The Insulin-Like Growth Factor-1 Receptor Is a Negative Regulator of Nitric Oxide Bioavailability and Insulin Sensitivity in the Endothelium

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OBJECTIVE—In mice, haploinsufficiency of the IGF-1 receptor (IGF-1R+) at a whole-body level, increases resistance to inflammation and oxidative stress, but the underlying mechanisms are unclear. We hypothesized that by forming insulin-resistant heterodimers composed of one IGF-1Rβ and one insulin receptor (IR), IRβ complex in endothelial cells (ECs), IGF-1R reduces free IR, which reduces EC insulin sensitivity and generation of the antioxidant/anti-inflammatory signaling radical nitric oxide (NO).

RESEARCH DESIGN AND METHODS—Using a number of complementary gene-modified mice with reduced IGF-1R at a whole-body level and specifically in EC, and complementary studies in EC in vitro, we examined the effect of changing IGF-1R/IR stoichiometry on EC insulin sensitivity and NO bioavailability.

RESULTS—IGF-1R+/− mice had enhanced insulin-mediated glucose lowering. Aortas from these mice were hypocorticotrope to phenylephrine (PE) and had increased basal NO generation and augmented insulin-mediated NO release from EC. To dissect EC from whole-body effects we generated mice with EC-specific knockdown of IGF-1R. Aortas from these mice were also hypocorticotrope to PE and had increased basal NO generation. Whole-body and EC deletion of IGF-1R reduced hybrid receptor formation. By reducing IGF-1R in IR-haploinsufficient mice we reduced hybrid formation, restored insulin-mediated vasorelaxation in aorta, and insulin stimulated NO release in EC. Complementary studies in human umbilical vein EC in which IGF-1R was reduced using siRNA confirmed that reducing IGF-1R has favorable effects on NO bioavailability and EC insulin sensitivity.

CONCLUSIONS—These data demonstrate that IGF-1R is a critical negative regulator of insulin sensitivity and NO bioavailability in the endothelium. Diabetes 60:2169–2178, 2011

Type 2 diabetes is a major cause of arterial atherosclerosis, leading to premature myocardial infarction (1), stroke (2), and peripheral vascular disease (3). A key feature of type 2 diabetes is insulin resistance, which is manifest by an inability of insulin to activate its complex signaling network in an appropriate temporospatial fashion (4). Recently it has emerged that in addition to its classic target tissues (muscle, liver, and fat), insulin resistance affects multiple cell types, including the vascular endothelium (5).

We have demonstrated that insulin resistance at a whole-body level (6,7), and specific to the endothelium (8), leads to reduced bioavailability of the anti-inflammatory/antioxidant, vasoactive signaling radical nitric oxide (NO), indicative of a critical role for insulin in regulating NO bioavailability. Consistent with this paradigm, a number of studies support an independent role for insulin resistance in the development of cardiovascular atherosclerosis and its complications (e.g., 9).

The IGF-1 receptor (IGF-1R) and insulin receptor (IR) are very similar tetrameric glycoproteins composed of two extracellular α and two transmembrane β subunits, linked by disulphide bonds (10). Because of this high homology, IGF-1R and IR can heterodimerize to form hybrid receptors composed of one IGF-1Rαβ complex and one IRαβ subunit complex (11,12). These hybrids have been shown to bind IGF-1, but not insulin, with high affinity (13). Hybrid receptors are more common in insulin-resistant individuals with type 2 diabetes and correlate with insulin sensitivity (14). In endothelial cells (ECs), IGF-1Rs substantially outnumber IRs (15), raising the possibility that IGF-1R may be a negative regulator of insulin signaling in the endothelium.

To explore this hypothesis and dissect the effect of manipulating IGF-1R numbers in the endothelium, we used four different gene-modified mice: 1) mice with whole-body haploinsufficiency of IGF-1R, 2) mice with different degrees of deletion of IGF-1R specific to the endothelium, and 3) mice with haploinsufficiency of IGF-1R IR at a whole-body level. These complementary models underpinned by in vitro studies support a novel mechanism by which IGF-1R is a critical negative regulator of insulin sensitivity and NO bioavailability in the EC.

RESEARCH DESIGN AND METHODS

Gene-modified mice. We used four different gene-modified mice to examine the role of IGF-1R in EC insulin sensitivity and NO production. All mice were...
bred onto a C57BL/6J background to at least 10 generations in a conventional animal facility with a 12-h light/dark cycle. Male mice aged 3 months were used. All experiments were conducted in accordance with accepted standards of humane animal care under U.K. Home Office Project license no. 402/088.

**Mice with whole-body haploinsufficiency of IGF-1R.** Mice with whole-body haploinsufficiency of IGF-1R (16) were obtained from The European Mutant Mouse Archive (Munich, Germany; http://www.emmanet.org) and maintained in the heterozygous state on a C57BL/6J background by mating IGF-1R+/+ males with 9–12-week-old C57BL/6J female wild-type mice.

**Mice with haploinsufficiency of both IGF-1R and IR.** To examine the effect of changing IGF-1R:IR stoichiometry on endothelial responses, we bred male IGF-1R−/− mice with female IGF-1R+/− mice to generate mice with whole-body haploinsufficiency of IR and IGF-1R (IR−/−; IGF-1R+/−).

**Mice with endothelium-specific deletion of IGF-1R.** To generate mice with endothelium-specific deficiency of IGF-1R, we used mice carrying a flexed IGF-1R allele (IGF-1R−/−), as previously described (17), and mice expressing the cre recombinase under control of the Tie-2 promoter (Tie-2; Jackson Laboratories, Bar Harbor, ME). A mating program of male Tie-2-cre/IGF-1RLox mice was used to avoid the possibility of inheritance of a null allele in the second generation, which is not tissue specific with female IGF-1R−/− was used. Male heterozygous and homozygous Tie-2-cre/IGF-1R−/− mice were compared with age-matched littermate controls.

**Metabolic tests.** Blood was sampled from tail vein. Glucose, insulin, and IGF-1 tolerance tests were performed by blood sampling after an intraperitoneal injection of glucose (1 mg/g; Sigma-Aldrich, Dorset, U.K.), human recombinant insulin (0.75 units/kg, Actrapid; Novo Nordisk, Bagsvaerd, Denmark), or human recombinant IGF-1 (0.75 μg/kg; GroPeP, Adelaide, Australia), as previously described (18). Glucose concentrations were determined in whole blood by a portable meter (Roche Diagnostics, West Sussex, U.K.). Plasma insulin concentrations were determined by enzyme-linked immunosassay (Ultraserase; BioCrystalChem, Downers Grove, IL). Plasma IGF-1 levels were determined by enzyme-linked immunoassay (19).

**Studies of vasomotor function in aortic rings.** Vasomotor function was assessed in aortic rings as previously described (6–8). Rings were mounted in an organ bath containing Krebs Henseleit buffer and equilibrated at a resting tension of 3 g for 45 min before the experiments. A cumulative dose-response to the constrictor phenylephrine (PE) (1 mM to 10 μM) was first performed. The aortic rings were then precontracted with PE (1 μM) for 30 min, followed by a 2 h preincubation with IGF-1 (100 mM) (19). Vasorelaxation in response to insulin was assessed by constructing PE dose–response curves before and after 2 h preincubation with insulin (100 μM/50 mM). Relaxation responses to cumulative addition of acetylcholine (1 nmol/L–1 μmol/L) were performed and responses expressed as percent decrement in preconstricted tension. Basal NO bioavailability in response to isometric tension was measured by recording the increase in tension elicited by 1-NO3-nitro-L-arginine methyl ester (l-NAME; 0.1 mM/L) in aortic segments maximally preconstricted with PE.

**NO synthase activity.** Endothelial NO synthase (eNOS) activity was determined by conversion of [14C]-arginine to [14C]-citrulline as we previously described (20). Aortic rings (5 mm in length) or ECs were incubated at 37°C for 20 min in HEPES buffer, pH 7.4 (in mM/L): 10 HEPES, 145 NaCl, 5 KCl, 1 MgSO4, 10 glucose, 1.25 CaCl2, containing 0.25% BSA. 0.5 μmol/L 1-[14C]-l-arginine was then added for 5 min and tissues were separated for analysis. Aortic rings were separated for further analysis and pooled together with 10 nmol/L L-NAME (l-NAME; Sigma-Aldrich) to prevent NO production. The vascular effect of IGF-1 was assessed by constructing PE dose–response curves before and after equilibration with 100 nmol/L IGF-1 (19). Vaso-relaxation in response to insulin was assessed by constructing PE dose–response curves before and after 2 h preincubation with insulin (100 μM/50 mM). Relaxation responses to cumulative addition of acetylcholine (1 nmol/L–1 μmol/L) were performed and responses expressed as percent decrement in preconstricted tension. Basal NO bioavailability in response to isometric tension was measured by recording the increase in tension elicited by 1-NO3-nitro-L-arginine methyl ester (l-NAME; 0.1 mM/L) in aortic segments maximally preconstricted with PE.

**Primary cells.** Primary cells were isolated from lungs of mice with female IGF-1R−/− male mating mice with female IR+/- mice (17) and mice expressing the cre recombinase under control of the Tie-2 promoter (Tie-2; Jackson Laboratories, Bar Harbor, ME). A mating program of male Tie-2-cre/IGF-1RLox mice was used. Male heterozygous and homozygous Tie-2-cre/IGF-1R−/− mice were compared with age-matched littermate controls.

**Gene expression.** mRNA was isolated using a commercial kit (Roche, Indianapolis, IN), and the levels of IGF-1R and IR mRNA quantified using real-time quantitative PCR (e.g., 6–8).

**Mouse pulmonary EC isolation and culture.** Primary ECs were isolated from lungs by immunoselection with CD146 antibody–coated magnetic beads (Miltenyi Biotech, Surrey, U.K.), as previously reported (21). Sheep anti–rat IgG (1:200) was used to coat the magnetic beads (BD Biosciences, Oxford, U.K.) according to the manufacturer’s instructions. Lungs were harvested, washed, finely minced, and digested in Hanks’ balanced salt solution containing 0.18 units/mL collagenase (10 mg/mL; Roche) for 45 min at 37°C. The digested tissue was filtered through a 70-μm cell strainer and centrifuged at 1,000 rpm for 10 min. The cell pellet was washed with DMEM/10% FCS, centrifuged, resuspended in 1 mL DMEM/10% FCS, and incubated with 1 × 106 CD31 antibody–coated beads at 4°C for 30 min. Bead–bound cells were separated from non–bead-bound cells using a magnet. Bead-bound (CD31 positive) and non–bead-bound cells were resuspended in 2 mL EGM-2-MV (Cambrex, East Rutherford, NJ), supplemented with human epi- dermal growth factor, hydrocortisone, vascular endothelial growth factor (VEGF), human fibroblast growth factor–basic, EGF, IGF-1, ascorbic acid, genta-mycins, amphotericin-B, and 5% FCS, and plated out. Only the EC population was tested positive for a range of endothelial markers, including eNOS, Tie2, and CD102 protein, measured using immunoblotting.

**siRNA-mediated downregulation of IGF-1R in human umbilical vein ECs.** The following validated oligonucleotides were synthesized from Sigma-Aldrich (SASI_Hs01_00126194 HUMAN NM_000875). Human umbilical vein endothelial cells (HUVECs) were grown in endothelial growth medium. Cells with 70% confluency were transfected with 10 nmol/L siRNA using the NTER transfection reagent (Sigma-Aldrich) according to the manufacturer’s protocol, optimized for use in HUVECs by the manufacturer. In brief, a mixture of target and scrambled siRNA (10 nmol/L), diluted in siRNA buffer and NTER transfection reagent, was preincubated at 37°C for 20 min. Growth medium was then removed and replaced with culture medium containing the target or scrambled siRNA (10 nmol/L), diluted in serum-starved medium for four hours in a humidified incubator at 37°C. After post-transfection, the medium was replaced with 0.2% serum-containing medium, and HUVECs were incubated overnight. The cells were then stimulated with 100 nmol/L insulin for 15 min at 37°C, after which the cells were harvested for protein analysis and Western blotting to analyze phosphorylation levels of eNOS. Assessment of eNOS activity was carried out on transfected and serum-starved HUVECs, as described above. After the overnight serum starvation, the medium was replaced with HEPES buffer, pH 7.4, for 30 min for equilibration.

**Fluorescence-based measurement of NO.** Insulin (100 nmol/L)-mediated NO release was measured as previously described (22). In brief, pulmonary endothelial cells (PECs) cultured in 96-well plates (90% confluent) in SBS (in mM/L: 135 NaCl, 5 KCl, 1 MgSO4, 8 glucose, 10 HEPES, and 1.5 CaCl2; in mmol/L: 135 NaCl, 5 KCl, 1 MgSO4, 8 glucose, 10 HEPES, and 1.5 CaCl2) were stimulated with DAF (DAF-FM acetate [Invitrogen, D-23844], stock 5 mM/L in DMSO; diluted DAF-FM acetate in SBS 2.1:000 to obtain 10 μmol/L DAF) in SBS by incubating at room temperature for 45 min. DAF was then removed and cells were washed in SBS twice. Cells were then left in SBS for 20 min at room temperature. SBS was replaced again and the plate was loaded into Flex station (FlexStation; Molecular Devices, Sunnyvale, CA). Fluorescence emission at 520 nm was recorded with a 485-nm excitation using the multiwell fluorescence plate reader system.

**Statistics.** Results are expressed as mean (SEM). Comparisons within groups were made using paired Student t tests and between groups using unpaired Student t tests or repeated-measures ANOVA, as appropriate; where repeated t tests were performed, a Bonferroni correction was applied. P < 0.05 was considered statistically significant.
RESULTS

Mice with whole-body haploinsufficiency of IGF-1R are glucose-intolerant but have enhanced insulin-mediated glucose lowering and increased basal and insulin-stimulated NO production. IGF-1R+/− mice had similar fasting glucose concentrations (5.4 mmol/L [0.3] vs. 4.9 mmol/L [0.1]), and as previously reported, were less efficient at dealing with a glucose bolus (Fig. 1A), with exaggerated postbolus hyperglycemia compared with wild-type mice. Despite this glucose intolerance, IGF-1R+/− mice had an exaggerated response to insulin, indicative of enhanced whole-body insulin sensitivity (Fig. 1B). IGF-1R+/− mice were resistant to IGF-1-mediated glucose lowering (Fig. 1C). Fasting plasma insulin and IGF-1 were similar in IGF-1R+/− and wild-type mice (Fig. 1D and E). There was no difference in vascular responses of IGF-1R+/− and wild-type mice to acetylcholine or sodium nitroprusside (data not shown). The vasorelaxant response to IGF-1 in IGF-1R+/− mice was significantly blunted (Fig. 2A and B), and IGF-1-mediated activation of eNOS, measured using the conversion of L-arginine to L-citrulline, was also blunted (Fig. 3C). In aortas from IGF-1R+/− mice, there was, as expected, significantly less IGF-1R protein expression than wild-type mice. There was no difference in IR, but a significant reduction in hybrid receptors, in keeping with an increase in the relative level of free IR homodimers (Fig. 3D–F).

Mice with EC-specific deficiency of IGF-1R have increased NO production: evidence for a gene dosage effect. As previously reported, mice homozygous for IGF-1R deletion were a knockdown model rather than complete deletion (17). “Holoinsufficient” mice, therefore, have some residual IGF-1R in the endothelium (Supplementary Fig. 1). EC-specific IGF-1R−/− mice had blunting of IGF-1R−/−-mediated aortic relaxation (Supplementary Fig. 2). Unlike mice with global IGF-1R deficiency, mice with haploinsufficiency and holoinsufficiency of the IGF-1R in the endothelium had similar responses in insulin and glucose tolerance tests as wild-type mice (Fig. 4A–D), and similar fasting glucose and insulin concentrations (Fig. 4E and F). Despite this, and consistent with our findings in whole-body IGF-1R−/− mice, endothelium-specific IGF-1R+/− and IGF-1R−/− mice had blunted constrictor responses to PE and an exaggerated constrictor response to L-NMMA, indicative of increased basal NO bioavailability (Fig. 5A–F). When comparing wild-type, endothelium-specific IGF-1R+/− and IGF-1R−/− mice, there was a clear gene dose effect on these vascular responses (Figs. 5G and H). Consistent with our findings in mice with whole-body haploinsufficiency of the IGF-1R hybrid receptor, expression was significantly lower in PEC from endothelium-specific IGF-1R−/− deficient mice (Fig. 5I).

**FIG. 1.** Haploinsufficiency of IGF-1R at a whole-body level leads to resistance to IGF-1-mediated glucose lowering and glucose intolerance but enhanced insulin sensitivity. A: Intraperitoneal glucose tolerance test. B: Intraperitoneal insulin tolerance test. C: Intraperitoneal IGF-1 tolerance test. Fasting plasma insulin (D) and fasting plasma IGF-1 (E) in mice with haploinsufficiency of IGF-1R and wild-type littermates. *P < 0.05; at least n = 10 per group for all experiments. Data shown as mean ± SEM.
Restoring insulin-mediated endothelial responses in mice with whole-body haploinsufficiency of IR by reducing IGF-1R. We have previously demonstrated that IR+/2 mice, while maintaining glucose competence, have significant blunting of insulin-mediated vasorelaxation and eNOS activation (6,20). To test our hypothesis that changing IR:IGF-1R stoichiometry would impact favorably on insulin-mediated NO production in vivo, we generated mice with whole-body haploinsufficiency of the IR and IGF-1R. IGF-1R+/2/IR+/2 and IR+/2 mice had similar fasting glucose concentrations and responses to a glucose bolus (Fig. 6A). IGF-1R+/2/IR+/2 mice had higher fasting glucose concentrations than IGF-1R+/2 and wild-type mice (Supplementary Fig. 3A). In insulin tolerance tests, IGF-1R+/2/IR+/2 and IR+/2 mice had similar responses (Fig. 6B and Supplementary Fig. 3B). IGF-1R+/2/IR+/2 mice had increased insulin-stimulated eNOS activation in aortic rings (Fig. 6C), restored aortic relaxation in response to insulin (Fig. 6D–F), and increased NO generation in PEC in response to insulin (Fig. 6G and H), and reduced numbers of hybrid receptors (Fig. 6I).

Reducing IGF-1R in ECs enhances basal and insulin-stimulated eNOS phosphorylation. To examine the specific effects of reducing IGF-1R expression on basal serine phosphorylation and activation of eNOS in response to insulin, we used siRNA-mediated knockdown of IGF-1R. siRNA-treated HUVEC had a significant reduction in IGF-1R but no change in IR (Fig. 7A–C), assessed using RT-PCR. This reduction in IGF-1R led to an increase in basal and insulin-stimulated serine-phosphorylated eNOS (Fig. 7D), and increased insulin-mediated eNOS activation (Fig. 7F).

DISCUSSION
The present report using a comprehensive in vivo and in vitro approach describes a novel mechanism by which the bioavailability of the endothelium-derived signaling radical NO is regulated. We have demonstrated using mice with
whole-body haploinsufficiency of IGF-1R and endothelium-specific deletion of IGF-1R that NO bioavailability is negatively regulated by IGF-1R in the endothelium.

To examine the therapeutic potential of manipulating IGF-1R numbers, we generated mice with whole-body haploinsufficiency of the IR and IGF-1R. We have previously demonstrated substantial abnormalities of EC function in IR+/- mice (6,7,20). Reducing IGF-1R numbers in IR+/- mice restored insulin-mediated vasorelaxation, enhanced insulin-stimulated eNOS activation, and enhanced insulin-stimulated NO release in ECs. In vitro experiments in human ECs confirmed that IGF-1R deletion enhances basal and insulin-stimulated serine phosphorylation of eNOS and eNOS activity in response to insulin. These data demonstrate for the first time that IGF-1R is a key negative regulator of insulin sensitivity and NO bioavailability in the endothelium.

**Insulin resistance, endothelial NO synthase, and atherosclerosis.** A well defined series of steps occur in the arterial wall during the development of atherosclerosis (23). Early in the development of atherosclerosis, before the onset of morphological changes, there are important alterations in EC phenotype, a situation described as endothelial dysfunction. A key feature of endothelial dysfunction is a reduction in NO bioavailability (24). In the endothelium, NO is generated by l-arginine to L-citrulline conversion principally by the NO synthase isoform eNOS (for review see Ref. 25). NO can influence multiple steps in the atherosclerotic process, including vascular smooth muscle cell phenotype (26), vascular inflammation (27), platelet aggregation (28), EC migration, proliferation and survival (29), and progenitor cell mobilization (30). In addition, NO has physiologically important antioxidant actions (31). eNOS activity is regulated at multiple different levels (for review see 25), including the availability of its substrate or cofactors, protein–protein interactions, post-translational modifications, and serine phosphorylation. Serine phosphorylation of eNOS accelerates the rate of electron flow through the enzyme, increases its calcium sensitivity, and augments NO biosynthesis and release (32). Insulin and IGF-1, via activation of their receptors, have been shown to increase levels of serine-phosphorylated eNOS in vivo (19), and in vitro (33), and to stimulate NO production (34). In humans, insulin sensitivity closely correlates with NO bioavailability in resistance vessels (35,36). In a novel murine model of endothelium-specific insulin
resistance, we demonstrated a significant reduction in insulin-mediated eNOS phosphorylation and NO bioavailability (8). In other preclinical models, eNOS phosphorylation status has been shown to play an important and favorable role in a range of pathological situations reminiscent of human vascular disease such as stroke (37) and lower limb ischemia (38).

**NO bioavailability in mice with whole-body haploinsufficiency of IGF-1R.** In this mouse, previously shown to be protected against oxidative stress (16), and age-related inflammatory degenerative brain disease (39), we demonstrated an intriguing phenotype. Using a range of complementary approaches we showed that despite whole-body glucose intolerance, haploinsufficiency of IGF-1R leads to the favorable vascular phenotype of reduced constriction to PE, increased basal NO production, and increased EC insulin sensitivity leading to augmented insulin-mediated NO generation.

**IGF-1R:IR stoichiometry and insulin sensitivity.** It is well established that IR and IGF-1R can heterodimerize to form insulin-resistant hybrid receptors (10–14). The formation of hybrid receptors is determined by the molar proportion of receptors. In ECs, the specific binding of insulin is significantly lower than IGF-1, indicative of a lower number of homodimeric IR than IGF-1R (41); studies suggest that IGF-1R outnumber IR tenfold (34). Crucially, for high-affinity binding of insulin to the IR and activation to occur, binding to both subunits is necessary, whereas IGF-1 requires only one subunit (13). This provides one explanation as to why hybrids have a low affinity for insulin but bind IGF-1 with the same high affinity as IGF-1R homodimers. In vitro studies of a range of cell types (42–45) have suggested that reduction
of IGF-1R density enhances insulin sensitivity. In cells from humans with polymorphisms that lead to a reduction in IGF-1R density, a similar pattern of enhanced insulin sensitivity has been demonstrated (46).

Reducing IGF-1R numbers in vivo to restore vascular function in a model of insulin resistance–related vascular disease: proof of concept. Insulin-resistant humans have a significant reduction in cell surface IR (14) and reduced insulin-mediated, NO–dependent vasorelaxation (47). We have previously used mice with whole-body haploinsufficiency of the IR as a model of non-obese whole-body insulin resistance. These mice, similar to insulin-resistant humans, have reduced insulin-mediated eNOS phosphorylation, reduced insulin-mediated eNOS activation, and reduced insulin-mediated vasorelaxation (6,7). More recently, consistent with reduced NO bioavailability, we have shown that these mice have reduced endothelial progenitor cell mobilization in response to VEGF and delayed endothelium regeneration after femoral artery wire injury (20). To examine whether reducing IGF-1R in IR−/− mice can restore insulin-mediated NO production, we generated IGF-1R−/−/IR−/− mice. IGF-1R−/−/IR−/− mice had enhanced insulin-mediated NO release in PEC and enhanced insulin-mediated vasorelaxation and eNOS activation in aortas compared with IR−/−. In complementary studies in HUVECs, we knocked down IGF-1R. HUVEC

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FIG. 5. Vascular responses in aortic rings of mice with haploinsufficiency and holoinsufficiency of IGF-1R in the endothelium. A: Constrictor responses to PE in rings preincubated with L-NMMA in wild-type mice. B: Enhanced constrictor responses to PE in mice with EC-specific haploinsufficiency of IGF-1R. C: Constrictor responses to PE in rings preincubated with L-NMMA in wild-type mice. D: Enhanced constrictor responses (indicative of increased NO bioavailability) in rings in mice with EC-specific holoinsufficiency of IGF-1R. E: Blunted constrictor responses to PE in mice with EC-specific haploinsufficiency of IGF-1R. F: Blunted constrictor responses to PE in mice with EC-specific holoinsufficiency of IGF-1R. G: Maximal change in tension in response to L-NMMA in wild-type mice, mice with EC-specific haploinsufficiency of IGF-1R, and mice with EC-specific holoinsufficiency of IGF-1R, demonstrating gene dose effect of IGF-1R on NO bioavailability. H: Maximal change in tension in response to PE in wild-type mice, mice with EC-specific haploinsufficiency of IGF-1R, and mice with EC-specific holoinsufficiency of IGF-1R, demonstrating gene dose effect of IGF-1R on constrictor responses to PE. I: Quantification of hybrid receptors in wild-type mice, mice with EC-specific haploinsufficiency of IGF-1R, and mice with EC-specific holoinsufficiency of IGF-1R. All experiments had at least n = 5 mice. *P < 0.05. (A high-quality color representation of this figure is available in the online issue.)
deficiency in IGF-1R, in keeping with our findings in vivo, had increased basal serine-phosphorylated eNOS and enhanced insulin-mediated serine eNOS phosphorylation and activation.

Potential mechanisms linking hybrid formation and type 2 diabetes. An increase in hybrid receptors is a hallmark of type 2 diabetes and obesity (14,48,49). Elegant studies from Sesti et al. provide important insights into how aspects of the type 2 diabetes phenotype may regulate the formation of hybrids. In a study of patients with primary, nongenetically determined hyperinsulinaemia (i.e., patients with insulinoma), Federici et al. (50) demonstrated that insulin-mediated downregulation of the IR, by shifting IR:IGF-1R stoichiometry in favor of IGF-1R led to a higher proportion of hybrid receptors in skeletal muscle in these patients. This suggests that hybrid receptor formation in patients with type 2 diabetes may be driven by changes in insulin concentration.

The role of hyperglycemia was also examined by this group (51). In diabetic rats rendered normoglycemic, Federici et al. showed that reducing glucose reduced hybrid receptor numbers. Moreover, the glucosamine pathway, at least in part, may mediate glucose-induced formation of hybrid receptors. These studies demonstrate that by driving the formation of hybrid receptors, different components of the type 2 diabetes phenotype may initiate a vicious cycle, leading to worsening of insulin sensitivity.

In summary we have demonstrated in complementary in vivo and in vitro models that IGF-1R is an important negative regulator of insulin sensitivity in the endothelium. Deletion of IGF-1R leads to a potentially favorable increase in NO bioavailability and, in a well characterized model of human insulin resistance, rescues the adverse phenotype. Our dataset raises the exciting possibility that IGF-1R may be a novel target for the treatment of insulin resistance–related vascular disease.

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