Adipocyte-derived cytokine resistin causes endothelial dysfunction of porcine coronary arteries

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Objective: **Resistin, a novel adipocyte-derived cytokine, is involved in the development of insulin resistance and diabetes mellitus. In this study, we determined whether resistin could affect vasomotor function, oxidative stress, and endothelial nitric oxide synthase (eNOS) expression in porcine coronary arteries.**

Methods: **Porcine coronary arteries were treated with resistin or antioxidant seleno-L-methionine (SeMet). Vasomotor function was studied by using a myograph system. Levels of superoxide anion (O_2^-) were detected by the lucigenin-enhanced chemiluminescence method. The eNOS mRNA and protein levels were determined by real-time polymerase chain reaction and immunohistochemistry, respectively. Culture of isolated porcine coronary artery endothelial cells (PCAECs) was also included.**

Results: **Endothelium-dependent relaxation in response to bradykinin was reduced by 15% and 30% for the rings treated with 10 and 40 ng/mL of resistin, respectively, as compared with controls ($P < .05$). Endothelium-independent relaxation in response to sodium nitroprusside (SNP) was also reduced by 11% after treatment with 40 ng/mL of resistin ($P < .05$). The O_2^- level was increased in the 40 ng/mL resistin-treated vessels by 88% as compared with controls ($P < .05$). SeMet reversed these effects. The eNOS mRNA levels in PCAEC cultures treated with resistin (10 and 40 ng/mL) were decreased by 27% and 55%, respectively ($P < .05$) and by 39% in the endothelial cells purified from porcine coronary artery rings after treatment with 40 ng/mL of resistin ($P < .05$). Immunoreactivity of eNOS in the resistin-treated vessel rings was also substantially reduced.**

Conclusions: **Resistin reduces the endothelium-dependent and endothelium-independent vasorelaxation. This effect is associated with increased superoxide radical production, decreased eNOS expression, and is effectively reversed by the antioxidant SeMet. (J Vasc Surg 2005;41:691-8.)**

Clinical relevance. **Obesity has been considered to be an independent risk factor for coronary artery disease and other vascular lesions. Resistin is a newly discovered adipocyte-derived cytokine, and its plasma levels are increased in obese individuals. However, it is not clear whether resistin could directly contribute to vascular disease formation. This study showed that resistin can cause endothelial dysfunction in porcine coronary arteries through oxidative stress and down-regulation of eNOS. Thus, this study may suggest a new mechanism of obesity-associated vascular disease and that antioxidants may effectively prevent vascular disease in obese individuals.**

Insulin-resistant metabolic syndrome affects a large number of North Americans and is associated with increased rates in cardiovascular disease that represents a major cause of morbidity and mortality for Western societies.[1] Despite divergent views regarding the exact pathogenesis of atherosclerosis,[2,3] it is generally accepted today that the fundamental problem that initiates and propagates the atherosclerotic lesion is the presence of an altered state of endothelial cell homeostasis, or endothelial dysfunction.[4]

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Competition of interest: none.

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Over the past few years, much effort has been made to comprehend the mechanisms that link insulin resistance with endothelial dysfunction.[5] Particular emphasis has been placed on the adipocyte-derived hormones (adipokines) that not only function prominently in the pathogenesis of the insulin-resistant syndrome but may also serve as important vasoactive factors, directly affecting endothelial function and vascular health.

It has been suggested that resistin, a recently described adipokine, has a role in the development of insulin resistance and obesity.[6] Resistin is produced during adipogenesis and inhibits glucose uptake in skeletal muscle cells in animal models.[7] Although a great deal of controversy surrounds its exact biologic roles in humans, recent studies suggest that resistin promotes the development of endothelial dysfunction. Verma et al[8] studied the effect of resistin on human saphenous vein endothelial cell activation. They found that resistin promotes endothelial cell activation by promoting endothelin-1 (ET-1) release. Resistin can up-regulate the vascular cell adhesion molecule (VCAM-1), intercellular adhesion molecule-1 (ICAM-1),

and the monocyte chemoattractant protein-1 (MCP-1), while down-regulating the expression of tumor necrosis factor–receptor-associated factor-3 (TRAF-3), an inhibitor of CD40 ligand signaling.[8,9] In addition, resistin can induce pentraxin 3, a marker of inflammation.[9]

In the present study, we hypothesized that the adipokine resistin adversely affects endothelial function and impairs vascular relaxation. To test this hypothesis we investigated the effects of human recombinant resistin in a porcine coronary artery model. Plausible mechanisms for the endothelial cell dysfunction via altered endothelial nitric oxide synthase (eNOS) levels or increased consumption of nitric oxide (NO) by superoxide radicals were also investigated. Our findings suggest vascular effects of the adipokine resistin and lend credence to the concept of adipocyte-endothelial cell interaction in states of insulin resistance and obesity.

METHODS

Chemicals and reagents. Thromboxane A_2 analogue U46619 (9,11-Dideoxy-11α,9α-epoxymethanoprostaglandin F2α), bradykinin, sodium nitroprusside (SNP), phosphate-buffered saline (PBS) solution, seleno-L-methionine (SeMet), and Tri-reagent kit were obtained from Sigma Chemical (St. Louis, Mo). Human recombinant resistin was obtained from Phoenix Pharmaceuticals, Inc (Belmont, Calif). Lucigenin was obtained from Molecular Probes (Eugene, Ore). Dulbecco modified Eagle's medium (DMEM) was obtained from Life Technologies, Inc (Grand Island, NY). Antibiotic-antimycotic solution was obtained from Mediatech Inc (Herndon, Va). iScript cDNA Synthesis Kit and the iQ SYBR Green SuperMix Kit were obtained from Bio-Rad Laboratories (Hercules, Calif). Antibody against human eNOS was obtained from BD Transduction Laboratories (Lexington, Ky). The biotinylated horse anti-mouse immunoglobulin G (IgG) and avidin-biotin complex kit were obtained from Vector Labs (Burlingame, Calif).

Tissue harvest and cell culture. Fresh porcine hearts were harvested from farm pigs (6 to 8 months old) at a local slaughterhouse. The hearts were rinsed with sterile PBS, the aortic arch and coronary arteries were perfused with cold PBS, and each was stored in ice cold PBS for transport back to the laboratory. The right coronary arteries were carefully dissected, the perivascular loose connective tissue was removed, and the arteries were cut into 5-mm rings. Several rings from each heart were allocated into groups: controls (dimethyl sulfoxide [DMSO]), those treated with resistin (10 and 40 ng/mL), and those treated with 40 ng/mL of resistin plus 100 μM of the commonly used antioxidant SeMet. The use of 100 μM of SeMet was based on experience and previous publications that showed potent antioxidant effects of SeMet at this concentration.[10-12] The rings were incubated in DMEM with each drug or DMSO with an equivalent amount in resistin solution (40 ng/mL) at 37°C and 5% CO_2 for 24 hours.

Porcine coronary endothelial cells (PCAECs) and culture medium were obtained from Cell Applications, Inc (San Diego, Calif). The cells were used at passage 6 to 7. Once the PCAECs grew to 80% to 90% confluence in 6-well culture plates, they were treated with the medium containing DMSO as control or with resistin (10 or 40 ng/mL) for 24 hours at 37°C. Cells were then harvested, and total mRNA was extracted for real-time polymerase chain reaction (PCR) study.

Myograph analysis. The myograph tension system used in our laboratory has been previously described.[13-16] Briefly, the rings were cultured in the medium for 24 hours and then were suspended between the wires of the organ bath chamber (Multi myograph system 700MO; Myo Technology, Aarhus N, Denmark) in 6 mL of Kreb's solution. After equilibration, each ring was precontracted with 20 μL of thromboxane A2 analogue U46619 (10^{-7} M). After 60 to 90 minutes of contraction, the relaxation dose-response curve was generated by adding 60 μL of five cumulative additions of the endothelium-dependent vasodilator bradykinin (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M) every 3 minutes. In addition, 60 μL of SNP (10^{-6} M) was added to the organ bath, and endothelium-independent vasorelaxation was recorded.

Detection of O_2^-. Levels of O_2^- produced by endothelial cells were detected by using the lucigenin-enhanced chemiluminescence method with a Sirius Luminometer and FB12 software from Berthold Detection System GmbH (Pforzheim, Germany) as previously described.[15] Briefly, vessel rings from each of the four experimental groups were used. The rings were cut open longitudinally and trimmed into ≈5- × 5-mm pieces. They were then rinsed briefly in a modified Kreb's HEPES buffer solution. An assay tube (12 × 75 mm) was filled with 500 μL buffer and 25 μL lucigenin, and after the vessel segments were gently vortexed, they were placed endothelium-side-down in the tubes so signals from the endothelial layer could be recorded. Time-based reading of the luminometer was recorded by FB12 software. The data, in relative light units per second (RLU/s) for each sample, were averaged between 5 and 10 minutes. Final data were represented as RLU/s/mm^2.

Real-time PCR. Total RNA from cultured PCAECs was isolated using Tri-Reagent following the manufacturer's instructions. In separate experiments, porcine coronary artery rings were incubated with different concentrations of resistin, SeMet, or both for 24 hours, and the endothelial cells were scraped off the vessel intima and processed using Tri-Reagent as previously described.[17,18] Briefly, the RNA from each well was resuspended in 20 μL of RNase-free water. cDNA was generated by reverse transcription from mRNA using the iScript cDNA Synthesis Kit. Next, the iQ SYBR Green SuperMix Kit was used for real-time PCR reaction. Porcine eNOS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers were designed using Beacon Designer. The eNOS (GeneBank #AY266137) primer sequences are: forward primer 5′-CCCTACAACGGCTCCCCTC-3′ and reversed primer 5′-GCTGTCTGTGTTACTGGATTCCTT-3′. The GAPDH (GeneBank #AF017079) primer sequences are: forward primer 5′-TGTACCACCAACTGCTTGGC-3′

and reversed primer 5′-GGCATGGACTGTGGTCATGAG-3′. Sample cycle threshold (Ct) values were determined from plots of relative fluorescence units (RFU) versus PCR cycle number during exponential amplification so that sample measurement comparisons were possible. The eNOS gene expression in each sample was calculated as 2^(40 – Ct) and further normalized to GAPDH expression as [2^($Ct_{[GAPDH]}$ – $Ct_{[eNOS]}$)].[19,20]

Immunohistochemistry of eNOS. Treated rings were fixed in 10% neutral buffered formalin and embedded in paraffin. Cross-sections were stained with monoclonal antibody against human eNOS (1:1000) using avidin-biotin complex immunoperoxidase procedure. Sections were counterstained and viewed on an Olympus BX41 microscope (Olympus USA, Melville, NY). Images were captured with an attached SPOT-RT digital camera and software (Diagnostic Instruments, Sterling Heights, Mich).

Statistical analysis. Statistic analysis was performed on the Data Analysis tool of the Microsoft Excel program (Microsoft Office 2000, Microsoft Inc, Seattle, Wash). Data were expressed as mean ± SEM. Significant difference of data between the control and treated groups was determined by the paired Student *t* test (two-tail). Comparison of vasorelaxation in response to a series of concentrations of bradykinin between controls and resistin-treated vessels was analyzed with a single factor analysis of variance (ANOVA) test. Furthermore, the final data points of all contractions and relaxations among different groups were also analyzed by ANOVA test. $P < .05$ was considered statistically significant.

RESULTS

Resistin impairs relaxation in porcine coronary arteries in a dose-dependent manner. Porcine coronary arteries were cultured for 24 hours with a clinically relevant concentration of 10 or 40 ng/mL of human recombinant resistin and subsequently subjected to contraction (U46619) and endothelium-dependent (bradykinin) and endothelium-independent (SNP) relaxation (n = 10 for each group). In response to U46619, the contraction of the vessel rings was increased by 4% and 13% for resistin (10 and 40 ng/mL), respectively, as compared with controls; however, this did not reach statistical significance ($P > .05$, *t* test, Fig 1, *A*). ANOVA analysis did not show a significant difference of contraction among groups. In response to bradykinin at 10^{-5} M, the endothelium-dependent relaxation was significantly reduced by 15% for the rings treated with 10 ng/mL of resistin and by 30% for the group treated with 40 ng/mL of resistin as compared with controls ($P < .05$, ANOVA, Fig 1, *B*). The endothelium-independent vasorelaxation in response to SNP was reduced by 7% in the rings treated with 10 ng/mL of resistin and 11% for the porcine arteries treated with 40 ng/mL of resistin as compared with controls ($P < .05$, *t* test and ANOVA, Fig 1, *C*).

Resistin increases superoxide anion production in porcine coronary arteries. Oxidative stress is one of the important mechanisms of endothelial dysfunction and vas-

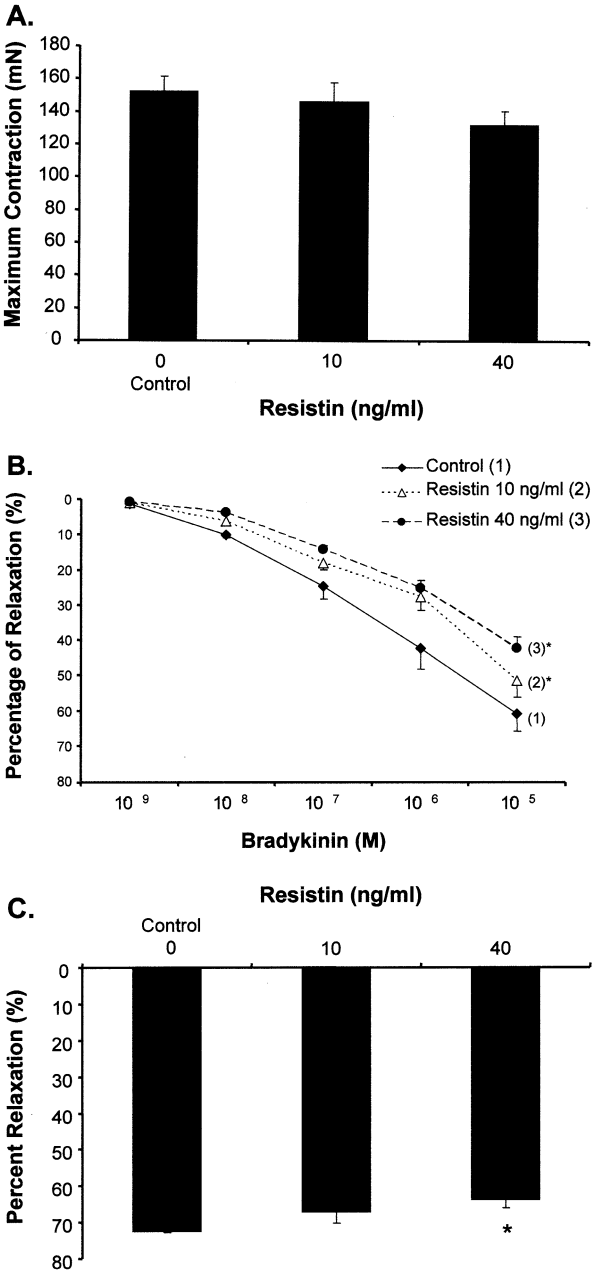

Fig 1. Effect of resistin on the vasomotor function of porcine coronary arteries. Pig right coronary arteries (n = 10) were cultured with medium-dimethyl sulfoxide (as control) or treated with resistin (10 or 40 ng/mL) for 24 hours. **A,** Maximal contraction of the vessel rings in response to thromboxane A2 analogue U46619 (10^{-7} M). Contractility was increased in the rings treated with resistin; however, this did not reach statistical significance ($P > .05$, *t* test and analysis of variance [ANOVA]) compared with controls. **B,** Precontracted vessels were tested for endothelium-dependent relaxation by addition of bradykinin (10^{-9} to 10^{-5} M). We observed a statistically significant reduction in the resistin-treated samples compared with controls (*$P < .05$, ANOVA). **C,** Endothelium-independent relaxation in response to sodium nitroprusside (10^{-6} M) was significantly reduced in the vessels treated with 40 ng/mL of resistin (*$P < .05$, *t* test and ANOVA). Data shown are means ± SEM.

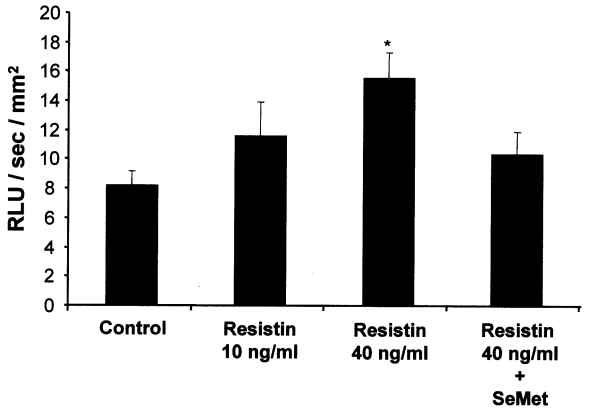

Fig 2. Effect of resistin on the O_2^- production in porcine coronary arteries. O_2^- levels in porcine coronary arteries were tested with lucigenin-enhanced chemiluminescence assay. The data were normalized with area (mm^2) of the ring and are expressed as relative light units *(RLU/sec/mm²)*. Resistin (40 ng/mL) significantly increased the O_2^- levels of vessel rings (*$P < .05$, n = 3). The antioxidant seleno-L-methionine *(SeMet)* blocked the resistin-induced O_2^- production.

cular injury. To determine whether this mechanism is involved in resistin-induced vasomotor dysfunction, O_2^- production was analyzed by lucigenin-enhanced chemiluminescence assay (n = 3). The O_2^- levels of the endothelial layer of vessel rings were significantly increased by 88% for 40 ng/mL of resistin as compared with control samples ($P < .05$, *t* test, Fig 2). The O_2^- level in the rings that were treated with 10 ng/mL of resistin increased by 40%; however, this was not statistically significant ($P > .05$). The addition of SeMet substantially reversed the effect of resistin. Rings treated with 40 ng/mL of resistin plus SeMet demonstrated a 25% increase in the O_2^- levels as compared with controls ($P > .05$, *t* test, Fig 2).

Antioxidant SeMet blocks resistin-induced vasomotor dysfunction in porcine coronary arteries. Known as an antioxidant, SeMet has been shown to increase the activity of glutathione peroxidase in endothelial cells.[10] To further confirm oxidative stress is involved in resistin-induced vessel dysfunction, vessel rings cultured with 40 ng/mL of resistin alone showed that the maximum bradykinin (10^{-5} M)-induced relaxation was significantly decreased; however, the addition of SeMet totally reversed this effect and, in fact, increased vasorelaxation by 11% compared with controls. ANOVA analysis showed a significant difference of bradykinin-induced vasorelaxation among groups ($P < .05$). In a similar manner, endothelium-independent vasorelaxation after exposure to SNP was decreased by 11% in the rings treated with 40 ng/mL of resistin. The addition of SeMet reversed this effect and increased the vasorelaxation by 3% as compared with controls (Fig 3, *A to C*).

Resistin reduces eNOS expression in porcine coronary arteries. To determine whether eNOS expression was correlated with the reduction of endothelium-

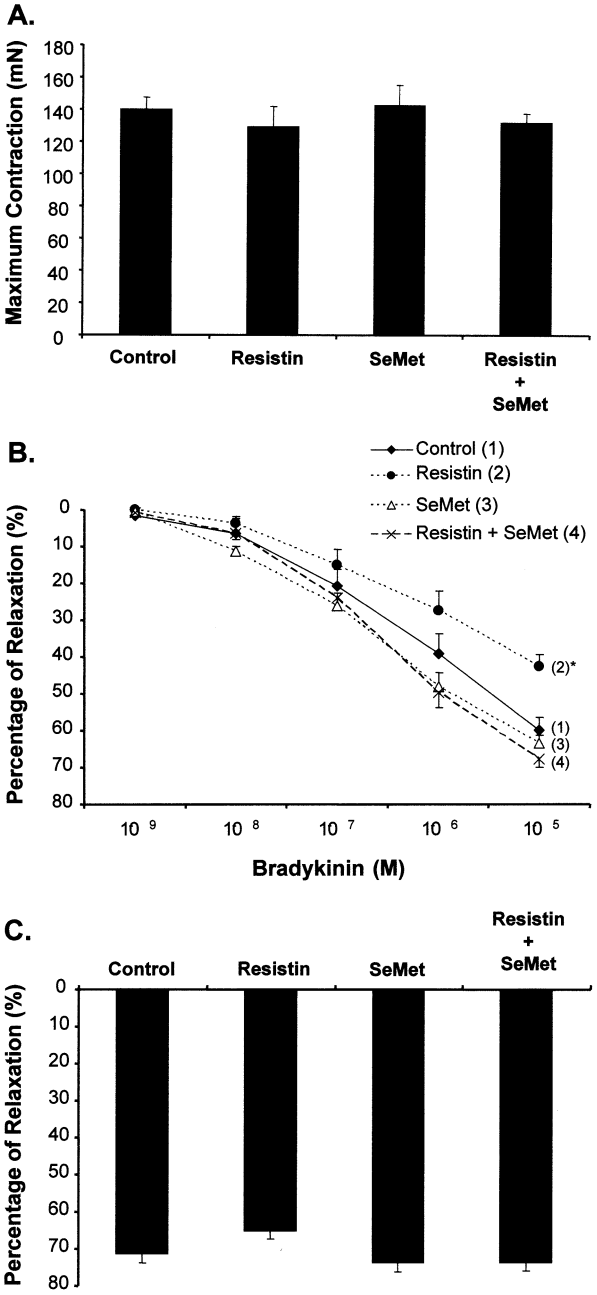

Fig 3. Effect of antioxidant seleno-L-methionine *(SeMet)* on the resistin-induced vasomotor dysfunction of porcine coronary arteries. Vessel rings were cultured with SeMet (100 μM), resistin (40 ng/mL), or both for 24 hours (n = 5). **A,** Maximal contraction of the vessel rings in response to U46619 (10^{-7} M). There was no statistically significant difference among the rings treated with resistin or SeMet, or both, compared with controls (*t* test and analysis of variance [ANOVA]). **B,** Endothelium-dependent vasorelaxation of the precontracted vessel rings in response to bradykinin (10^{-9} to 10^{-5} M). Resistin significantly inhibited bradykinin-induced vasorelaxation (*$P < .05$, ANOVA). SeMet blocked this effect. **C,** Endothelium-independent vasorelaxation in response to SNP (10^{-6} M). Resistin 40 ng/mL interfered with the sodium nitroprusside-induced vasorelaxation (*$P < 0.05$, t-test, and ANOVA), and SeMet reversed this effect.

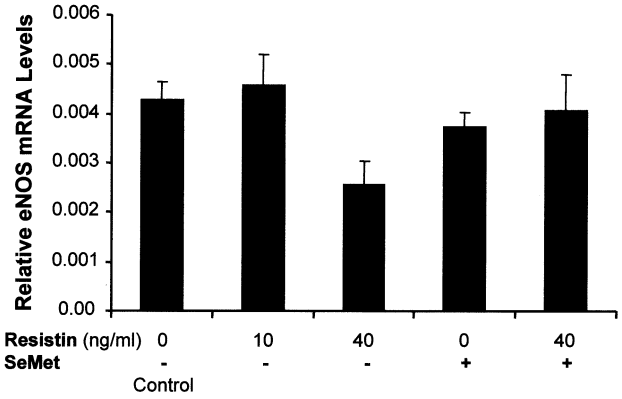

Fig 4. Effect of resistin on the endothelial nitric oxide synthase *(eNOS)* mRNA level of porcine coronary arteries. Porcine coronary rings were cultured for 24 hours in medium (dimethyl sulfoxide [control]) with resistin at concentrations of 10 and 40 ng/mL, with seleno-L-methionine *(SeMet)*, or with resistin (40 ng/mL) plus SeMet. The total mRNA was purified from endothelial layers of the rings. The eNOS mRNA expression was quantified with real-time PCR. The eNOS mRNA level in each sample was normalized to that of glyceraldehyde phosphate dehydrogenase (GAPDH). Relative eNOS mRNA level was presented as $[2\text{^}(Ct_{[GAPDH]} - Ct_{[eNOS]})]$. Treatment with resistin (40 ng/mL) significantly decreased the eNOS mRNA expression compared with controls (*$P < .05$, n = 6, *t* test). This effect was reversed with the addition of SeMet.

dependent vasorelaxation after treatment with resistin, porcine coronary artery rings were incubated with resistin (10 or 40 ng/mL), SeMet, or resistin (40 ng/mL) and SeMet simultaneously (n = 6). The eNOS levels were then calculated using real-time PCR and were found to be significantly reduced by 39% ($P < .05$) after treatment with 40 ng/mL of resistin as compared with controls. After cotreatment of the rings with SeMet and 40 ng/mL of resistin, the noted decrease in eNOS mRNA expression was only 4% as compared with controls, indicating a complete reversal of the resistin effect (Fig 4).

Immunohistochemistry analysis (n = 3) for eNOS was also performed in porcine coronary artery rings. Immunoreactivity of eNOS of the endothelial cells in resistin-treated vessel rings was also substantially reduced (Fig 5) as compared with controls.

Resistin reduces eNOS expression in PCAECs. To determine the impact of resistin on eNOS expression, eNOS mRNA levels were calculated using real-time PCR in endothelial cells in culture (n = 6 for each group). The eNOS mRNA levels were significantly reduced by 27% ($P < .05$, *t* test) and 55% ($P < .05$, *t* test) in the group, as compared with controls treated with resistin (10 and 40 ng/mL), respectively (Fig 6).

DISCUSSION

The present study demonstrated for the first time the direct effects of resistin on coronary artery dysfunction and the association with eNOS expression and O_2^- production. Using the model of porcine coronary artery rings, we have shown that resistin has a detrimental effect that results in vasomotor dysfunction, eNOS down-regulation, and O_2^- over production. Furthermore, antioxidant therapy could completely block these adverse effects of resistin.

Resistin is a member of a newly discovered family of cysteine-rich secretory proteins called *resistin-like molecules* (RELM) or *found in the inflammatory zone* (FIZZ). It was initially discovered in a screen to identify potential targets of the thiazolidinedione (TZD) class of insulin sensitizers in 3T3-L1 adipocytes.[6] Resistin is encoded by the *Retn* gene and secreted as a disulfide-linked dimer.[21] In mice, the *Retn* gene is expressed almost exclusively in white adipose tissue, and the protein is detectable in adipocytes and in the blood. Unlike murine resistin, human resistin is expressed at low levels in adipocytes but is readily detectable in mononuclear blood cells.[22,23]

The pharmacokinetic properties of resistin have been assessed. The mean plasma resistin levels in volunteers have been reported to be from 4 to 13 ng/mL, depending on the assay used,[24,25] whereas the peak levels may be as high as 38.35 ng/mL.[26] The doses of 10 and 40 ng/mL that we selected simulate these clinical situations. Although plasma levels in porcine are not known this model may still be relevant to human obesity. Both human and porcine resistin genes have been cloned and share over 90% homology of protein sequences. Our data showed human recombinant resistin have cross reactions for porcine coronary arteries and endothelial cells. Since resistin is a newly discovered adipokine, its potential receptors are not clear yet.

Impaired vasorelaxation in response to pharmacologic agents has been used as an indicator of vascular dysfunction. In the present study, resistin significantly impaired endothelium-dependent vasorelaxation in a dose-dependent manner. For the arteries incubated with 10 and 40 ng/mL of resistin, the decrease in bradykinin-induced vasorelaxation as compared with controls was 15% and 30%, respectively, indicating resistin-induced endothelial cell dysfunction. Impaired relaxation to a lesser extent took place in the presence of SNP; resistin at 10 and 40 ng/mL caused a decrease in the endothelium-independent vasorelaxation by 7% and 11%, respectively, indicating a reduced efficiency of NO released from SNP.

Endothelium produces molecular oxygen during aerobic respiration and generates a substantial amount of O_2^-. Imbalance in the production and regulation of oxygen radicals is known to lead to oxidative stress and contribute to vascular disease.[27] NO reacts with O_2^- to form peroxynitrite anion ($ONOO^-$), which subsequently decomposes to form the highly reactive hydroxyl radical (OH). This interaction occurs faster than the rate of O_2^- production with superoxide dismutase, thus a portion of NO can constantly be utilized to react with O_2^- and become unavailable for its biologic functions, including vasorelaxation.[28] Evidence from cell culture and animal studies suggests that overproduction of O_2^- and subsequent oxidative inactivation of NO may both be important in the pathogenesis of atherosclerosis.[28]

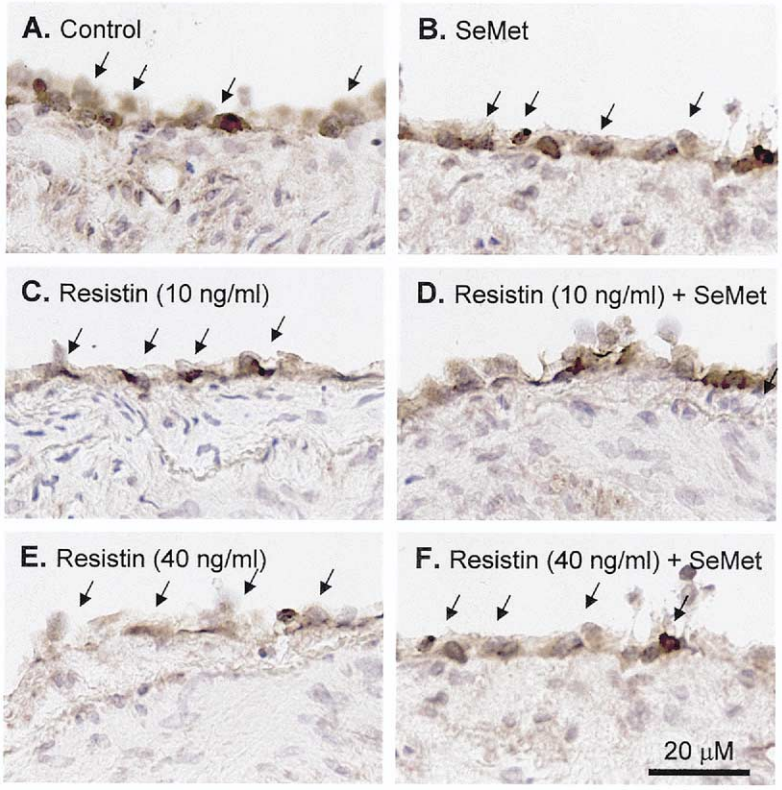

Fig 5. Effect of resistin on the endothelial nitric oxide synthase (eNOS) immunoreactivity of porcine coronary arteries. Avidin-biotin complex immunoperoxidase procedure was used to detect eNOS protein levels in both control and resistin-treated vessels. **A,** Control. **B,** SeMet (seleno-L-methionine) alone (control). **C,** Resistin (10 ng/mL). **D,** Resistin (10 ng/mL) plus SeMet. **E,** Resistin (40 ng/mL). **F,** Resistin (40 ng/mL) plus SeMet. High dose resistin (40 ng/mL) treatment substantially reduced eNOS immunoreactivity compared with controls, but the antioxidant SeMet reversed this effect (n = 3). Arrows indicate endothelial cells.

In this study, an 88% increase of O_2^- production was shown in the resistin-treated samples as compared with controls. Furthermore, when rings were co-cultured with resistin and antioxidant SeMet, endothelium-dependant as well as endothelium-independent vasorelaxation returned to levels similar to those seen in control vessels, which were correlated with a decrease in the O_2^- levels. This finding indicates that oxidative stress may be the major mechanism in resistin-induced vascular dysfunction. In a future study, we will examine nitrotyrosine production in resistin-treated vessels.

The detailed mechanisms of resistin's action and sources of O_2^- were not determined in the current study. O_2^- could be generated from NADPH oxidase, xanthine oxidase-uncoupled eNOS, and the mitochondria.[29,30] Once produced, free radicals could be removed by antioxidant defenses including enzyme catalase, glutathione peroxidase, and superoxide dismutase.[29,30] Identification of the potential sources and characteristics of free radicals, as well as antioxidant enzymes in resistin-treated cells warrants further investigations.

A key process in the early pathogenesis of atherosclerosis is diminished bioavailability of the endothelium-derived signaling molecule NO, which is generated by eNOS and is a potent vasodilator with multiple additional cardiovascular functions.[31] Many lines of evidence indicate that eNOS has a very important role in cardiovascular disease.[32,33] Higher rates of myocardial ischemia or infarction have been reported in patients with polymorphisms of eNOS that reduce the enzyme activity.[34] From our findings we believe that the changes in endothelium-dependent relaxation may also be due to the decrease in eNOS expression. Indeed, the real-time PCR data showed a 55% decrease of eNOS mRNA expression in resistin-treated vessel rings.

Effects of resistin on endothelial integrity and eNOS protein levels were studied by immunohistochemical staining. Resistin substantially decreased eNOS immunoreactivity, whereas the change of endothelial integrity was very minimal. However, these data could not be quantified for statistical analysis because of the insensitivity of the intensity measurement and small sample size. In future studies, Western blot analysis could be used to semi-quantitatively measure eNOS protein levels. Scanning or transmission electromicroscopy would help to confirm the integrity of endothelial cells and is warranted for future investigation.

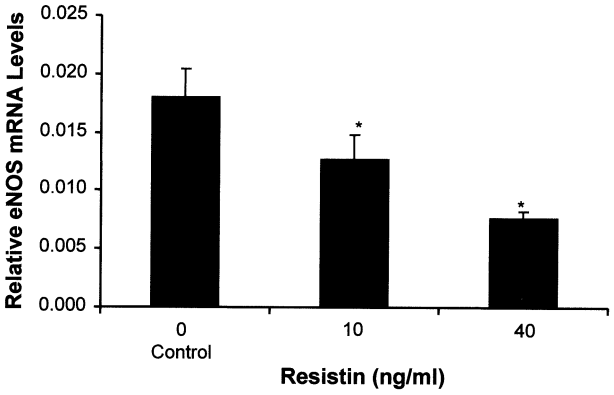

Fig 6. Effect of resistin on the endothelial nitric oxide synthase *(eNOS)* mRNA levels of porcine coronary artery endothelial cells in culture. The cells were incubated with resistin (10 or 40 ng/mL) for 24 hours. Real-time polymerase chain reaction was used to assess eNOS mRNA expression. The eNOS mRNA level in each sample was normalized to that of glyceraldehyde-s-phosphate dehydrogenase (GAPDH). Relative eNOS mRNA level was presented as $2^{\wedge}[Ct_{(GAPDH)} - Ct_{(eNOS)}]$. Addition of resistin (10 or 40 ng/mL) substantially decreased the eNOS mRNA expression (* $P < 0.05$, n = 6, *t* test). Data shown are means ± SEM.

In addition, NO inhibitor and control vessels without endothelial cells could also be included in a future study.

In recent years, we have developed and characterized the porcine coronary artery culture model, which represents a useful tool for the study of vascular biologic and endothelial functions.[10-13] Porcine right coronary arteries were exclusively used in this study because these vessels are relatively superficial and have a uniform diameter >5 cm with fewer branches. Optimal pretension for porcine coronary artery rings was determined by performing serial experiments with a wide range of pre-tension. The 30 mN pre-tension gave rise to the maximal tension change in response to a given dose of U46619. The variability was within a minimal range. Thus, pre-tension of 30 mN was used for all porcine coronary artery rings for myograph analysis.

In addition, porcine coronary arteries were harvested from a local slaughterhouse, stored in 4°C PBS, and transferred into the lab for experiments. In our experience, the vessel rings do not respond well in acute study because of cold ischemia. The vessels cultured for 24 hours at 37°C show the optimal response; thus, we did not perform an acute study because the data might not have been reliable.

Prostaglandins are thought to be involved in the vascular system through both vasoconstrictor and vasodilator actions. Endoperoxides are converted to the vasoconstrictor thromboxane through the cyclo-oxygenase pathway, which may also generate the vasodilators, prostacyclin and prostaglandin E-2.[35] The effect of resistin on prostaglandin activity is not known and should be interesting to investigate.

Our findings are consistent with those from the above-mentioned studies and indicate that resistin activates endothelial cells and therefore may promote the initiation or perpetuation of the atherosclerotic state. Further questions remain unanswered, however. For instance, interspecies variation could limit the usefulness of the observations in animal models. It is still unclear whether there is a significant difference in the serum resistin levels between healthy and obese or diabetic individuals.[25,26,36] The exact mechanism of action remains unknown at the cellular and the molecular level. It is likely that resistin activates a receptor on endothelial cells. However, further studies are needed to clarify this interaction.

In summary, we have demonstrated that resistin can cause vasomotor dysfunction, decrease eNOS expression, and increase O_2^- production in porcine coronary arteries. Antioxidant SeMet can successfully block these resistin-induced effects, indicating a possible molecular mechanism and a potential therapeutic strategy in patients who are at high risk for vascular disease. Thus, these data are of clinical significance although in vivo experiments may be warranted.

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