Dissociation between metabolic and vascular insulin resistance in aging

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Schulman IH, Zhou M-S, Jaimes EA, Raij L. Dissociation between metabolic and vascular insulin resistance in aging. Am J Physiol Heart Circ Physiol 293: H853–H859, 2007. First published April 13, 2007; doi:10.1152/ajpheart.00138.2007.—Physiological actions of insulin in the vasculature serve to couple regulation of hemodynamic and metabolic homeostasis. Insulin resistance, endothelial dysfunction, and hypertension increase in prevalence with aging. We investigated the metabolic and endothelial actions of insulin in 24- vs. 3-mo Sprague-Dawley rats. With the use of the hyperinsulinemic euglycemic clamp, the rate of glucose infusion necessary to maintain equivalent plasma glucose (5.5 mmol/l) was similar in 24- vs. 3-mo rats, as was fasting glucose (5.2 ± 0.33 vs. 4.4 ± 0.37 mmol/l; mean ± SE) and insulin (0.862 ± 0.193 vs. 1.307 ± 0.230 mg/l). Systolic blood pressure was higher in 24-mo rats (133 ± 5 vs. 110 ± 4 mmHg; P = 0.005). Endothelial nitric oxide (NO)-dependent relaxation to insulin was impaired in aortas of 24- vs. 3-mo rats (maximal response 8.9 ± 4.3 vs. 34.9 ± 3.9%; P = 0.002); Nω-nitro-l-arginine methyl ester abolished insulin-mediated relaxation in 3-but not 24-mo rats. Endothelium NO-dependent (acetylcholine) and -independent (sodium nitroprusside) relaxation, as well as NADPH oxidase activity, were similar in 3- and 24-mo rats. Insulin increased aortic serine phosphorylation of Akt in 3-mo rats by 120% over 24-mo rats (P < 0.05) and serine phosphorylation of endothelial NO synthase (eNOS) in 3-mo rats by 380% over 24-mo rats (P < 0.05). Aortic expression of phosphorylated c-Jun NH2-terminal kinase-1 and serine phosphorylated insulin receptor substrate-1, known mediators of metabolic insulin resistance, was similar in 3- and 24-mo rats. Expression of caveolin-1, a regulator of eNOS activity and insulin signaling, was 55% lower in 24- than 3-mo rats (P = 0.002). In summary, impaired vasorelaxation to insulin in aging was independent of metabolic insulin sensitivity and associated with impaired insulin-mediated activation of the Akt/eNOS pathway, but intact activation of the acetylcholine-mediated Ca2+-calmodulin/eNOS pathway. Vascular insulin resistance in aging may add to the increased susceptibility of this population to vascular injury induced by traditional cardiovascular risk factors.

endothelium; nitric oxide; cell signaling; metabolic syndrome

INSULIN RESISTANCE, ENDOTHELIAL dysfunction, and hypertension (HTN), central features of the metabolic syndrome, are more prevalent among the aging population (5, 13, 14, 31, 33). Indeed, older age has been shown to be associated with increased odds of the metabolic syndrome in both men and women (31). Among men and women 65 yr of age and older, the odds ratio for the metabolic syndrome was approximately fivefold higher than among those aged 20–34 yr (31).

Physiological actions of insulin in the vasculature serve to couple regulation of metabolic and hemodynamic homeostasis (25). The vascular endothelium is the first organ that insulin encounters after it is secreted into the circulation. Insulin activates the insulin receptor (IR) tyrosine kinase, leading to tyrosine phosphorylation of IR substrate (IRS)-1, which binds and activates phosphatidylinositol 3-kinase (PI3K). PI3K phosphorylates Akt at serine 473, which directly activates endothelial nitric oxide (NO) synthase (eNOS) via phosphorylation at serine 1177, leading to increased blood flow (10, 39, 48). The increased blood flow occurs via capillary recruitment first, followed by vasodilation of larger blood vessels. There is evidence that constitutive production of NO by the endothelium maintains the vasculature in a state of vasodilation and that insulin may participate in basal NO release (7, 21, 29, 34).

It is intriguing that the same pathway through Akt activation is responsible for the metabolic action of insulin in skeletal muscle, adipose tissue, as well as vascular smooth muscle, leading to recruitment of the glucose transporter GLUT-4 to the cell surface, resulting in increased glucose uptake (30, 36). Therefore, impairment of insulin action in the vascular endothelium may be a link between HTN and metabolic dysfunction in obesity and Type 2 diabetes (22). Indeed, obese insulin-resistant individuals and patients with Type 2 diabetes have impaired insulin-stimulated activation of PI3K (5, 8, 14, 28). In these patients, both insulin-mediated vasodilation as well as endothelium-dependent vasorelaxation (EDR) in response to acetylcholine are often blunted (40, 45). Angiotensin (ANG) II, oxidative stress, and inflammation play a causal role in numerous settings of insulin resistance, including obesity, Type 2 diabetes, and HTN (2, 20, 37, 41). In these settings, the underlying mechanisms involve activation of c-Jun NH2-terminal kinase-1 (JNK1) and serine phosphorylation of IRS-1, resulting in inhibition of PI3K activation by insulin (1, 2, 19, 41, 43). In the present study, we investigated the mechanisms underlying the endothelial and metabolic actions of insulin in normal aging.

METHODS

Animals and experimental protocols. Male Sprague-Dawley (SD) rats were obtained from Harlan Laboratories (Indianapolis, IN). The animals were housed in the animal care facility with 12:12-h light-dark cycles and allowed free access to standard rat chow (Harlan Teklad Laboratories) and water. For the organ bath studies, described below, SD rats aged 3 and 24 mo (n = 5) were killed by decapitation, and the aorta and heart were harvested immediately and weighed. Aortic rings (thoracic) were obtained for the organ bath studies, described below, and immediately stored in 80°C for subsequent analysis. For the hyperinsulinemic-euglycemic clamp procedure, SD rats aged 3 and 24 mo (n = 5) were fasted overnight, studied as described below, and subsequently killed by inhalation of 3% isoflurane in room air. Nephrology and Hypertension Section, Veterans Affairs Medical Center, and Division of Nephrology and Hypertension and Vascular Biology Institute, University of Miami Miller School of Medicine, Miami, Florida

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decapitation. The aorta and heart were harvested immediately, weighed, frozen with liquid nitrogen, and stored at −80°C for subsequent Western blot analysis as described below. Tibia length was measured in all animals after death. Body weight was recorded at the time of the studies. Systolic blood pressure (SBP) was measured by cannulating the left femoral artery with polyethylene tubing (PE-10). The tubing was connected to a pressure transducer, and SBP was continuously measured for 60 min. Plasma insulin levels were determined using Mercodia Ultrasensitive Rat Insulin ELISA, Alpco Diagnostics. The Institutional Animal Care and Use Committee at the Miami Veterans Affairs Medical Center approved all of the animal studies.

Hyperinsulinemic-euglycemic clamp study. Metabolic insulin sensitivity was quantified using the hyperinsulinemic-euglycemic clamp, as previously described by others (9, 38), in 3- and 24-mo-old SD rats. All animals were fasted overnight for 16 h before the experiments were conducted. Animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and maintained at 37°C with a heating pad. The right femoral vein and left femoral artery were catheterized and used for glucose and insulin infusion and blood sampling, respectively. Baseline plasma samples were obtained, after which insulin (Sigma) at a constant rate of 30 mU·kg⁻¹·min⁻¹ and glucose (17.5 g/100 ml saline) at varying infusion rates were continuously infused for 120 min. The blood glucose concentration, measured with an automatic blood glucose meter (Accu-Chek Advantage Blood Glucose Meter), was clamped at 5.5 mmol/l (Fig. 1B). Euglycemic clamp was achieved by 60 min and maintained for 60 min. The glucose infusion rate was adjusted according to the blood glucose levels at 5-min intervals during the first 60-min period and, once stable, at 15-min intervals during the second 60-min period. The samples obtained over the second 60-min period were averaged and reported as the mean steady-state glucose infusion rate (mg·kg⁻¹·min⁻¹) required for maintaining euglycemic conditions in the face of hyperinsulinemia (Table 1 and Fig. 1A).

Determination of endothelium-dependent and independent relaxation in aorta. EDR to insulin and acetylcholine in aortic rings was examined using an organ chamber bath, as we and others have previously described (17, 26, 46, 49, 50). EDR in response to insulin (10⁻⁹ to 10⁻⁶ mol/l), EDR in response to acetylcholine (10⁻⁹ to 10⁻⁵ mol/l), and vasorelaxation to sodium nitroprusside (SNP, 10⁻⁵ mol/l) were assessed in aortic rings precontracted to 70% of maximal contraction to norepinephrine (10⁻⁷ mol/l). In separate aortic rings, EDR to insulin after pretreatment with N⁶-nitro-L-arginine methyl ester (L-NAME; 10⁻⁴ mol/l), a NO synthase inhibitor, or superoxide dismutase (8 mg/ml) and EDR to acetylcholine after pretreatment with L-NAME (10⁻⁴ mol/l) were also assessed.

Phosphorylated and total Akt, eNOS, JNK, and IRS-1 protein expression. The baseline and insulin clamp-stimulated aortic protein expression of serine phosphorylated and total Akt and eNOS and the baseline aortic protein expression of phosphorylated and total JNK1 and 6% gel for eNOS and IRS-1) and transferred to nitrocellulose membranes. The membranes were incubated with specific rabbit polyclonal anti-phospho (Ser 1177)-eNOS, anti-phospho JNK1/2, or anti-phospho (Ser 307)-IRS-1 antibodies (1:300 or 1:500; Cell Signaling) overnight at 4°C, followed by incubation with a peroxidase-conjugated host-specific secondary antibody (1:1,000; Santa Cruz Biotechnology). The immunoreactive bands were detected by enhanced chemiluminescence followed by exposure to an autoradiography film and were quantified by densitometry analysis. Membranes were subsequently stripped and re-probed with anti-Akt, anti-eNOS, anti-JNK1/2, or anti-IRS-1 antibodies (1:500; Cell Signaling), respectively. Anti-actin was used as a loading control (1:500; Santa Cruz Biotechnology). Protein phosphorylation is expressed as the ratio of phosphorylated protein to total protein.

ANG II type 1 receptor and monocyte chemoattractant protein-1 mRNA expression. Total RNA (2 μg) was extracted from aortas using TRIzol Reagent (Invitrogen, Carlsbad, CA) and reverse-transcribed using the SuperScript II RT First Strand Synthesis kit (Gibco, BRL), according to the manufacturer’s instructions (50). Primers and probes for rat angiotensin II type 1 receptor (AT1R) and monocyte chemoattractant protein-1 (MCP-1) were designed by using Primer Express software [Applied Biosystems (ABI)]. Real-time PCR was performed in a 50-μl reaction mixture containing 100 ng cDNA solution, 0.1 μmol/l of each primer, 0.2 μmol/l probe, and PCR Master Mix assay kit (ABI) under the following conditions: 50°C for 2 min, 95°C for 10 min, and 40 cycles at 95°C for 15 s, and 60°C for 1 min. A housekeeping gene (GAPDH) was also determined as an internal control.

IR, AT1R, gp91phox, and caveolin-1 protein expression. Protein expression in aortas was evaluated by Western blot. Aortas were dissected and homogenized in Eppendorf tubes (containing lysis buffer, a protease inhibitor mixture to inhibit serine, cysteine, and aspartic proteases to prevent degradation; Sigma) using a tissue homogenizer. The Bradford assay was used to measure protein concentration. Forty micrograms of protein were loaded onto an SDS-
analyses. Results were considered significant when Tukey’s tests for multiple comparisons where appropriate for post hoc
mined from the concentration-response curve, using the best fit to a
vs. 0.449
SBP, aged rats exhibited cardiac hypertrophy (0.259
remained normotensive (Table 1). Consistent with the higher
rats aged 24 mo manifested significantly increased SBP, but
(Table 1) necessary to maintain equivalent plasma glucose (5.5
mmol/l) was similar in 24- compared with 3-mo-old SD rats
Table 1) and was completely inhibited by l-NAME. These findings suggest that the impaired vasorelaxation to insulin in aged rats is due to selective impairment of NO bioavailability in response to insulin, despite normal NO-dependent vascular response to acetylcholine. Superoxide dismutase did not improve vascular relaxation to insulin in aged SD rats (E_max 8.2 ± 3.9%). Endothelium-independent relaxation to SNP was intact in both young and aged SD rats (E_max 101 ± 2.0 vs. 103 ± 1.7%; P = NS).

Effect of insulin on phosphorylation of Akt and eNOS in aorta. Akt is a serine/threonine kinase that is phosphorylated at serine 473 and activated by insulin through the PI3K pathway. In response to insulin, Akt directly activates eNOS by phosphorylation at serine 473. The phosphorylation of Akt and eNOS is shown in Fig. 4.

**Table 1. Characteristics of 3-mo-old and 24-mo-old Sprague-Dawley rats**

<table>
<thead>
<tr>
<th></th>
<th>3-mo-old</th>
<th>24-mo-old</th>
<th>P</th>
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<tbody>
<tr>
<td>Body weight, g</td>
<td>334±10</td>
<td>507±15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>110±4</td>
<td>133±5</td>
<td>0.005</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>4.4±0.37</td>
<td>5.17±0.33</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin, µg/l</td>
<td>1.307±0.230</td>
<td>0.862±0.193</td>
<td>NS</td>
</tr>
<tr>
<td>GIR, mg·kg⁻¹·min⁻¹</td>
<td>10.5±0.4</td>
<td>9.9±0.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. SBP, systolic blood pressure; GIR, mean steady-state glucose infusion rate during the last 60 min of the clamp; NS, not significant. P value for 3-mo-old vs. 24-mo-old is from unpaired t-tests between the two groups.

PAGE 10% gel and transferred to a nitrocellulose membrane. Membranes were then probed with rabbit polyclonal anti-IR (IR-β, 1:500; Cell Signaling), anti-AT1R antibody (1:300; Santa Cruz Biotechnology), reduced P_Akt (1:500; kindly provided by Dr. Mark T. Quinn) (4), or anti-caveolin-1 (1:500; Cell Signaling). The primary antibody was then detected with a peroxidase-conjugated host-specific secondary antibody (1:1,000; Santa Cruz Biotechnology). The immunoreactive bands were detected by luminol chemiluminescence (enhanced chemiluminescence) and quantified by densitometry using a UMAX Astra 2200 scanner and ImageJ 1.33 software. After initial exposure to these antibodies, membranes were stripped (Re-probe solution) and then probed with anti-actin (as a loading control; 1:500; Santa Cruz Biotechnology).

**Determination of NADPH oxidase activity in aorta.** NADPH oxidase activity in aortic homogenates was determined as described previously. In brief, 20 ml of aortic homogenate were added into 50 mmol/l phosphate assay buffer (pH 7.4) containing 1.0 mmol/l EDTA and 5 mmol/l lucigenin. The reaction was triggered by addition of 100 mmol/l NADPH as substrate, and superoxide (O_2⁻) production was determined by lucigenin-enhanced chemiluminescence. In the present studies, as in previous studies, NADPH oxidase-derived O_2⁻ was confirmed using the flavoprotein inhibitor diphenyleneiodinium, which reduced production of O_2⁻ by >95% in the homogenate (data not shown).

**Data analysis.** The results are expressed as means ± SE. Western blot data are expressed as relative to control, assigning a value of 1 to the 3-mo-old baseline mean. Relaxation of aortic rings is expressed as a percentage inhibition of norepinephrine-induced constriction. The maximal response to acetylcholine (E_max), insulin, or SNP was determined from the concentration-response curve, using the best fit to a logistic sigmoid function. Differences between groups were examined for statistical significance using Student’s t-test or ANOVA, with Tukey’s tests for multiple comparisons where appropriate for post hoc analyses. Results were considered significant when P < 0.05.

**RESULTS**

**SBP and cardiac weight.** Compared with 3-mo-old rats, SD rats aged 24 mo manifested significantly increased SBP, but remained normotensive (Table 1). Consistent with the higher SBP, aged rats exhibited cardiac hypertrophy (0.259 ± 0.014 vs. 0.449 ± 0.012 g/cm, 3 vs. 24 mo old; P < 0.001), as measured by organ weight-to-tibia length ratios.

**Metabolic insulin sensitivity.** Using hyperinsulinenic euglycemic clamp, the rate of glucose infusion (mg·kg⁻¹·min⁻¹) necessary to maintain equivalent plasma glucose (5.5 mmol/l) was similar in 24- compared with 3-mo-old SD rats (Table 1 and Fig. 1). Similarly, fasting plasma glucose and fasting plasma insulin did not differ between the young and aged rats (Table 1). These findings suggest that metabolic (peripheral) insulin sensitivity was not impaired in the 24-mo-old SD rats.

EDR to insulin and acetylcholine in aortic rings. Aortic EDR to insulin (E_max 34.9 ± 3.9 vs. 8.9 ± 4.3%; 3- vs. 24-mo rats; P = 0.002; Fig. 2) was impaired in 24-mo-old rats compared with 3-mo-old rats. l-NAME abolished insulin-mediated relaxation in young rats but not in aged rats [E_max 7.6 ± 2.6 vs. 8.5 ± 4.5%; P = nonsignificant (NS); 3- vs. 24-mo rats; Fig. 2]. On the other hand, EDR to acetylcholine did not significantly differ between the 3- and 24-mo-old rats (E_max 95.7 ± 4.0 vs. 85.8 ± 3.1% NS; 3- vs. 24-mo rats; Fig. 2). Using hyperinsulinenic euglycemic clamp, increased serine phosphorylation of Akt by 3.5-fold over the basal levels (Fig. 4A). However, in the aorta from 24-mo-old SD rats, insulin infusion, administered during the clamp, increased serine phosphorylation of Akt by 3.5-fold over the basal levels (Fig. 4A). In the aorta from 3-mo-old SD rats, insulin infusion, administered during the clamp, increased serine phosphorylation by only 2.3-fold over the basal levels, representing a 120% decrease compared with their young counterparts (P < 0.05, 3 vs. 24 mo old after insulin; Fig. 4A). Similarly, insulin infusion increased serine phosphorylation of eNOS by 6.7-fold over the basal levels in aorta from 3-mo-old SD rats, compared with 2.9-fold over the basal levels in aorta from 24-mo-old rats.
represents a 380% decrease (P < 0.05, 3 vs. 24 mo old after insulin; Fig. 4B).

Expression of IR and IRS-1 in aorta. We investigated whether the decrease in insulin-stimulated phosphorylation of Akt and eNOS in aged rats was due to decreased aortic expression of the IR or IRS-1. There were no significant differences between 3- and 24-mo-old SD rats in the basal aortic protein expression of the IR (1.07 ± 0.14; P = NS) or IRS-1 (1.35 ± 0.20; P = NS).

Expression of AT1R, gp91phox, JNK1, serine phosphorylated IRS-1, and MCP-1 in aorta. Activation of the renin-ANG system and increased oxidative stress have been linked with the development of insulin resistance (2, 20, 37, 41). A major source of endothelial reactive oxygen species (ROS) generation is the NAD(P)H oxidase complex, composed of a membrane-bound flavocytochrome b558 consisting of gp91phox (Nox2) and p22phox and two cytosolic subunits (p47phox and p67phox) (15, 23). The subunit gp91phox seems to be the limiting subunit of the NAD(P)H oxidase complex in endothelial cells (11, 32). ANG II and NADPH oxidase-derived ROS are associated with the activation, via phosphorylation, of the serine/threonine kinase JNK1. ANG II, ROS, and JNK1 have been demonstrated to phosphorylate IRS-1 at serine 307, which results in its dissociation from the IR and triggers proteasome-dependent degradation, impairing insulin signaling through the PI3K pathway (1, 2, 19, 37, 41, 43).

In aortas from SD rats aged 3 vs. 24 mo, AT1R mRNA (0.406 ± 0.064 vs. 0.327 ± 0.065; P = NS) and protein expression (1.06 ± 0.09; P = NS) were similar. Protein expression of gp91phox did not differ between the young and aged SD rats (1.02 ± 0.13; P = NS). Furthermore, no significant differences were found in the basal aortic expression of phosphorylated JNK1 (1.11 vs. 1.7 ± 0.37; P = NS) or serine phosphorylated IRS-1 (1.12 vs. 0.72 ± 0.08; P = NS) between young and aged SD rats. The mRNA expression of MCP-1, a proinflammatory molecule (12) also shown to contribute to the development of insulin resistance (24), did not differ between the young and aged SD rats (0.63 ± 0.37 vs. 0.44 ± 0.217; P = NS).

NADPH oxidase activity in aorta. We determined NADPH oxidase-derived O2 production in the aortic homogenate by lucigenin chemiluminescence and found no difference in NADPH oxidase activity between young and aged SD rats (Fig. 5).

**DISCUSSION**

The major novel findings of the present study are that, despite normal fasting plasma levels of insulin and glucose, as
well as normal metabolic insulin sensitivity, as determined by hyperinsulinemic euglycemic clamp, 24-mo-old SD rats exhibited impaired aortic EDR to insulin, but not to acetylcholine, indicating selective vascular insulin resistance. Compared with their 3-mo-old counterparts, 24-mo-old rats also manifested a higher SBP, which was accompanied by cardiac hypertrophy. Insulin signaling pathways in the endothelium leading to the activation of eNOS and increased production of NO have been demonstrated in vitro and in vivo to be independent from classical calcium-dependent mechanisms used by G protein-coupled receptors, such as the acetylcholine receptor (26, 27). Endothelial dysfunction, manifested by impairment in acetylcholine-mediated EDR, has been observed clinically and experimentally in more advanced aging (42, 44). Collectively, these studies suggest that decreased EDR to insulin, in conjunction with other cardiovascular risk factors, may contribute to the increase in susceptibility to vascular injury that occurs with age.

Insulin plays a central role in the regulation of metabolic and hemodynamic homeostasis (14, 25). To elucidate the mechanisms underlying vascular insulin resistance in aging, we investigated basal and insulin-stimulated expression of phosphorylated (activated) Akt and eNOS. IR activation of the PI3K pathway leads to phosphorylation of Akt; phosphorylated Akt directly phosphorylates eNOS, resulting in vasodilation, and promotes glucose uptake via GLUT-4 in muscle and adipose tissue. However, the metabolic and hemodynamic actions of insulin do not completely overlap, since it has been shown that eNOS inhibition abolishes vasodilation but reduces glucose uptake by only 30% (3). Consistent with these findings, vascular insulin resistance in our aged rats was associated with decreased insulin-stimulated activation (phosphorylation) of Akt and eNOS and impaired vascular relaxation, despite normal whole body sensitivity to insulin-mediated glucose uptake. Furthermore, the decrease in insulin-stimulated activation of Akt and eNOS in aged rats was not due to decreased aortic expression of the IR or IRS-1.

We subsequently investigated mechanisms that could potentially underlie the impairment in insulin-stimulated activation of Akt and eNOS. The renin-ANG system and oxidative stress have been implicated in the pathogenesis of insulin resistance (2, 19, 37). ANG II, via the AT1R, increases NADPH oxidase-derived oxidative stress, induces vasoconstriction, and promotes inflammation and monocyte migration into the vessel wall through increased expression of adhesion molecules and proinflammatory molecules, such as MCP-1, which is implicated in atherogenesis (12, 16) and insulin resistance (24). Moreover, activation of JNK1 by ANG II or oxidative stress leads to inhibition of insulin-mediated PI3K pathway signaling, via phosphorylation of IRS-1 on serine 307, and insulin resistance (2, 19, 37, 41, 43). Experimental studies have shown that aged rats manifest increased AT1R gene expression in left ventricular myocardium (18). Thus we investigated whether the development of vascular insulin resistance with aging was associated with AT1R upregulation. We found that 24-mo-old SD rats did not exhibit increased mRNA or protein expression of aortic AT1R; however, this does not preclude aging-induced activation of the renin-ANG system through other mechanisms that do not involve AT1R upregulation. We subsequently assessed NADPH oxidase activity and protein expression of the NADPH oxidase subunit gp91phox and found no differences between the young and aged rats. Furthermore, we found no significant difference in the basal aortic expression of phosphorylated (activated) JNK1 or phosphorylated (serine 307) IRS-1 between the young and aged SD rats. Finally, aortic gene expression of MCP-1 was not increased in 24-mo-old rats. Thus, although NADPH oxidase-derived oxidative stress and inflammation play a causal role in numerous settings of insulin resistance (20), the development of vascular insulin resistance was not linked to increased expression of the proinflammatory markers AT1R, gp91phox, activated JNK1, or MCP-1 or increased NADPH oxidase activity in this early stage of aging.

Caveolin-1 plays an important regulatory role in insulin signaling (6) and eNOS activity (47), and its aortic expression has been shown to decrease with aging (35). We, therefore,
investigated caveolin-1 expression. We found a significant (55\%) reduction in caveolin-1 in aorta of aged SD rats. Recent elegant studies revealed that acute flow-dependent carotid artery dilation was reduced in caveolin-1 knockout mice, despite normal eNOS protein levels and enhanced acetylcholine-stimulated EDR (47). These altered responses were normalized by reexpression of caveolin-1 in the endothelium. These studies suggested that, in the absence of caveolin-1 and caveolae, flow-dependent activation of caveolar-associated eNOS is reduced, leading to an impairment of flow mechanotransduction, whereas acetylcholine-induced eNOS activation is increased due to the loss of the direct inhibitory influence of caveolin-1 on eNOS (47). Furthermore, eNOS phosphorylation on serine 1176 in mice, a key regulatory site of phosphorylation by many kinases including Akt, was reduced in aortic extracts from caveolin-1 knockout mice, suggesting that flow activation of PI3K, among other upstream kinases, may be impaired. Similar to laminar flow, insulin activates eNOS via the PI3K signaling pathway. Based on these studies, we hypothesize that loss of caveolin-1 with aging may contribute to the impairment in vascular insulin signaling. Further investigations are needed to verify the relevance of this hypothesis.

Endothelial dysfunction, HTN, and insulin resistance are part of the metabolic syndrome, which increases in prevalence with aging. The present study suggests that, in aging, during the early stages, development of vascular insulin resistance is independent of metabolic insulin sensitivity and is associated with impaired activation of Akt/eNOS by insulin, but intact activation of the Ca\(^{2+}\)-calmodulin pathway. We thus surmise that vascular insulin resistance in aging may contribute to the development of HTN and may be an early phenomenon that precedes the development of metabolic insulin resistance, systemic oxidative stress, and endothelial dysfunction (44). Furthermore, impaired vascular relaxation to insulin in aging may add to the increased susceptibility of this population to vascular injury induced by cardiovascular risk factors, such as obesity, diabetes, HTN, smoking, and dyslipidemia.

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REFERENCES


