG-3’. Mice were euthanized according to approved institutional animal care and use committee protocol with a compressed-air carbon dioxide chamber.

Quantitative Real-Time Polymerase Chain Reaction

RNA was isolated from murine aorta with Qiagen’s RNeasy kit and reverse transcribed to cDNA according to manufacturer’s directions (Clontech). Transcript abundance was analyzed using the Applied Biosystems primer probe sets and the Applied Biosystems 7900HT instrument (see Supplemental Table I, available online at http://atvb.ahajournals.org). Target amplification and detection were performed in replicates, following, for minimizing the occurrence of experimental variability and calculation of ΔΔCt based on the corresponding reference control, hypoxanthine phosphoribosyltransferase 1 (HPRT1) (ie, target ΔCt/cell)12.

Western Blotting

Western blotting was performed on murine aorta by standard methods as previously described14 using the following primary antibodies: GLUT1 (a gift from Frank Brosius, University of Michigan and Chemicon), myosin light chain (catalog no. 3672, Cell Signaling Technology), calponin (catalog no. sc-28545, Santa Cruz Biotechnology), smooth muscle α-actin (catalog no. A2547, Sigma-Aldrich), osteopontin (catalog no. AB10910, Millipore), caldesmon (catalog no. 2980, Cell Signaling Technology), rock I (Cat #4035, Cell Signaling Technology), rock II (catalog no. sc-5561, Santa Cruz Biotechnology), fibronectin (catalog no. ab27350, Abcam), tubulin (catalog no. 2148, Cell Signaling Technology), and vinculin (catalog no. V4139, Sigma-Aldrich). Secondary antibodies included goat anti-rabbit IgG–horseradish peroxidase (catalog no. sc-2004, Santa Cruz Biotechnology), goat anti-rabbit IgG–horseradish peroxidase (catalog no. sc-2030, Santa Cruz Biotechnology), and goat anti-mouse horseradish peroxidase (catalog no. 1858413, Pierce). Substrates used for developing the blot included SuperSignal West Dura Extended Duration Substrate (catalog no. 34075, Thermo Scientific), SuperSignal West Femto Maximum Sensitivity Substrate (catalog no. 34095, Thermo Scientific), SuperSignal West Pico Chemiluminescent Substrate (catalog no. 34080, Thermo Scientific), and Pierce ECL Western Blotting Substrate (catalog no. 32106, Thermo Scientific).

Metabolic Measurements

Metabolic measurements were performed as previously described.14-17 Briefly, fasting blood glucose was monitored with a handheld clinical glucometer (Roche), fasting NEFA were measured with a handheld clinical glucometer (Roche), and insulin was measured with an ultrasensitive mouse insulin ELISA kit from Alpco Diagnostics. Glucose uptake was measured with radiolabeled 2-deoxyglucose in primary VSMCs in the presence and absence of cytochalasin B, as we have described.14 Samples were incubated for 30 minutes in glucose-free minimum essential medium (MEM), 2-[3H]Deoxyglucose (0.33 μCi) was added for an additional 5 to 15 minutes (we confirmed that transport was linear during this interval), washed with ice-cold buffer containing phloretin (0.1 mmol/L) to terminate transport, solubilized in 1 N NaOH, neutralized, and counted in a beta scintillator. Glucose uptake was normalized to protein content.

Isolation of VSMCs

Aortic smooth muscle cells were isolated from 16-week-old mice according to the protocol by Ray et al.18 Briefly, aortas were harvested and digested in collagenase type 2. Cell were plated and maintained in high-glucose Dulbecco’s modified Eagle’s medium supplemented with 1% penicillin and streptomycin and 10% fetal calf serum.

Vascular Contractility

Aortic ring segments (nondenuded) were mounted on stirrups in ring baths in Earle’s balanced salts solution as previously described19 and allowed to equilibrate at a resting tension of 400 mg for at least 60 minutes. Aortic segments were exposed 3 times to 60 mmol/L KCl at 30-minute intervals. The contractile responses to 1 μmol/L phenylephrine (PE) and 10 μmol/L serotonin were then assessed in random order with washout and reequilibration periods of 30 minutes. Vascular relaxation measurements were also performed in nondenuded aortic rings in response to 10-6 mol/L acetylcholine. No differences were identified in vascular relaxation properties between groups (data not shown).

Surgical Intervention to Induce Vascular Lesion

All protocols were in accordance with institutional animal care and use committee guidelines. Briefly, a straight guide wire (0.38 mm in diameter) was inserted into the left femoral artery of anesthetized mice via a small muscular branch as described.20,21 The wire was left in the lumen for 1 minute to denude and dilate the artery. After the wire was removed, the small branch was tied off, and blood flow was restored in the injured vessel.

Morphometric Measurements

Morphometric measurements of femoral arteries collected at 7, 14, and 28 days after wire injury. Mice were perfused with PBS followed by 10% neutral-buffered formalin (In Vivo Rodent Perfusion System, Automate Scientific). Morphometric evaluation of femoral arteries was performed by staining serial 5-μm-thick paraffin-embedded sections with hematoxylin-eosin and examination under an Olympus BX41 microscope. Sections (8 to 10 per mouse) covering 500 μm of femoral artery length were measured for internal elastic lamina length, external elastic lamina length, and intimal and medial areas. All the measurements were performed using NIH Imagej software.20

Immunostaining

Five-micrometer sections of paraffin-embedded femoral arteries from injured and uninjured animals was stained for GLUT1 (a gift from Dr Brosius). Macrophages (anti-Mac3, BD Biosciences; anti-F4/80, Serotec) and neutrophils (anti-Ly6G antibody, Novus Biologicals) were identified by using the respective IgG ABC Vectastain kit according to the manufacturer’s protocol (Vector Laboratories). Elastin was assessed by Verhoeff Van Giesen stain. VSMCs were identified by immunostaining with smooth muscle α-actin (clone 1A4, Sigma-Aldrich). Proliferating VSMCs were identified by costaining with Ki-67 (catalog no. RM-9106, Thermo Scientific). Apoptotic cells were detected by terminal deoxynucleotidyl transferase dUTP nick-end labeling (catalog no. 11 684 795 910, Roche Diagnostics). Neutrophils, macrophages, and VSMCs (terminal deoxynucleotidyl transferase dUTP nick-end labeling– and Ki67-positive) in the sections were imaged and quantified at ×60 on a Zeiss AxioVision Upright microscope (n=4 animals, 3 or 4 sections per animal, per cohort) and are represented as total cells in the lesion at 7 days postinjury. PhosphoSMAD-stained nuclei were identified by using an anti-phosphoSMAD2/3 antibody (ab65847, Abcam), imaged and quantified at ×100 on a Zeiss AxioVision Upright microscope.
Confocal Microscopy

Confocal microscopy of MCP-1 staining was performed using an anti-MCP-1 antibody (Santa Cruz Biotechnology) followed by secondary incubation in Alexa Fluor 488 (Invitrogen) in sections of vessels 7 days postinjury (Olympus Fluoview Confocal microscope, 60X magnification, n=3 animals, 3 or 4 sections per animal, per cohort).

Measurement of Circulating Factors

Measurements of circulating factors in the serum of 16-week-old male mice were performed using the following kits: haptoglobin (ELISA, Alpco Diagnostics, 41-HAPMS-E01), MCP-1 (ELISA, R&D Systems), and glutathione (GSH)/total GSH (Abcam). All assays were run according to the manufacturers’ instructions.

Statistical Analysis

Values are represented as mean±SE. An ANOVA with a Tukey post hoc test was used for multiple comparisons. A Student t test was used for comparisons between 2 groups.

Results

The goals of this study were to determine whether increasing glucose uptake in smooth muscle specifically contributed to changes in vascular phenotype at baseline and in response to injury. We established a novel murine model in which the non–insulin-dependent glucose transporter protein, GLUT1, was upregulated in smooth muscle. Increased immunostaining of GLUT1 was seen in the medial wall of sm22α-GLUT1 femoral arteries compared with controls (Figure 1a and 1b). GLUT1 protein levels in aorta of sm22α-GLUT1 mice were also increased (Figure 1c). The increase in GLUT1 resulted in

Table 1. Fasting Blood Glucose, Insulin, and NEFA Levels in Serum From 8- to 12-Week-Old Control and sm22α-GLUT1 Mice

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Fasting Blood Glucose (mg/dL)</th>
<th>Insulin (mIU/ml)</th>
<th>NEFA (mEq/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>135±7</td>
<td>3.6±0.3</td>
<td>1.3±0.8</td>
</tr>
<tr>
<td>sm22α-GLUT1</td>
<td>128±7</td>
<td>3.7±0.6</td>
<td>1.1±0.03</td>
</tr>
</tbody>
</table>

Data represented as mean±SE, n=3 to 16 per group per test, P=not significant.

Figure 2. Contractility of aortic ring segments from sm22α-GLUT1 mice was significantly reduced in the presence of PE (P<0.03) or serotonin (5-HT) (P<0.04). Aortic ring segments (control, n=10, and sm22α-GLUT1, n=10) were allowed to equilibrate at a resting tension of 400 mg for at least 60 minutes before exposure to 60 mmol/L potassium chloride (KCl) at 30-minute intervals, three times. The contractile responses to 1 μmol/L PE and 10 μmol/L 5-HT were then assessed in random order with washout and reequilibration periods of 30 minutes.

Figure 3. a, Western analysis of proteins in aorta from control and sm22α-GLUT1 mice. b, Fibronectin expression in sm22α-GLUT1 (n=4) was significantly decreased compared with control aorta (n=4, P=0.003).
increased glucose uptake as measured by radiolabeled 2-deoxyglucose in VSMCs isolated from male adult mice (P<0.0001) (Figure 1d). Fasting blood glucose, insulin, and NEFA concentrations were not significantly different in sm22α-GLUT1 mice compared with controls (see Table 1). Medial thickness (data not shown) and smooth muscle cell number in the medial wall of the femoral artery were unaffected (54±9 VSMCs versus 52±5 VSMCs in control and sm22α-GLUT1 respectively, not significant). Neutrophils and macrophages in the vessel wall were also not different (data not shown).

Next, we assessed vascular contractility in aortic rings. Overexpression of GLUT1 was associated with a significant decrease in vascular contractility in response to PE (P<0.03) and serotonin (P<0.04) (Figure 2). To examine the potential mechanisms whereby vascular contractility was decreased in response to increased glucose uptake, we assessed expression of extracellular matrix proteins, contractile proteins, matrix, and calcium handling targets via Western blotting (Figure 3a) and gene expressions via quantitative real-time polymerase chain reaction (Supplemental Table I). Fibronectin, a component of the basal lamina of the smooth muscle layer was decreased in the sm22α-GLUT1 aorta as measured by densitometry (Figure 3a and 3b) (n=4, P<0.003). The matrix gene, decorin harboring a glucose response element in the
promoter region, exhibited a minor yet statistically significant increase in sm22α-GLUT1 aorta (Supplemental Table 1) (n = 8 to 9/group, P < 0.04). No significant differences were detected in decorin at the protein level (data not shown). Thus, increased glucose uptake resulted in decreased expression protein expression of fibronectin and an increase in the RNA expression of the matrix gene decorin, which contains a glucose response element in the promoter region.

We used a well-established femoral artery wire injury model to determine whether increased glucose uptake in smooth muscle affected the process of vascular remodeling. Femoral arteries were harvested 7, 14, and 28 days postinjury. Seven days after injury, a significant increase in the number of inflammatory cells was seen in the lesion of sm22α-GLUT1 mice compared with controls (n = 4 per group, P < 0.05) (Figure 4a to 4c). A specific increase in neutrophils was detected in the sm22α-GLUT1 mice by staining with anti-Ly6G antibody (Figure 4d to 4f). The number of macrophages in the lesion 7 days postinjury was not different between the groups (Figure 4g to 4i). The number of VSMCs in the lesion from sm22α-GLUT1 mice was significantly lower as compared with controls 7 days postinjury (control, 53 ± 4, and sm22α-GLUT1, 24 ± 1*, P < 0.002). Despite the reduced total number of VSMCs in the lesions of sm22α-GLUT1, a greater percentage of these VSMCs were proliferating, as assessed by Ki67 staining, compared with control mice (15 ± 3 and 6 ± 1, respectively, *P < 0.02). Apoptosis measurements of VSMCs in the intima were not different between the sm22α-GLUT1 and control mice. The increase in neutrophils in the sm22α-GLUT1 mice was associated with an increase in MCP-1 staining in the vessel (Figure 5a to 5c). In addition, circulating MCP-1 was significantly higher in sm22α-GLUT1 in response to injury (Figure 5d).

In response to vascular injury, the sm22α-GLUT1 mice also exhibited an increase in circulating haptoglobin and GSH (Table 2). The significant increase in these 2 factors suggests increased flux through the pentose phosphate shuttle in the sm22α-GLUT1 mice in response to injury.

No change in the intimal area was detected between the control and sm22α-GLUT1 mice at 14 or 28 days postinjury (Table 3). Sm22α-GLUT1 mice exhibited a significant increase in medial area at 28 days (medial hypertrophy) (Table 3). We hypothesized that the increase in medial area in the sm22α-GLUT1 mice was due to activation of the transforming growth factor-β (TGF-β) signaling pathway in the nucleus of medial VSMCs. To test this hypothesis, we stained injured vessels from sm22α-GLUT1 and control mice for phosphoSMAD2/3 – a molecular marker of activated TGF-β signaling. Figure 6a to 6c shows a significant increase in the percentage of VSMC nuclei staining positive for phosphoSMAD2/3 in the media of sm22α-GLUT1 mice compared with control mice at 28 days postinjury. The number of medial VSMCs at 28 days was not significantly different between the groups (control 42 ± 3 and sm22α-GLUT1 48 ± 6, n = 4, P = not significant). Thus, the increase in medial area combined with the increase in phosphoSMAD2/3 staining in the media supports a hypertrophic signal in the vessel wall in response to injury.

**Discussion**

The major findings from this study are that increasing glucose uptake in vascular smooth muscle via overexpression of GLUT1 impairs contractility of the vessel wall and potentates inflammation and medial hypertrophy in response to vascular injury. The decreased contractility was accompanied by a significant loss of fibronectin in the sm22α-GLUT1 mice. In response to vascular injury, sm22α-GLUT1 mice exhibited a significant increase in neutrophils in the lesion.

![Figure 5](image_url)

**Table 2. Circulating Haptoglobin and GSH Levels in Serum From Control and sm22α-GLUT1 Mice**

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>sm22α-GLUT1</th>
<th>GSH/Total GSH (µg/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Haptoglobin (µg/mL)</td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>85 ± 1 (n = 6)</td>
<td>92 ± 19 (n = 6)</td>
<td>0.072 ± 0.004 (n = 8)</td>
</tr>
<tr>
<td>7</td>
<td>302 ± 194 (n = 4)</td>
<td>1322 ± 596 (n = 4), P &lt; 0.05</td>
<td>0.095 ± 0.020 (n = 3)</td>
</tr>
</tbody>
</table>

Serum was collected from control and sm22α-GLUT1 mice at 7 days postinjury. Haptoglobin was assessed by ELISA (Alpco Diagnostics). GSH was detected by a fluorometric assay performed according to the manufacturer’s instructions (Abcam). GSH levels are represented as a ratio of GSH to total GSH. Values are mean ± SE, and statistical significance is compared with corresponding sm22α-GLUT1 values at day 0.
that was associated with elevated circulating MCP-1, as well as increased localized MCP-1 staining in the vessel wall. Finally, increased expression of GLUT1 in smooth muscle resulted in a significant increase in medial hypertrophy that was associated with an increase in phospho-SMAD2/3 staining, indicative of enhanced TGF-β activity.

The decrease in vascular contractility in a mouse model with significant increases in glucose uptake has been reported in previous models of diabetic animal models.22–24 This impaired vascular contractility was associated with a decrease in fibronectin. Recent work has identified a role for multiple extracellular matrix proteins and arterial stiffness and contractility, including fibronectin.25–28 Fibronectin binds to many different integrin binding proteins that regulate both contraction and relaxation. A limitation of this study is that we did not perform this experiment on vessels with denuded endothelium. We did, however, perform a small number of experiments in which we assessed vascular relaxation in response to acetylcholine and did not detect differences between the sm22α-GLUT1 and control vessels (data not shown). Finally, we found no loss of elastin by Verhoeff Van Giesen staining (data not shown), which has been reported in previous models of diabetes.29,30 More work will be needed to determine the mechanisms governing the decrease in contractility in response to increased glucose metabolism.

We identified a significant increase in circulating haptoglobin in response to injury in sm22α-GLUT1 mice. Haptoglobin is a serum protein that binds to extracorporeal hemoglobin. Released from red blood cells within a hemorrhaging plaque, extracorporeal hemoglobin induces a potent stimulus for inflammation.31 Haptoglobin binding to extracorporeal hemoglobin attenuates the inflammatory and oxidative potential.31 Haptoglobin is significantly increased in both type 1 and type 2 diabetic individuals32,33 and also in response to acute coronary syndrome, inflammation, tissue destruction, and neoplasia.34–36 The increase in circulating haptoglobin in diabetic individuals and in mice with increased GLUT1 suggests a common mechanism of increased glucose metabolism. A landmark genetic finding in 1963 identified an association between deficiency in the enzyme glucose-6-phosphate dehydrogenase and decreased serum haptoglobin.37 Glucose-6-phosphate dehydrogenase is a rate-determining enzyme in the pentose phosphate shuttle. The link between glucose metabolism and haptoglobin appears to be through the pentose phosphate pathway. Suzuki et al showed that flux through the pentose phosphate pathway is minimal under resting conditions in vascular smooth muscle cells (4% under low-glucose conditions and 8% under high-glucose conditions).38 In response to vascular injury, haptoglobin is significantly increased in the sm22α-GLUT1 mice, suggesting that flux through the pentose phosphate pathway is significantly increased postinjury. GSH levels are also increased in response to injury in the sm22α-GLUT1 mice, a second indication that flux through the pentose phosphate pathway may be enhanced. Regardless, these data suggest that increased glucose flux in VSMCs promotes 2 antioxidative signaling pathways, suggesting 2 protective mechanisms of glucose flux.

We postulated that MCP-1 was significantly elevated in sm22α-GLUT1 mice. This hypothesis was based on previous work showing increased MCP-1 in diabetic animal models and in response to high glucose.39,40 In line with this hypothesis, MCP-1 immunostaining in the vessel wall and circulating MCP-1 levels were significantly elevated in sm22α-GLUT1 mice. Burke et al reported that the inflammatory cell composition of lesions in humans with type 1 and type 2 diabetes is increased compared with nondiabetics.41 Booth et al demonstrated increased leucocyte rolling and adherence in response to glucose infusion in the rat.42 A novel model to study the effect of glucose on vascular inflammation has also been developed in which diabetes is induced through a virus in an low-density lipoprotein receptor–deficient background. In this model, inflammation is enhanced through increased macrophage accumulation.43,44 Olive et al demonstrated the involvement of inflammatory cells, including neutrophils and macrophages, in neointimal burden following vascular injury in C57BL/6 mice maintained on a normal diet.45 Similarly, in our model of overexpressing GLUT1, in a C57BL/6 background, we identified macrophages, as well as neutrophils, in the lesion at 7 days postinjury. We did not detect an increase in macrophage accumulation in injured vessels but instead identified an increase in neutrophils in the vessel wall that was associated with an increase in MCP-1 staining in the vessel and circulating in the blood. These findings support and extend earlier work by Torreggiani et al in which a transgenic mouse model with decreased circulating levels of advanced glycosylated end products exhibited decreased inflammation.46 Few studies have assessed the role of glucose in vascular inflammation in the absence of alterations in insulin or lipids. The work presented in this study adds new information to the field and suggests that increased glucose flux in smooth muscle promotes proinflammatory signaling pathways mediated in part through MCP-1 that recruit neutrophils to the vessel wall.
Finally, the medial wall of the GLUT1-overexpressing mice underwent significant hypertrophy, but only in response to injury. Previous work has shown an essential role for TGF-β–Smad3 signaling in vascular smooth muscle cell hypertrophy.47,48 We hypothesized that the increase in medial area seen in the sm22α-GLUT1 mice was mediated in part through activation of the TGF-β–Smad3 pathway. We identified an increase in phospho-SMAD2/3 immunostaining specific for VSMCs within the media of sm22α-GLUT1 mice compared with control mice in response to injury. This correlated with the increased medial thickness in the sm22α-GLUT1 mice. Previous studies have shown a role of TGF-β–Smad3 pathway in glucose-induced hypertrophy.49,50 The increase in number of phospho-SMAD2/3 stained nuclei, together with the absence of any significant difference in the number of medial VSMCs in sm22α-GLUT1, supports a role for the TGF-β–Smad3 pathway in glucose-dependent hypertrophic signal in the vessel wall in response to injury.

We did not detect any differences in intimal hyperplasia in response to vascular injury. Early work by Kunjathoor et al51 showed that the effect of diabetes on lesion formation (atherosclerosis) is strain dependent (BALB/c versus C57BL/6), suggesting that the role of glucose, fat, and cholesterol in lesion development is dependent on the underlying genetic structure of these mouse strains. C57BL/6 mice showed extensive lesion development in response to fat feeding alone.52 Earlier work by Nishina et al using the C57BL6 strain showed that mutations in genes that predisposed these mice to diabetes or obesity did not alter lesion formation when fed a high-fat diet.52 In children with type 1 diabetes, aortic/intima53 media thickness but not carotid intima/media thickness was found to correlate with glycosylated hemoglobin.53 A study by Larsen et al also found a significant correlation between HbA1c and carotid intima/media thickness.54 Jensen-Urstad et al found that intensified conventional insulin treatment in patients with type 1 diabetes decreased carotid intima/media thickness and arterial wall stiffness compared with standard insulin therapy.55 Thus, our data would suggest that increased glucose uptake in VSMCs in the absence of vascular intervention does not induce hypertrophy or proliferation of VSMCs. These findings are in agreement with our previous in vitro studies supporting a role for increased glucose metabolism and increased cell cycle progression.56 In large-animal diabetic models, previous work has shown that high glucose does not directly stimulate proliferation in the absence of injury.38 Our work in the uninjured vessels supports this.

In summary, we have used a genetic approach to provide new insights into the role of glucose in vascular complications. This study specifically identifies a role for increased glucose uptake into VSMCs in contributing to altered vascular contractility. In addition, the increased glucose uptake in VSMCs potentiated inflammatory signaling pathways in the vessel wall. However, increased glucose uptake restricted to VSMCs did not alter the size of the lesion in response to vascular intervention, but it did result in medial hypertrophy at 28 days.

**Acknowledgments**

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**Disclosures**

None.

**References**

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### Supplemental Table I. RNA abundance in control and sm22α-GLUT1 aorta.

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<tr>
<th>Gene</th>
<th>Primer ID</th>
<th>Control</th>
<th>sm22α-GLUT1</th>
<th>p value</th>
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<tr>
<td>Decorin</td>
<td>Mm00514535_m1</td>
<td>0.047 ± 0.007</td>
<td>0.065 ± 0.003</td>
<td>p &lt; 0.04</td>
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<td>Versican</td>
<td>Mm00514535_m1</td>
<td>0.868 ± 0.234</td>
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<td>Mm00483888_m1</td>
<td>0.457 ± 0.240</td>
<td>0.155 ± 0.044</td>
<td>ns</td>
</tr>
<tr>
<td>Procollagen</td>
<td>Mm01302043_g1</td>
<td>0.083 ± 0.017</td>
<td>0.105 ± 0.031</td>
<td>ns</td>
</tr>
<tr>
<td>Integrin α1</td>
<td>Mm01306364_m1</td>
<td>1.033 ± 0.085</td>
<td>1.137 ± 0.094</td>
<td>ns</td>
</tr>
<tr>
<td>Integrin α5</td>
<td>Mm00439797_m1</td>
<td>0.095 ± 0.020</td>
<td>0.079 ± 0.016</td>
<td>ns</td>
</tr>
<tr>
<td>Integrin αV</td>
<td>Mm01339545_m1</td>
<td>2.106 ± 0.586</td>
<td>2.759 ± 0.858</td>
<td>ns</td>
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<tr>
<td>Integrin β3</td>
<td>Mm00443980_m1</td>
<td>1.994 ± 0.408</td>
<td>2.332 ± 0.467</td>
<td>ns</td>
</tr>
<tr>
<td>Integrin β1</td>
<td>Mm01253227_m1</td>
<td>0.037 ± 0.004</td>
<td>0.047 ± 0.006</td>
<td>ns</td>
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<tr>
<td>SM MHC</td>
<td>Mm01344360_m1</td>
<td>0.045 ± 0.007</td>
<td>0.063 ± 0.014</td>
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<tr>
<td>SMα-actin</td>
<td>Mm01546133_m1</td>
<td>0.002 ± 0.001</td>
<td>0.003 ± 0.001</td>
<td>ns</td>
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<tr>
<td>Transgelin</td>
<td>Mm00441660_m1</td>
<td>0.008 ± 0.001</td>
<td>0.010 ± 0.002</td>
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<tr>
<td>CCR2</td>
<td>Mm01275521_m1</td>
<td>0.251 ± 0.024</td>
<td>0.250 ± 0.034</td>
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<tr>
<td>CXCL2 (MIP2)</td>
<td>Mm00436450_m1</td>
<td>0.072 ± 0.012</td>
<td>0.065 ± 0.019</td>
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<tr>
<td>CXCR4</td>
<td>Mm01292123_m1</td>
<td>0.291 ± 0.060</td>
<td>0.209 ± 0.025</td>
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<td>EGFR</td>
<td>Mm01187858_m1</td>
<td>0.474 ± 0.069</td>
<td>0.359 ± 0.049</td>
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<tr>
<td>CXCR2</td>
<td>Mm00438258_m1</td>
<td>0.230 ± 0.105</td>
<td>0.140 ± 0.051</td>
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<tr>
<td>PDGFRb</td>
<td>Mm0126248_m1</td>
<td>2.486 ± 0.321</td>
<td>1.854 ± 0.221</td>
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<tr>
<td>COX 1</td>
<td>Mm00477214_m1</td>
<td>4.176 ± 0.455</td>
<td>3.705 ± 0.334</td>
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<tr>
<td>MPO</td>
<td>Mm00447886_m1</td>
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<td>0.059 ± 0.008</td>
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<tr>
<td>Ptgs1</td>
<td>Mm00477214_m1</td>
<td>4.176 ± 0.455</td>
<td>3.705 ± 0.334</td>
<td>ns</td>
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<tr>
<td>TGFβ RI</td>
<td>Mm03024015_m1</td>
<td>7.672 ± 1.055</td>
<td>6.337 ± 1.112</td>
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<td>Gene</td>
<td>Hs003024091_m1</td>
<td>Expression 1</td>
<td>Expression 2</td>
<td>P-value</td>
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<td>TGFβ RII</td>
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<td>4.488 ± 0.377</td>
<td>4.036 ± 0.260</td>
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<td>VEGFR1</td>
<td>Mm00438983_m1</td>
<td>0.816 ± 0.128</td>
<td>1.038 ± 0.093</td>
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</tbody>
</table>

**Abbreviations.** SM MHC, smooth muscle myosin heavy chain; SMα-actin, smooth muscle alpha actin; CCR2, chemokine receptor 2; CXCL2 (MIP2), chemokine ligand 2; CXCR4, chemokine receptor 4; EGFR, epidermal growth factor receptor; CXCR2, chemokine receptor 2; PDGFR, platelet derived growth factor receptor; COX1, cyclooxygenase 1; MPO, myeloperoxidase; Ptgs1, prostaglandin-endoperoxide synthase 1; TGFβ R1; transforming growth factor beta receptor 1; TGFβ RII, transforming growth factor receptor beta receptor 2, VEGFR1, vascular endothelial growth factor receptor 1; ns, not significant.

Values are expressed as mean ± SE, normalized to HPRT1 and expressed as ΔΔCT, n = 8-9 vessels per group.