Down-regulation of Insulin Receptor Substrate 1 during Hyperglycemia Induces Vascular Smooth Muscle Cell Dedifferentiation*

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Diabetes is a major risk factor for the development of atherosclerosis, but the mechanism by which hyperglycemia accelerates lesion development is not well defined. Insulin and insulin-like growth factor 1 (IGF-I) signal through the scaffold protein insulin receptor substrate 1 (IRS-1). In diabetes, IRS-1 is down-regulated, and cells become resistant to insulin. Under these conditions, the IGF-I receptor signals through an alternate scaffold protein, SHPS-1, resulting in pathophysiologic stimulation of vascular smooth muscle cell (VSMC) migration and proliferation. These studies were undertaken to determine whether IRS-1 is functioning constitutively to maintain VSMCs in their differentiated state and, thereby, inhibit aberrant signaling. Here we show that deletion of IRS-1 expression in VSMCs in non-diabetic mice results in dedifferentiation, SHPS-1 activation, and aberrant signaling and that these changes parallel those that occur in response to hyperglycemia. The mice showed enhanced sensitivity to IGF-I stimulation of VSMC proliferation and a hyperproliferative response to vascular injury. KLF4, a transcription factor that induces VSMC dedifferentiation, was up-regulated in IRS-1−/− mice, and the differentiation inducer myocardin was undetectable. Importantly, these changes were replicated in wild-type mice during hyperglycemia. These findings illuminate a new function of IRS-1: that of maintaining cells in their normal, differentiated state. Because IRS-1 is down-regulated in states of insulin resistance that occur in response to metabolic stresses such as obesity and cytokine stimulation, the findings provide a mechanism for understanding how patients with metabolic stress and/or diabetes are predisposed to developing vascular complications.

Diabetes is a major predisposing factor for the development of atherosclerosis. The diabetes control complications trial showed that patients with type 1 diabetes who maintained a hemoglobin A-1 C value 1.2% lower than control subjects for 7 years had a significantly reduced rate of vascular disease events during the 20-year follow-up period (1). Similarly the United Kingdom prospective trial demonstrated a significant benefit of lowering glucose in type 2 diabetics on cardiovascular risk (2). Despite intense analysis, the molecular mechanism by which glucose lowering results in a clinical benefit has remained poorly defined. Insulin and insulin-like growth factor 1 (IGF-I)2 signal through a scaffold protein termed insulin receptor substrate 1 (IRS-1) (3). The insulin and IGF-I receptor tyrosine kinases directly phosphorylate IRS-1, and these phosphoryrosines recruit the p85 subunit of PI3K and Grb-2, thereby activating the PI3K and MAPK pathways (4). In differentiated cells, these IRS-1-linked signaling cascades promote glucose influx; glycogen, lipid, and protein synthesis; as well as changes in gene expression (5). Under normal physiologic conditions, both insulin and IGF-I stimulate differentiated cell functions through IRS-1 activation (6–7). In response to hyperglycemia, proinflammatory cytokines, or nutrient excess, IRS-1 is down-regulated in multiple tissues, and insulin signaling is impaired (8–9). In cell types that are capable of undergoing dedifferentiation, such as vascular smooth muscle cells (VSMCs) and endothelial cells, IGF-I utilizes an alternative mechanism to stimulate PI3K and MAPK activation (10). IGF-I stimulates tyrosine phosphorylation of the scaffolding protein Src homology 2 domain-containing protein tyrosine phosphatase substrate 1 (SHPS-1), which recruits signaling elements that activate both the PI3K and MAPK pathways, leading to enhanced cell migration and proliferation (11). In contrast, under the same hyperglycemic conditions, overexpression of IRS-1 down-regulates SHPS-1 expression, thereby restoring signaling through IRS-1, which inhibits these pathophysiologic changes (12). Dedifferentiation of VSMCs has been implicated as a major event in atherosclerotic lesion formation (13). Several signaling events by which external stimuli such as hyperlipidemia and cytokine expression control differentiation have been reported, but the changes in signaling that lead to dedifferentiation of VSMCs during hyperglycemia have not been characterized. Based on those observations, we investigated whether IRS-1-mediated signaling was required to maintain VSMC differentiation and whether decreased expression of IRS-1 led to dedifferentiation and altered sensitivity to stimulation of cell proliferation.

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2 The abbreviations used are: IGF, insulin-like growth factor; IRS, insulin receptor substrate; VSMC, vascular smooth muscle cell; AKT, protein kinase B; PCNA, proliferating cell nuclear antigen; UNC, University of North Carolina.
Results

To determine the effect of loss of expression of IRS-1, we prepared mice in which expression had been selectively deleted in VSMCs. As shown in Fig. 1, IRS-1 expression was eliminated in VSMCs but not in the liver, kidney, heart, skeletal muscle (Fig. 1A), or endothelium (Fig. 1B). Similarly, IRS-2 was selec-
tively deleted in the aorta but not in the liver (Fig. 1C). Furthermore, no compensatory increase in IRS-2 expression was detected in the aortas of IRS-1−/− mice and vice versa (Fig. 1, D and E). There was no difference in body weight or organ size among the groups. However, smooth muscle-specific deletion of IRS-1 or IRS-2 resulted in a significant reduction in the weight of the aorta compared with control animals, and the effect of IRS-1 deletion was significantly greater than deleting IRS-2 (Fig. 1F). Similar results were obtained when the aortic wall thickness (e.g., control, 97 ± 3; IRS-1−/−, 89 ± 2; IRS-2−/−, 92 ± 3 μm) and total nuclei (e.g., control, 393 ± 32; IRS-1−/−, 303 ± 33; IRS-2−/−, 334 ± 30) in the aortic ring sections were analyzed (Fig. 1, G and H). These results are consistent with the known effects of IRS-1 and IRS-2 in mediating fetal growth (4).

Our prior studies have shown that, in response to hyperglycemia and IRS-1 down-regulation, the transmembrane protein SHPS-1 is up-regulated and functions to transduce IGF-1 signaling (10–11). The IGF-1 receptor phosphorylates SHPS-1, which forms a scaffold for signaling complex localization, and this complex mediates IGF-1 actions in VSMCs during hyperglycemia (10, 12). Therefore, we analyzed SHPS-1 complex activation in the different settings. Our results showed that IGF-1 stimulated a major increase in SHPS-1 tyrosine phosphorylation in aortae from IRS-1 VSMC knockout non-diabetic mice, and wild-type animals that had been made diabetic showed a similar response, whereas normal control mice showed no change in SHPS-1 phosphorylation (Fig. 2A). This change was specific for IRS-1 because IRS-2 VSMC knockout animals showed no SHPS-1 phosphorylation response. In contrast, IRS-1 tyrosine phosphorylation in response to IGF-1 could only be detected in normal wild-type and IRS-2 knockout animals (Fig. 2B). There was minimal IRS-2 protein, and no IRS-2 tyrosine phosphorylation was detected in control, diabetic, wild-type animals or IRS-1 knockout animals (Fig. 2C). This signaling switch was associated with enhanced downstream signaling responses. When the four groups of mice were compared, IGF-1 induced 7.5 ± 0.8-fold and 9.3 ± 0.7-fold increases in protein kinase B (AKT) phosphorylation in normoglycemic IRS-1−/− and diabetic IRS-1−/+ animals, respectively, whereas control and IRS-2−/− animals showed minimal changes (Fig. 2, D and E). When MAPK activation was analyzed, there were 7.0 ± 0.8-fold and 2.7 ± 0.2-fold increases in IRS-1−/− and IRS-1−/+ diabetic mice, respectively, and no increase in MAPK activation in IRS-2−/− control animals (Fig. 2, D and E). SHPS-1-mediated activation of downstream signaling requires recruitment of SHP-2 to the phosphotyrosines on SHPS-1 (12). SHP-2 recruitment results in assembly of a signaling complex composed of Src, p52 Shc, and Grb-2, which subsequently activates MAPK and AKT (12). Therefore, to confirm that the changes in downstream signaling that occurred in the IRS-1−/− mice were mediated through SHPS-1, we utilized a cell-permeable peptide that disrupts SHP-2/SHPS-1 association (11). The peptide significantly diminished SHP-2 recruitment in diabetic (IRS-1−/+ or) non-diabetic IRS-1−/− mice (Fig. 3A). Importantly, it also inhibited IGF-I induction of AKT and MAPK activation (Fig. 3B).

Next we determined the significance of these changes in signaling for cellular replication. IGF-1 induced no change in replication in aortae obtained from control animals; however, following IRS-1 deletion, there was a 2.0 ± 0.3-fold increase in Ki67 labeling that was comparable with the 1.6 ± 0.1-fold change that occurred in wild-type diabetic animals (Fig. 4). However, in IRS-2 knockout animals, this increase could only be detected in diabetic animals, and the magnitude of the increase was similar to wild-type animals (1.8 ± 0.6-fold versus 1.6 ± 0.1-fold increases). VSMCs in adult animals are capable of undergoing dedifferentiation, and this results in a hyperproliferative response to growth stimuli. Therefore, we determined whether IRS-1 knockdown was associated with a change in VSMC differentiation. As shown in Fig. 5A, the transcription factor Kruppel-like factor 4 (KLF-4), which inhibits the expression of genes that stimulate smooth muscle cell differentiation, was significantly increased in the IRS-1−/− mice. This increase was also noted in diabetic animals, but there was no change in wild-type non-diabetic or IRS-2−/− animals. This change was associated with a marked decrease in the expression of myocardin, a protein whose expression is required for VSMC differentiation (13) (Fig. 5A), and SM22, a marker of smooth muscle cell differentiation (Fig. 5D). Similar to wild-type animals, these changes were only detected in the diabetic animals but not in non-diabetic animals with IRS-2 deletion (Fig. 5, B and E). Importantly, these changes could be reversed using SHPS-1/SHP-2-disrupting peptide in diabetic wild-type mice and IRS-1−/− mice; that is, KLF4 expression was inhibited, and myocardin and SM22 were stimulated in diabetic and IRS-1−/− animals (Fig. 5, C and F). This finding strengthens the conclusion that the SHPS-1 signaling pathway predominated under these conditions.

To determine the functional consequences of decreased IRS-1 expression and VSMC dedifferentiation, the three groups of mice were wounded, and VSMC proliferation was determined. There was a major increase in arterial wall thickness in femoral arteries from IRS-1−/− animals (Fig. 6A). Quantitative analysis showed a 4.7 ± 0.9-fold increase in IRS-1−/− mice and a 6.6 ± 1.1-fold change in diabetic mice compared with a 2.4 ± 0.6-fold change in control animals. To determine whether this change was in part due to increased proliferation of VSMCs, Ki67 labeling and α-actin were analyzed. There was a 46.3% ± 7.4% increase in IRS-1−/− mice and a 50.3% ± 6.0% increase in diabetic animals (Fig. 6B), whereas this change was significantly less (e.g., 15.0% ± 5.2%) in control animals. Biochemical analyses verified that KLF4 expression was markedly up-regulated following wounding, and myocardin expression was undetectable in the IRS-1−/− and diabetic mice (Fig. 7A). In contrast, there was no change in control animals. Similarly, MAPK and AKT phosphorylation were markedly enhanced in IRS-1−/− mice and the diabetic (IRS-1−/+ ) animals but changed only minimally in the non-diabetic controls (Fig. 7B). To determine whether these changes might affect vascular function, several markers, such as SM22, α-actin, and proliferating cell nuclear antigen (PCNA), were analyzed. The results showed that wounding treatment induced PCNA in diabetic wild-type and non-diabetic IRS-1−/− mice, whereas SM22 and α-actin expression were remarkably reduced (Fig. 7C).
IRS-1 Prevents VSMC Dedifferentiation

Panel A: Western blot analysis of SHPS-1 and IRS-1 expression in mouse aortic SMCs with or without IGF-1 treatment. The blots show a dose-dependent increase in SHPS-1 and IRS-1 expression with IGF-1 treatment.

Panel B: Western blot analysis of IRS-1 and IRS-2 expression in mouse aortic SMCs with or without IGF-1 treatment. The blots show a dose-dependent increase in IRS-1 and IRS-2 expression with IGF-1 treatment.

Panel C: Western blot analysis of pAkt expression in mouse aortic SMCs with or without IGF-1 treatment. The blots show a dose-dependent increase in pAkt expression with IGF-1 treatment.

Panel D: Western blot analysis of pAkt and pErk1/2 expression in mouse aortic SMCs with or without IGF-1 treatment. The blots show a dose-dependent increase in pAkt and pErk1/2 expression with IGF-1 treatment.

Panel E: Western blot analysis of pErk1/2 expression in mouse aortic SMCs with or without IGF-1 treatment. The blots show a dose-dependent increase in pErk1/2 expression with IGF-1 treatment.
IRS-1 Prevents VSMC Dedifferentiation

FIGURE 3. Disruption of SHPS-1 signaling complex formation prevents enhanced IGF-I-dependent MAPK and AKT activation. A, aortic lysates from diabetic wild-type mice (WT HG) and normal IRS-1 knockout mice (IRS-1−/− NG) that had been treated with a control peptide (CP) or a SHPS-1/SHP-2-disrupting peptide (AP) were immunoprecipitated (IP) with an anti-SHPS-1 antibody and immunoblotted (IB) with an anti-SH-2 antibody. The same amount of each aortic lysate was immunoblotted with an anti-SHPS-1 antibody as a loading control. Scanning densitometry values obtained from of the aortic extracts from three mice in two separate experiments showed a 5.8 ± 0.9-fold increase in wild-type diabetic mice exposed to the control peptide and a 1.6 ± 0.3-fold (p < 0.01) change with the disrupting peptide. The increases were 6.4 ± 0.7-fold and 1.4 ± 0.2-fold (p < 0.01), respectively, in IRS-1−/− mice. B, the aortic extracts were immunoblotted with anti-pAKT (Ser-473) and pErk1/2 antibodies. The blots were reprobed with an anti-β-actin antibody to control for loading. The increases AKT activation were 9.5 ± 1.7-fold and 1.7-fold compared with 1.3 ± 0.2-fold (p < 0.01) in wild-type diabetic and 9.1 ± 1.5-fold compared with 2.0 ± 0.3-fold (p < 0.01) in IRS-1−/− mice. The changes in MAPK were 6.0 ± 1.0-fold and 1.2 ± 0.2-fold (p < 0.01) in diabetic wild-type mice and 7.9 ± 1.6-fold compared with 1.3 ± 0.3-fold (p < 0.01) in IRS-1−/− mice.

Discussion

During normoglycemia, adult VSMCs remain differentiated and do not proliferate. Under these conditions, IRS-1 mediates normal physiologic responses following insulin or IGF-I stimulation, including glucose transport, protein synthesis, and inhibition of apoptosis (3). In contrast, during hyperglycemia or insulin-resistant states, IRS-1 is down-regulated (8–10). In cultured VSMCs exposed to high glucose, IRS-1 downstream signaling is dysregulated and mediated through the scaffolding protein SHPS-1 (11–12). Overexpression of IRS-1 under these conditions inhibits SHPS-1 tyrosine phosphorylation in response to IGF-I, and this is associated with attenuated VSMC migration and proliferation (12). This study was undertaken to determine whether IRS-1 functions constitutively in a cell-autonomous manner to inhibit these aberrant responses under normoglycemic conditions in vivo. The findings demonstrate unequivocally that this signaling switch occurs in intact arteries in mice following deletion of IRS-1 expression. Specifically, in VSMCs, IRS-1 deletion resulted in enhancement of the ability of IGF-I to stimulate SHPS-1 tyrosine phosphorylation during normoglycemia. This function was cell-autonomous and not dependent on deletion of IRS-1 expression in other vascular cell types, such as endothelium. This up-regulation of SHPS-1 tyrosine phosphorylation during normoglycemia resulted in significant enhancement of AKT and MAPK phosphorylation as well as cell proliferation. Of greater importance is that knockdown of IRS-1 led to increased expression of the dedifferentiation-associated transcription factor KLF-4 and down-regulation of myocardin and SM22, two important stimuli of VSMC differentiation. Therefore, we conclude that IRS-1 expression is necessary to maintain VSMCs in their normal, differentiated state. The loss of IRS-1 results in dedifferentiation that is accompanied by aberrant signaling through SHPS-1 and enhancement.
**FIGURE 5.** Smooth muscle-specific knockout of IRS-1 but not IRS-2 stimulates the dedifferentiation of aortic smooth muscle cells. A, B, D, and E, aortae from normal wild-type mice (WT+/− NG), diabetic wild-type mice (WT+/− HG), normal IRS-1 knockout mice (IRS-1−/− NG), IRS-2 knockout mice (IRS-2−/− NG), or diabetic IRS-2 knockout mice (IRS-2−/− HG) were prepared following the procedure described under “Experimental Procedures.” The lysates were immuno-blotted (IB) with anti-KLF4, anti-myocardin (A and B) or anti-SM22 antibody (D and E). The blots were reprobed with an anti-β-actin antibody as a loading control. The value of each column is the ratio of the mean ± S.D. value of the scanning units obtained from 6 mice/group for KLF4, myocardin, or SM22 divided by the scanning units of corresponding β-actin, respectively. *, p < 0.05; **, p < 0.01; ***, p < 0.001; significant differences between two mouse types. C and F, aortic lysates from diabetic wild-type mice (WT+/− HG) or non-diabetic IRS-1 knockout mice (IRS-1−/− NG) treated with SHPS-1/SHP2-disrupting peptide (AP) or a control peptide (CP) for 5 days were immunoblotted with anti-KLF4, anti-myocardin (C), or anti-SM22 antibody (F). The blots were reprobed with an anti-β-actin antibody as a loading control. M1 and M2 denote two representative mice for each group.
of VSMC proliferation in response to IGF-I or vascular injury (Fig. 8).

In addition to SHPS-1 activation, we investigated the possibility that up-regulation of IRS-2 could compensate for loss of IRS-1. However, the results clearly show that, following IRS-1 deletion or induction of diabetes, there is no increase in IRS-2 and that deletion of IRS-2 does not induce SHPS-1 activation in the aorta. Therefore, we conclude that the loss of IRS-1 does not induce IRS-2 and that the switch in signaling that occurs with hyperglycemia from IRS-1 to SHPS-1 is IRS-2-independent.

IRS-1 is down-regulated in multiple cell types in response to hyperglycemia, including VSMCs (11), skeletal muscle (14), endothelium (15), pre-adipocytes (16), hepatocytes (16), and cardiomyocytes (17). Several mechanisms have been postulated to mediate this change, including serine phosphorylation of IRS-1 (18), direct proteolytic degradation (19), and ubiquitination with targeting to proteasomes (20). Multiple serine/threonine kinases, including JNK, mechanistic target of rapamycin (mTOR), MAPK, and several protein kinase C isoenzymes that phosphorylate IRS-1 are activated in response to hyperglycemia (21–22). Cytokine activation of the NF-κB pathway (23), dyslipidemia (24), and hormones that stimulate endothelial dysfunction, such as angiotensin II (25) and aldosterone (26), induce IRS-1 down-regulation, and this is a mechanism by which these factors confer insulin resistance. Whether each of these factors can induce SHPS-1 activation has not been reported, but a recent study showed that TNFα stimulation of skeletal muscle activated SHPS-1 expression and down-regulated IRS-1 (23). In that model, knockdown of SHPS-1 restored insulin signaling and IRS-1 tyrosine phosphorylation. This suggests that there is a dynamic interplay between IRS-1 and SHPS-1 signaling to maintain insulin sensitivity and that dys-

![FIGURE 6. Smooth muscle-specific knockout of IRS-1 enhances femoral arterial cell proliferation after injury.](image)

### FIGURE 6. Smooth muscle-specific knockout of IRS-1 enhances femoral arterial cell proliferation after injury. Femoral arterial sections after injury or sham treatment from normal wild-type, diabetic wild-type, and normal IRS-1−/− mice were prepared following a procedure described under “Experimental Procedures.” A, the sections were stained with Masson trichrome elastin stain, and images were captured and analyzed as under “Experimental Procedures.” Representative cross-sections are shown. The value of each column is the mean value ± S.D. obtained from 12 mice from each group. B, the sections were stained with anti-Ki67 and anti-α-actin antibodies and DAPI following a procedure described under “Experimental Procedures.” The value of each column is the mean value ± S.D. of the percentage increase of Ki67-positive nuclei after injury above the sham treatment value for each mouse group (n = 12). **, p < 0.01; *, p < 0.05; significant differences when the two treatments or two mouse types were compared. NS, no significant difference. Error bars denote the standard deviation.
regulation of insulin/IRS-1 signaling in response to cytokines is mediated through SHPS-1.

Unlike IGF-I, peptide growth factors, such as PDGF, that are potent stimuli of VSMC proliferation down-regulate IRS-1 expression even during normoglycemia (27). Similarly, loss of insulin receptor signaling through genetic manipulation results in decreased IRS-1 activation and leads to enhanced serum stimulation of smooth muscle cell proliferation (28). A prior study demonstrated that mice that were globally heterozygous for IRS-1 were more sensitive to vascular dysfunction in the presence of genetically induced lipoprotein excess (29). However, in those mice, the change in IRS-1 was present in multiple cell types that contribute to atherogenesis, including endothelial cells and macrophages as well as smooth muscle cells. In contrast, our study clearly demonstrates that a cell-autonomous reduction of IRS-1 results in markedly altered intracellular signaling, VSMC dedifferentiation, and abnormal proliferation, suggesting that these changes enhance cellular sensitivity to IGF-I stimulation by a mechanism that is independent of changes in these other cell types.

The key finding in this study is that IRS-1 is functioning to inhibit dedifferentiation and, thereby, maintain VSMCs in the differentiated state. This appears to be mediated by its ability to attenuate the expression of KLF-4, a transcription factor that induces smooth muscle cell dedifferentiation (30). Suppression of KLF-4 resulted in maintenance of myocardin expression, which is essential for smooth muscle cell differentiation (13). Myocardin suppression may also regulate the proliferative response to injury because myocardin expression was robust in wild-type animals but nearly absent in IRS-1−/− mice that had a hyperproliferative response to injury, and myocardin is known to modulate this response (31). Other variables, such as TNFα, PDGF, and oxidized LDL, that regulate VSMC differentiation stimulate KLF-4 expression, thereby resulting in enhancement of VSMC dedifferentiation (30–33). During vascular injury, KLF-4 is up-regulated, and this is accompanied by dedifferentiation (34–35). This finding was replicated in our study, and we noted an enhanced dedifferentiation response in the absence of IRS-1, suggesting that IRS-1 is also functioning during injury to attenuate smooth muscle cell proliferation by this mechanism. KLF-4 can also be induced by other atherogenic stimuli, such oxidized phospholipids and advanced glycation end products (32–36).

Cyclosporine, which induces KLF-4 expression, also induces smooth muscle cell dedifferentiation (37). In contrast, myocardin overexpression promotes VSMC differentiation and increases the expression of other VSMC marker proteins (38). Factors that inhibit myocardin expression are associated with dedifferentiation (35). Therefore, our finding of loss of myocardin expression in IRS-1−/− mice strongly supports the conclusion that loss of the ability of IRS-1 to suppress dedifferentiation is mediating the abnormal growth-regulatory response.

Although this study was confined to the analysis of vascular smooth muscle cell differentiation and growth, down-regulation of IRS-1 occurs in multiple cell types in the presence of hyperglycemia (4, 14–16). This raises the possibility that IRS-1 could regulate the state of differentiation in other cell types, such as vascular endothelium, podocytes, and pericytes, whose differentiated functions undergo pathophysiologic changes that lead to diabetic complications (39). Therefore, the reduction in IRS-1 that occurs in these cell types because of hyperglycemia or insulin resistance could lead to abnormal angiogenesis or podocyte dysfunction (40–41). New strategies that focus on ways of controlling this dysregulation of IRS-1 expression hold promise for developing medical treatments that reverse or stabilize the development of these diabetic complications.

**Experimental Procedures**

Human IGF-I was a gift from Genentech (South San Francisco, CA). Immobilon-P membranes, antibodies against IRS-1 (catalog no. 05-1085) and SHP-2 (catalog no. 06-118) were purchased from EMD-Millipore (Billerica, MA). Antibodies against phospho-AKT (Ser-473) (catalog no. 9271) and phospho-Erk1/2 (catalog no. 4370) were purchased from Cell
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Signaling Technology Inc. (Beverly, MA). Anti-phospho-tyro-
sine (PY99) (catalog no. sc7020), α-actin (catalog no. sc32251),
and KLF-4 antibodies (catalog no. sc20691) were purchased
from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Anti-β-
actin antibody (catalog no. A1978) was purchased from Sigma-
Aldrich (St. Louis, MO). Antibodies against Ki67 (catalog no.
ab66155) and SM22 (catalog no. ab10135) were purchased from
Abcam (Cambridge, MA). An anti-myocardin antibody ( cata-
log no. MAB4028) was purchased from R&D Systems (Minne-
apolis, MN). An anti-PCNA antibody (catalog no. 610664) was
purchased from BD Biosciences (San Jose, CA). A rabbit anti-
IRS-2 antiserum was raised by our laboratory using a peptide,
CLNINKRADAKHKYLIALYT, linked to keyhole limpet hemocya-
nin as an immunogen (42). A mouse anti-IRS-2 antibody was
provided by Dr. White (Harvard Medical School). An anti-SPHS-1
antiserum was prepared as described previously (43). The horse-
radish peroxidase-conjugated mouse anti-rabbit, goat anti-mouse,
and mouse anti-rabbit light chain-specific antibodies were pur-
based from Jackson Immunoresearch Laboratories (West Grove,
PA). The synthetic peptide YARAAARQARATLTYADLDM (a
SHPS-1/SHP-2-disrupting peptide) and YARAAARQARVQA-
LYAVSEE (a control peptide) were synthesized by the Protein
Chemistry Core Facility at the University of North Carolina at
Chapel Hill. The purity and the sequences were confirmed by mass
spectrometry. All other reagents were obtained from Sigma-Ald-
rich unless otherwise stated.

Mice—All mouse experiments were approved by the Institu-
tional Animal Care and Use Committees of the University of
North Carolina at Chapel Hill. The floxed IRS-1 mice and
floxed IRS-2 mice were provided by Dr. Morris White (Harvard
Medical School). They were created on C57BL/6 background
mice following the procedures described previously (44–45).

To generate smooth muscle-specific knockout mice, floxed
IRS-1 (IRS-1fl/fl) or floxed IRS-2 (IRS-2fl/fl) mice were crossed
with SM22-Cre mice (The Jackson Laboratory, Bar Harbor,
ME) for four generations to obtain homozygous SM22-Cre
mice (Jackson Laboratory, Bar Harbor, ME) or IRS-1fl/fl or
floxed IRS-2 (IRS-2fl/fl) mice were crossed with SM22-Cre mice
(The Jackson Laboratory, Bar Harbor, ME) for four generations
to obtain homozygous SM22-Cre+ IRS-1fl/fl or IRS-2fl/fl mice.

Mice were maintained at 22 °C with a 12-h light/dark cycle and
given free access to regular chow (2018 Teklad global rodent
diet containing 18.6% crude fiber and 3.5% crude fiber and water.
All groups of mice maintained normal nutrient intake and growth
during the experiment.

Induction of Hyperglycemia in Mice and Preparation of Tis-
sues for Biochemical Analysis—Hyperglycemia was induced in
wild-type (IRS-1fl/fl) and smooth muscle specific IRS-2 knock-
out mice using low-dose streptozotocin (46). All mice had
serum glucose concentrations that were >250 mg/dl, and the
levels were maintained during the experiments. There were 12
mice/group (wild-type, wild-type with diabetes, and smooth
muscle-specific IRS-1 or IRS-2 knockout mice) for the bio-
chemical analyses and Ki67 staining studies, respectively. There
were 16 mice/group for the femoral artery injury study and 8
mice/group for sham treatment. IGF-1 (1 mg/kg) (n = 6) or PBS
( n = 6) was administered i.p. 15 min before sacrifice for bio-
chemical analyses and 24 h and 15 min before sacrifice for
assumption of Ki67 labeling. For the SHPS-1/SHP-2-disrupting
cell signaling experiment, the disrupting peptide (2 mg/kg) and
control peptide (2 mg/kg) were injected 24 h and 1 h before
sacrifice in diabetic wild-type mice (n = 12) and IRS-1 knock-
out mice (n = 12), respectively. IGF-1 (1 mg/kg) (n = 6) or PBS
(n = 6) was administered i.p. to each group of mice 15 min
before sacrifice. In experiments to assess the effect of disrupting
SHPS-1/SHP-2 on the expression of differentiation markers,
the disrupting or control peptide (2 mg/kg) was injected every
24 h for 5 days in diabetic wild-type mice (n = 6) and IRS-1
knockout mice (n = 6). The mice were euthanized by injection
of ketamine (100 mg/kg)/xylazine (10 mg/kg). The major
organs were cleaned by removing the connective tissues and
then weighed. The aortic endothelial cells were collected by
opening the aorta longitudinally and scraping the inside of the
vessel with a scalpel. The cells were pooled from several aortas.

For all other biochemical analyses, the aortas were opened lon-
gitudinally, the endothelial cells were removed by scraping, and
the remaining tissue was homogenized in ice-cold buffer (20
mm Tris, 150 mm sodium chloride, 2 mm EDTA, and 0.05%
Triton X-100 (pH 7.4)) using a glass tissue grinder. The lysates
were centrifuged at 13,000 g for 15 min. Protein concentra-
tions of tissue extracts were measured using a BCA assay
(Thermo Scientific, Rockford, IL). Equal amounts of protein
were used in each analysis.

Femoral Artery Injury—The procedures were performed in
the Rodent Advanced Surgical Models Core Facility at UNC
Chapel Hill. Briefly, 15-week-old mice were anesthetized with
inhaled isoflurane and placed in the supine position with their
paws fixed on the table and their lower extremities abducted
and extended. The femoral vessels were exposed by an 1-cm
longitudinal groin incision and viewed with the aid of a surgical
microscope. The segment of the saphenous artery below the
branching of the epigastric and femoral arteries was dissected
free from the adjacent nerve and vein. The distal portion of the
saphenous artery was encircled with a nylon suture, a vascular
clip was placed proximally at the level of the inguinal liga-
ment, and a 0.010 (0.25-mm) diameter angioplasty guide wire
(Advanced Cardiovascular Systems) was introduced into the
arterial lumen through an arteriotomy made just distal to the
suture. After release of the clamp, the guide wire was advanced
to the level of the aortic bifurcation and immediately pulled
back. This process was repeated two additional times to denude
the endothelium. The guide wire was then removed, and the
arteriotomy site was ligated by tying the previously placed
suture. For sham treatment, the procedure was the same but
without guide wire treatment. The skin incision was closed with
non-absorbable sutures. After surgery, mice were returned
back to animal housing and routinely monitored for 3 weeks
before being sacrificed for specimen collection.

Histology and Morphometry—The vasculature was cleared by
transcardial perfusion with 40 ml of PBS, followed by 40 ml of
freshly prepared 4% paraformaldehyde in PBS. Femoral arter-
ies were post-fixed for 24 h in 4% paraformaldehyde at 4 °C.
The sections were prepared by the UNC Histology Core Facility.
Briefly, tissue samples containing the centermost 10-mm sec-
tion distal to the inguinal ligament were blocked and embedded
in paraffin. Twelve adjacent 8-μm sections were cut every 500
μm, extending through the length of the vessel block. Serial
sections from each artery were stained with Masson trichrome
elastic stain. Images were captured using Aperio-5072 and/or
Olympus BX61 microscopes. Images were analyzed using ImageJ (1.47N, National Institutes of Health) by measuring 10 points of thickness spaced evenly around the vessel wall for each section in a blinded manner.

**Immunohistochemistry**—The aortae and femoral arteries from mice were fixed with 4% paraformaldehyde overnight, and paraffin-embedded sections were prepared by the UNC Histology Core facility. An immunohistochemistry paraffin protocol provided by Abcam was followed, and the procedures were described previously for Ki67 and DAPI staining (46–47). A similar procedure was used for α-actin staining. The Ki67-positive and total nuclei in a whole aortic ring were quantified using ImageJ (1.47N, National Institutes of Health) and expressed as the percentage of total nuclei.

For measurement of aortic thickness, sections of aortae from each group of mice were stained with hematoxylin and eosin. Images were captured using an Olympus BX61 microscope. Images were analyzed using ImageJ (1.47N, National Institutes of Health) by measuring 10 points of thickness spaced evenly around the vessel wall for each section in a blinded manner. Total DAPI-stained nuclei in each aortic section from different mice were counted.

**Immunoprecipitation and Immunoblotting**—The immunoprecipitation and immunoblotting procedures were performed as described previously (48). Immunoprecipitation was performed by incubating 0.5 mg of cell lysate protein with 1 μg of anti-SHPS-1 antibody at 4 °C overnight. Immunoblotting was performed using a dilution of 1:1000 for anti-pAkt (Ser-473), anti-SHPS-1 antibody at 4 °C overnight. Immunoblotting was performed using a dilution of 1:1000 for anti-pAkt (Ser-473), anti-SHPS-1 antibody at 4 °C overnight. Immunoblotting was performed using a dilution of 1:500 for anti-KLF-4, IRS-1, IRS-2, and myocardin antibodies. The proteins were visualized using enhanced chemiluminescence (Thermo Fisher Scientific).

**Statistical Analysis**—The results that are shown in all experiments are representative of at least three independent experiments and expressed as the mean ± S.D. Analysis of variance was applied for all data obtained from in vivo studies when multiple points were compared. In addition, repeated measures analysis of variance was used where appropriate. p < 0.05 was considered statistically significant.

**Author Contributions**—G. X. designed and performed many of the experiments. He planned the mouse breeding program and supervised the technical work that was necessary to complete the manuscript. D. R. C. helped with design of the experiments and planning of the studies. He reviewed the data extensively and prepared the manuscript. M. F. W. prepared and provided the floxed IRS-1 mice. C. W. maintains the mouse breeding program and assisted with preparation of the tissues for biochemical and immunohistochemical analysis.

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**References**

1. Nathan, D. M., Cleary, P. A., Backlund, J. Y., Genuth, S. M., Lachin, J. M., Orchard, T. J., Raskin, P., Zinman, B., and Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N. Engl. J. Med.* 353, 2643–2653.

2. Holman, R. R., Paul, S. K., Bethel, M. A., Matthews, D. R., and Neil, H. A. (2008) 10-year follow-up of intensive glucose control in type 2 diabetes. *N. Engl. J. Med.* 359, 1577–1589.

3. White, M. F. (1998) The IRS-signalling network: a network of docking proteins that mediate insulin action. *Mol. Cell. Biochem.* 182, 3–11.

4. Sun, X. J., Crimmins, D. L., Myers, M. G., Jr., Miraipleix, M., and White, M. F. (1993) Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. *Mol. Cell. Biol.* 13, 7418–7428.

5. Navé, B. T., Ouwens, M., Withers, D. J., Alessi, D. R., and Shepherd, P. R. (1999) Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.* 344, 427–431.

6. Longobardi, L., Granero-Moltó, F., O’Rear, L., Myers, T. J., Li, T., Kregor, P. J., and Spagnoli, A. (2009) Subcellular localization of IRS-1 in IGF-1-mediated chondrogenic proliferation, differentiation and hypertrophy of bone marrow mesenchymal stem cells. *Growth Factors* 27, 309–320.

7. Ding, M., Xie, Y., Wagner, R. J., Jin, Y., Carrao, A. C., Liu, L. S., Guzman, A. K., Powell, R. J., Hwa, J., Rzucidlo, E. M., and Martin, K. A. (2011) Adiponectin induces vascular smooth muscle cell differentiation via repression of mammalian target of rapamycin complex 1 and FoxO4. *Arterioscler. Thromb. Vasc. Biol.* 31, 1403–1410.

8. Saad, M. J., Araki, E., Miraipleix, M., Rothenberg, P. L., White, M. F., and Kahn, C. R. (1992) Regulation of insulin receptor substrate-1 in liver and muscle of animal models of insulin resistance. *J. Clin. Invest.* 90, 1839–1849.

9. Nemoto, S., Matsumoto, T., Taguchi, K., and Kobayashi, T. (2014) Relationships among protein tyrosine phosphatase 1B, angiopoietin II, and insulin-mediated aortic responses in type 2 diabetic Goto-Kakizaki rats. *Atherosclerosis* 233, 64–71.

10. Maile, L. A., Capps, B. E., Ling, Y., Xi, G., and Clemmons, D. R. (2007) Hyperglycemia alters the responsiveness of smooth muscle cells to insulin-like growth factor-I. *Endocrinology* 148, 2435–2443.

11. Radhakrishnan, Y., Shen, X., Maile, L. A., Xi, G., and Clemmons, D. R. (2011) IGF-I stimulates cooperative interaction between the IGF-1 receptor and CSK homologous kinase that regulates SHPS-1 phosphorylation in vascular smooth muscle cells. *Mol. Endocrinol.* 25, 1636–1649.

12. Radhakrishnan, Y., Maile, L. A., Ling, Y., Graves, L. M., and Clemmons, D. R. (2008) Insulin-like growth factor-I stimulates Shc-dependent phosphatidylinositol 3-kinase activation via Grb2-associated p85 in vascular smooth muscle cells. *J. Biol. Chem.* 283, 16320–16331.

13. Zheng, X. L. (2014) Myocardin and smooth muscle differentiation. *Arch. Biochem. Biophys.* 543, 48–56.

14. Ordóñez, P., Moreno, M., Alonso, A., Llanza, P., Díaz, F., and González, C. (2008) 17β-Estradiol and/or progesterone protect from insulin resistance in STZ-induced diabetic rats. *J. Steroid Biochem. Mol. Biol.* 111, 287–294.

15. Salt, I. P., Morrow, V. A., Brandle, F. M., Connell, J. M., and Petrie, J. R. (2003) High glucose inhibits insulin-stimulated nitric oxide production without reducing endothelial nitric-oxide synthase Ser1177 phosphorylation in human aortic endothelial cells. *J. Biol. Chem.* 278, 18791–18797.

16. Carvalho, E., Jansson, P. A., Nagaev, I., Wenthzel, A. M., and Smith, U. (2008) 17β-Estradiol and/or progesterone protect from insulin resistance in STZ-induced diabetic rats. *J. Steroid Biochem. Mol. Biol.* 111, 287–294.

17. Yu, Q., Zhou, N., Nan, Y., Zhang, L., Li, Y., Hao, X., Xiong, L., Lau, W. B., Ma, X. L., Wang, H., and Gao, F. (2014) Effective glycaemic control critically determines insulin cardioprotection against ischaemia/reperfusion injury in anaesthetized dogs. *Cardiovasc. Res.* 103, 238–247.

18. Hançer, N. J., Qiu, W., Cherella, C., Li, Y., Copps, K. D., and White, M. F. (2014) Insulin and metabolic stress stimulate multisite serine/threonine
phosphorylation of insulin receptor substrate 1 and inhibit tyrosine phosphor-
ylation. J. Biol. Chem. 289, 12467–12484.
19. Stöhr, O., Hahn, J., Moll, L., Leeser, U., Freude, S., Bernard, C., Schilbach,
K., Markl, A., Udelhoven, M., Krone, W., and Schubert, M. (2011) Insulin
receptor substrate-1 and -2 mediate resistance to glucose-induced
caspase-3 activation in human neuroblastoma cells. Biochim. Biophys.
Acta 1812, 573–580.
20. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-mediated
degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. J. Biol. Chem. 275, 22558–22562.
21. Copps, K. D., and White, M. F. (2012) Regulation of insulin sensitivity by
serine/threonine phosphorylation of insulin receptor substrate proteins
IRS1 and IRS2. Diabetologia 55, 2565–2582.
22. Lee, S., Lynn, E. G., Kim, J. A., and Quon, M. J. (2008) Protein kinase C-
dependent regulation of insulin receptor substrate-1 by angiotensin II.
Am. J. Physiol. Heart Circ. Physiol. 293, H319–319.
23. Thomas, S. S., Dong, Y., Zhang, L., and Mitch, W. E. (2013) Signal regula-
tion of insulin receptor substrate-1 by angiotensin II. J. Biol. Chem.
288, 2451–2458.
24. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-medi-
ated degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. Biochim. Biophys. Acta 1812,
573–580.

IRS-1 Prevents VSMC Dedifferentiation

phosphorylation of insulin receptor substrate 1 and inhibit tyrosine phosphor-
ylation. J. Biol. Chem. 289, 12467–12484.
19. Stöhr, O., Hahn, J., Moll, L., Leeser, U., Freude, S., Bernard, C., Schilbach,
K., Markl, A., Udelhoven, M., Krone, W., and Schubert, M. (2011) Insulin
receptor substrate-1 and -2 mediate resistance to glucose-induced
caspase-3 activation in human neuroblastoma cells. Biochim. Biophys.
Acta 1812, 573–580.
20. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-mediated
degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. J. Biol. Chem. 275, 22558–22562.
21. Copps, K. D., and White, M. F. (2012) Regulation of insulin sensitivity by
serine/threonine phosphorylation of insulin receptor substrate proteins
IRS1 and IRS2. Diabetologia 55, 2565–2582.
22. Lee, S., Lynn, E. G., Kim, J. A., and Quon, M. J. (2008) Protein kinase C-
dependent regulation of insulin receptor substrate-1 by angiotensin II.
Am. J. Physiol. Heart Circ. Physiol. 293, H319–319.
23. Thomas, S. S., Dong, Y., Zhang, L., and Mitch, W. E. (2013) Signal regula-
tion of insulin receptor substrate-1 by angiotensin II. J. Biol. Chem.
288, 2451–2458.
24. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-medi-
ated degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. Biochim. Biophys. Acta 1812,
573–580.

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ylation. J. Biol. Chem. 289, 12467–12484.
19. Stöhr, O., Hahn, J., Moll, L., Leeser, U., Freude, S., Bernard, C., Schilbach,
K., Markl, A., Udelhoven, M., Krone, W., and Schubert, M. (2011) Insulin
receptor substrate-1 and -2 mediate resistance to glucose-induced
caspase-3 activation in human neuroblastoma cells. Biochim. Biophys.
Acta 1812, 573–580.
20. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-mediated
degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. J. Biol. Chem. 275, 22558–22562.
21. Copps, K. D., and White, M. F. (2012) Regulation of insulin sensitivity by
serine/threonine phosphorylation of insulin receptor substrate proteins
IRS1 and IRS2. Diabetologia 55, 2565–2582.
22. Lee, S., Lynn, E. G., Kim, J. A., and Quon, M. J. (2008) Protein kinase C-
dependent regulation of insulin receptor substrate-1 by angiotensin II.
Am. J. Physiol. Heart Circ. Physiol. 293, H319–319.
23. Thomas, S. S., Dong, Y., Zhang, L., and Mitch, W. E. (2013) Signal regula-
tion of insulin receptor substrate-1 by angiotensin II. J. Biol. Chem.
288, 2451–2458.
24. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-medi-
ated degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. Biochim. Biophys. Acta 1812,
573–580.

IRS-1 Prevents VSMC Dedifferentiation

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ylation. J. Biol. Chem. 289, 12467–12484.
19. Stöhr, O., Hahn, J., Moll, L., Leeser, U., Freude, S., Bernard, C., Schilbach,
K., Markl, A., Udelhoven, M., Krone, W., and Schubert, M. (2011) Insulin
receptor substrate-1 and -2 mediate resistance to glucose-induced
caspase-3 activation in human neuroblastoma cells. Biochim. Biophys.
Acta 1812, 573–580.
20. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-mediated
degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. J. Biol. Chem. 275, 22558–22562.
21. Copps, K. D., and White, M. F. (2012) Regulation of insulin sensitivity by
serine/threonine phosphorylation of insulin receptor substrate proteins
IRS1 and IRS2. Diabetologia 55, 2565–2582.
22. Lee, S., Lynn, E. G., Kim, J. A., and Quon, M. J. (2008) Protein kinase C-
dependent regulation of insulin receptor substrate-1 by angiotensin II.
Am. J. Physiol. Heart Circ. Physiol. 293, H319–319.
23. Thomas, S. S., Dong, Y., Zhang, L., and Mitch, W. E. (2013) Signal regula-
tion of insulin receptor substrate-1 by angiotensin II. J. Biol. Chem.
288, 2451–2458.
24. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-medi-
ated degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. Biochim. Biophys. Acta 1812,
573–580.

IRS-1 Prevents VSMC Dedifferentiation

phosphorylation of insulin receptor substrate 1 and inhibit tyrosine phosphor-
ylation. J. Biol. Chem. 289, 12467–12484.
19. Stöhr, O., Hahn, J., Moll, L., Leeser, U., Freude, S., Bernard, C., Schilbach,
K., Markl, A., Udelhoven, M., Krone, W., and Schubert, M. (2011) Insulin
receptor substrate-1 and -2 mediate resistance to glucose-induced
caspase-3 activation in human neuroblastoma cells. Biochim. Biophys.
Acta 1812, 573–580.
20. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-mediated
degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. J. Biol. Chem. 275, 22558–22562.
21. Copps, K. D., and White, M. F. (2012) Regulation of insulin sensitivity by
serine/threonine phosphorylation of insulin receptor substrate proteins
IRS1 and IRS2. Diabetologia 55, 2565–2582.
22. Lee, S., Lynn, E. G., Kim, J. A., and Quon, M. J. (2008) Protein kinase C-
dependent regulation of insulin receptor substrate-1 by angiotensin II.
Am. J. Physiol. Heart Circ. Physiol. 293, H319–319.
23. Thomas, S. S., Dong, Y., Zhang, L., and Mitch, W. E. (2013) Signal regula-
tion of insulin receptor substrate-1 by angiotensin II. J. Biol. Chem.
288, 2451–2458.
24. Zhang, H., Hoff, H., and Sell, C. (2000) Insulin-like growth factor I-medi-
ated degradation of insulin receptor substrate-1 is inhibited by epidermal
growth factor in prostate epithelial cells. Biochim. Biophys. Acta 1812,
573–580.