Numerous studies have established a strong link between obesity and cardiovascular disease (1). For example, the Pathobiological Determinants of Atherosclerosis in Youth research group recently provided direct evidence associating accelerated coronary atherosclerosis with obesity in adolescent and young adult men (14). Predictably, these obese subjects exhibited a variety of cardiovascular risk factors, including insulin resistance, dyslipidemia, and hypertension, i.e., the metabolic syndrome (5, 6). However, despite the known relationship between obesity and coronary disease, the underlying cellular and molecular mechanisms have not been fully elucidated.

Coronary heart disease, or atherosclerosis, is an inflammatory process that begins with dysfunction of the vascular endothelium (20). Experimental evidence suggests that mediators released from adipose tissue (adipokines) directly affect endothelial function and could be molecular links between obesity and cardiovascular disease (10, 13). Recently, our laboratory (8) demonstrated that one adipokine, leptin, significantly impairs coronary endothelium-dependent vasodilation both in vivo and in vitro. Resistin is another adipokine implicated in endothelial dysfunction and coronary heart disease (19). Endothelial effects of resistin include augmented endothelin-1 release and increased expression of VCAM-1 and monocyte chemoattractant chemokine-1 (21). In addition, resistin treatment in vitro has been shown to impair porcine coronary artery endothelial function by augmenting superoxide (O$_2^-$) production (9). Importantly, however, controversy surrounds the pathophysiological implications of this adipokine, as medical and surgical weight loss improves endothelial function in humans without correlation to plasma resistin levels (4). Thus it is impossible to predict agreement between clinical associations and in vitro and in vivo studies.

The goal of this investigation was to elucidate the effects of resistin on coronary and cardiovascular hemodynamics in vivo and coronary artery reactivity in vitro. We examined the hypothesis that resistin impairs coronary endothelial function by augmenting O$_2^-$ production. In addition, experiments were conducted to investigate whether resistin impairs the production and/or responses to nitric oxide (NO) or prostaglandins (e.g., prostacyclin; PGI$_2$), and we performed experiments with NO-nitro-L-argi-nine methyl ester (L-NAME) and indomethacin. The effect of resistin to attenuate bradykinin-induced vasodilation persisted in the presence of L-NAME or indomethacin, suggesting resistin may act at a cell signaling point upstream of NO or PGI$_2$ production. Resistin-induced endothelial dysfunction is not generalized, and it is not consistent with effects mediated by O$_2^-$ or interference with NO or PGI$_2$ signaling. The site of the resistin-induced impairment is unknown but may be at the bradykinin receptor or a closely associated signal transduction machinery proximal to NO synthase or cyclooxygenase.

Endothelium-dependent dilation to bradykinin, but not acetylcholine, in the coronary circulation. Am J Physiol Heart Circ Physiol 291: H2997–H3002, 2006. First published August 11, 2006; doi:10.1152/ajpheart.01035.2005.—Elevated plasma levels of adipokine; reactive oxygen species; hormone; obesity


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In vivo coronary dose-response experiments. Male mongrel dogs were sedated with morphine (3 mg/kg sc) and anesthetized with α-chloralose (100 mg/kg iv). After intubation, the animals were ventilated with room air supplemented with oxygen. A catheter was placed into the thoracic aorta via the right femoral artery to measure aortic blood pressure. A catheter was also inserted into the right femoral vein for injection of supplemental anesthetic, heparin, and sodium bicarbonate. The left femoral artery was catheterized to supply blood to a pump perfusing the left anterior descending coronary artery. A left lateral thoracotomy was performed to expose the heart. The left anterior descending coronary artery was isolated distal to its first major diagonal branch, cannulated, and connected to the extra-corporal perfusion system. Coronary perfusion pressure was maintained constant at 100 mmHg throughout the experimental protocol by a servo-controlled roller pump (8).

To assess effects on coronary blood flow and cardiovascular hemodynamics, resistin (0.15–9.6 μg/min) was infused into the coronary perfusion line. Each resistin dose was infused for at least 3 min, and values were recorded when stable. To determine effects on endothelium-dependent vasodilation, the vehicle for resistin (1% DMSO in saline; 100 μl/min) was infused into the coronary perfusion line for 10 min before responses to intracoronary acetylcholine (0.3–30 μg/min) or bradykinin (0.03–3.0 μg/min) were assessed. Each dose of acetylcholine or bradykinin was infused for at least 2 min, and data were recorded when coronary blood flow was stable. A 10-min recovery allowed coronary hemodynamics to return to baseline before a continuous intracoronary infusion of human resistin (Phoenix Pharmaceuticals; ~250 ng/min) was started. Ten minutes later, the acetylcholine or bradykinin dose-response protocol was repeated in the presence of resistin. Our laboratory previously documented that repeated infusion of these doses of acetylcholine does not produce tachyphylaxis (8). In the present study, we performed similar time controls for bradykinin dose-response experiments (see Fig. 3A, inset).

Functional assessment of isolated epicardial coronary rings. We further tested the mechanism by which resistin induces endothelial dysfunction in isolated left circumflex coronary artery rings. Experiments followed the in vivo studies described in the previous section: dog hearts were excised and immediately immersed in cold lactated Ringer solution. Coronary arteries were dissected from the heart and cleaned of periadventitial fat. Arteries were cut into 3-mm rings and mounted in organ baths for isometric tension studies. Optimal length was found by assessing contraction to 60 mM KCl. Passive tension was increased in gram increments until there was <10% change in active KCl contractions.

Control rings were treated with the vehicle for resistin (0.01% DMSO) for 10 min. Rings were then precontracted with 1 μM U-46619 (thromboxane A2 mimetic; BioMol), and graded concentrations of acetylcholine (1 nM to 10 μM) or bradykinin (0.1 nM to 10 μM) were added to the baths in a cumulative manner. In other rings, resistin (10 or 40 ng/ml) was added to the baths 10 min before preconstriction with U-46619, and acetylcholine or bradykinin concentration-response protocols were performed in the presence and absence of Nω-nitro-l-arginine methyl ester (l-NAME; 300 μM) or indomethacin (10 μM). Responses are expressed as percent maximum relaxation, where 100% is equivalent to loss of all tension developed in response to U-46619.

DHE staining. DHE (Molecular Probes) staining for O2 was carried out as previously described (16, 22). Arteries were allowed to equilibrate for 30 min in the organ baths and set to a passive tension of 2 g. Viability of the coronary rings was assessed with KCl (60 mM). The rings were then incubated with 10 μM DHE at 37°C for 30 min. After DHE loading, the rings were washed and given their various treatments. Experiments were performed to determine whether 1) resistin increased DHE fluorescence; 2) any such increase compared with that elicited by pyrogallol (a source of O2; positive control); and 3) increases in DHE fluorescence were sensitive to Tempol; and 4) DHE staining techniques are sensitive enough to detect agonist-induced changes (e.g., bradykinin). Arteries were embedded in OCT and flash frozen in liquid nitrogen. Tissue sections (10 μm) were cut with a cryostat and thaw-mounted on slides. Ethidium fluorescence was assessed with 508-nm excitation and 615-nm emission.

Statistical analyses. Data are presented as means ± SE of n experiments. The effects of graded doses of resistin on coronary blood flow, aortic blood pressure, and heart rate were assessed by one-way repeated-measures ANOVA. Two-way repeated-measures ANOVA was used to test the effects of resistin on acetylcholine- and bradykinin-induced coronary vasodilation, as responses were determined before and after resistin treatment. Two-way ANOVA was used to compare the vasodilator responses to acetylcholine and bradykinin in isolated coronary rings in the presence or absence of resistin. When statistical differences were found, a Student-Newman-Keuls multiple comparison test was performed. The criterion for statistical significance was P < 0.05 in all tests.

RESULTS

In vivo coronary dose-response experiments. Experiments were conducted to examine the coronary and cardiovascular hemodynamic effects of resistin (0.15–9.6 μg/min ic) in open-chest, anesthetized dogs (n = 5). Blood-gas parameters were maintained within normal physiological limits throughout the experimental protocol (arterial values: pH = 7.40 ± 0.01, PO2 = 39 ± 1 Torr, PO2 = 110 ± 8 Torr, hematocrit = 35 ± 2%). These infusion rates of resistin produced coronary plasma concentrations ranging from 3.0 ± 0.2 to 195 ± 24 ng/ml. Resistin had no effect on coronary blood flow, aortic pressure, or heart rate (Fig. 1). Thus resistin itself had no direct coronary vascular or hemodynamic effects. Resistin, however, is reported to impair endothelium-dependent vasodilation in vitro (9); therefore, our goal was to determine whether resistin exerts a similar effect in vivo.

Resistin and coronary endothelial function. To test the hypothesis that resistin impairs coronary endothelial function in vivo, we examined the effects of resistin (~250 ng/min ic) on acetylcholine-mediated dilation in open-chest, anesthetized dogs (n = 5). Baseline coronary blood flows were 0.68 ± 0.09 and 0.71 ± 0.07 ml·min⁻¹·g⁻¹ before and during resistin treatment, respectively, in paired experiments. An acetylcholine dose-response curve was performed before (with a vehicle control) and during intracoronary infusion of resistin. Resistin was infused continuously, and the average plasma concentra-
Resistin and coronary $O_2^-$ production. Kougias et al. (9) linked the inhibitory effect of resistin to $O_2^-$ production; therefore, we determined whether this mechanism underlies the effect of resistin in the canine coronary circulation. To examine whether resistin increases coronary $O_2^-$ production, DHE studies were performed on isolated coronary arteries with resistin (10 ng/ml), pyrogallol (20 μM), and Tempol (10 μM). We found that resistin did not appreciably increase coronary $O_2^-$ production over vehicle control (Fig. 4A and B). In contrast, pyrogallol significantly increased DHE fluorescence (Fig. 4C); Tempol largely prevented the effect of pyrogallol to increase $O_2^-$ production (Fig. 4D). To determine whether DHE techniques possess the sensitivity to detect agonist-induced changes in $O_2^-$ production, we performed another set of experiments with bradykinin and resistin at a higher magnification (Fig. 4E). Bradykinin increased DHE fluorescence associated with the endothelial layer; however, resistin did not augment this effect. As another experimental approach, we determined whether Tempol could prevent the attenuation of bradykinin-induced dilation by resistin. The effect of resistin to impair bradykinin-induced vasodilation persisted in the presence of Tempol (10 μM; Fig. 5). Together, the DHE and Tempol dilation data suggest that $O_2^-$ is not a major factor in the effect of resistin to impair bradykinin-induced vasodilation in the canine coronary circulation. This finding is different from what was observed by Kougias et al. (9) in the porcine coronary artery.

Resistin and nitric oxide and cyclooxygenase products. We performed experiments to determine whether resistin impairs the production of or/and smooth muscle sensitivity to nitric oxide (NO) or cyclooxygenase products. Our rationale was that, if resistin impairs the production of NO or the response to NO, then inhibition of NO synthase with L-NAME should eliminate the difference in bradykinin relaxation between vehicle- and resistin-treated arteries. L-NAME impaired bradykinin-induced relaxation; however, the inhibitory effect of resistin remained (Fig. 6). Similarly, if resistin impaired the production of (or response to) cyclooxygenase products, then indomethacin should eliminate the effect of resistin to impair bradykinin-induced relaxation. However, resistin impaired bradykinin-induced relaxation in the presence of indomethacin (Fig. 7).

DISCUSSION

The present investigation was designed to determine whether the adipokine resistin affects coronary and cardiovascular hemodynamics in vivo and to examine the hypothesis that
resistin impairs coronary endothelium-dependent vasodilation by augmenting $O_2^-$ production. The major new findings of this investigation are as follows. 1) Intracoronary infusion of physiological, pathophysiological, and pharmacological concentrations of resistin have little effect on coronary blood flow, arterial pressure, or heart rate in open-chest, anesthetized dogs. 2) Concentrations of resistin (10 ng/ml) comparable with those observed in obese, Type 2 diabetic individuals impair endothelium-dependent vasodilation to bradykinin but not to acetylcholine. 3) Pathophysiologically relevant concentrations of resistin (10 ng/ml) do not substantially augment coronary $O_2^-$ production. Finally, 4) the inhibitory effect of resistin is not due to disruption of NO or prostacyclin (PGI$_2$) signaling. Together, these findings indicate that resistin specifically impairs coronary endothelium-dependent vasodilation to bradykinin. In addition, our data suggest that resistin-induced endothelial dysfunction is not generalized nor is it associated with changes in $O_2^-$ production or NO/PGI$_2$ signaling.

Resistin and coronary blood flow. Overall, very little is known about the vascular effects of the adipokine resistin. Although recent studies have reported that resistin may exert vasoactive effects by augmenting endothelin-1 production from endothelial cells (21), no study has directly examined whether resistin affects vascular resistance in vivo. Accord-

Fig. 4. Resistin has no effect on dihydroethidium (DHE) fluorescence, an indicator of superoxide ($O_2^-$) production. Representative DHE-stained coronary artery sections are shown to reflect $O_2^-$ production with the different treatments. Arrows point to the endothelium. Resistin (10 ng/ml; B) did not increase DHE fluorescence over control (vehicle; A). Pyrogallol (20 μM; C), a source of $O_2^-$, dramatically increased DHE fluorescence, and this was reduced by Tempol (10 μM; D), a mimetic of superoxide dismutase. E: higher magnification photographs, demonstrating the sensitivity of DHE to detect agonist-induced changes in fluorescence associated with the endothelium.

Fig. 5. Effect of resistin to attenuate bradykinin-induced relaxation persists in the presence of Tempol, a mimetic of superoxide dismutase. Experiments were performed in the presence of 10 μM Tempol. Group data show that resistin (10 ng/ml) impaired bradykinin-induced endothelium-dependent relaxation in the presence of Tempol. Data are from 4 dogs. *$P < 0.05$ vs. vehicle at same concentration.

Fig. 6. Effect of resistin to attenuate bradykinin-induced relaxation persists in the presence of $N^\omega$-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide synthase. Experiments were performed in the presence of 300 μM L-NAME. Group data show that resistin (10 ng/ml) impaired bradykinin-induced endothelium-dependent relaxation in the presence of L-NAME. Data are from 3 dogs. *$P < 0.05$ vs. vehicle at same concentration.
ingly, in the present study, we conducted resistin dose-response experiments in open-chest, anesthetized dogs to determine whether physiological, pathophysiological, and/or pharmacological concentrations of resistin affect coronary blood flow. Resistin was infused into the extracorporeal perfusion circuit, producing coronary plasma concentrations ranging from 3.0 ± 0.2 to 195 ± 24 ng/ml. Humans are reported to have plasma resistin concentrations ranging from 3 to 38 ng/ml (11, 15, 18). Exogenous resistin at these concentrations had no effect on coronary blood flow, aortic blood pressure, or heart rate (Fig. 1). This finding is significant because it demonstrates that resistin is unlikely to activate endothelin-1 release from endothelial cells in vivo, unlike what has been observed in cultured endothelial cells (21), as endothelin-1 is a potent coronary vasoconstrictor but blood flow did not change. In addition, the limited effect of resistin on coronary blood flow is similar to that of another adipokine, leptin, which our laboratory (7, 8) recently found to have little, if any, direct effect on coronary vasomotor tone in vivo. Together, these data indicate that resistin does not activate mechanisms that directly regulate coronary vascular resistance in vivo.

**Resistin and coronary endothelial function.** This study was also designed to examine the hypothesis that resistin impairs coronary endothelium-dependent vasodilation. To test this hypothesis, we conducted experiments in vivo (open-chest, anesthetized dogs) and in vitro (in isolated coronary arteries). We found that acetylcholine-mediated coronary vasodilation was unaffected by resistin both in vivo (Fig. 2A) and in vitro (Fig. 2B). This negative finding is unlikely to be related to resistin concentration, as neither 10 ng/ml nor 40 ng/ml resistin had a significant effect on acetylcholine-induced relaxation. In contrast, resistin did significantly impair bradykinin-induced coronary vasodilation both in vivo (Fig. 3A) and in vitro (Fig. 3B). The reason for the different effect of resistin on endothelium-dependent vasodilation to acetylcholine and bradykinin is unknown but may be related to the fact that acetylcholine-induced coronary dilation in dogs is almost entirely mediated by NO (8), whereas bradykinin elicits coronary vasodilation via NO and other vasoactive mediators [e.g., PGI\textsubscript{2} and endothelial-derived hyperpolarizing factor(s)] (17). It is unlikely that the inhibitory effect of resistin on bradykinin-induced vasodilation is related to impaired NO or PGI\textsubscript{2} production, as the effect of resistin persisted in the presence of L-NAME or indomethacin (Figs. 6 and 7). Thus the selective impairment of bradykinin-mediated dilation may suggest that resistin attenuates coronary vasomotor responses to endothelium-derived hyperpolarizing factor or other paracrine mediators. Alternatively, resistin signaling to bradykinin receptors or associated G proteins may explain the difference between acetylcholine and bradykinin vasodilation. However, further studies are needed to address these hypotheses.

The first group of investigators to examine the effect of resistin on vascular reactivity [Kougias et al. (9)] found that resistin produced dose-dependent reductions in bradykinin-induced relaxation in isolated porcine coronary arteries. They also found that resistin depressed endothelial NO synthase mRNA expression in cultured endothelial cells and reduced endothelial NO synthase protein levels in porcine coronary rings. We observed no apparent change in NO signaling, as the inhibitory effect of resistin persisted in the presence of 300 μM L-NAME (Fig. 6). This could be related to the time of resistin exposure; Kougias et al. incubated coronary rings in resistin for 24 h, whereas we infused/incubated with resistin for 10 min. However, we contend that 10 min of exposure to resistin is sufficient to damage endothelial function, as it attenuated endothelium-dependent vasodilation to bradykinin (Fig. 3). In fact, the ~30% decrease in resistin-induced vasodilation to bradykinin that we found after 10 min of treatment in open-chest, anesthetized dogs was similar to the decrease in bradykinin-induced relaxation reported by Kougias et al. (9) after 24 h. Furthermore, our group (8) recently documented that a 10-min treatment with another adipokine, leptin, substantially diminished endothelium-dependent vasodilation both in vivo and in vitro. In this earlier study, we found that acetylcholine produced a 100% increase in coronary blood flow after 10 min of intracoronary leptin infusion (8); compare this to the 178% increase in coronary blood flow after 10 min of intracoronary resistin infusion. Thus, under these conditions, leptin produces a much more substantial impairment of coronary endothelial function (in vivo and in vitro) than does resistin.

**Resistin and coronary O\textsubscript{2}\textsuperscript{-} production.** Another aim of this investigation was to examine whether pathophysiological concentrations of resistin (10 ng/ml) augment coronary O\textsubscript{2}\textsuperscript{-} production to diminish endothelial function. To examine this hypothesis, we 1) performed DHE staining experiments in isolated coronary arteries and 2) determined whether Tempol, a superoxide dismutase mimetic, could prevent the inhibitory effect of resistin. Resistin did not alter DHE fluorescence, indicating that clinically relevant concentrations of resistin do not significantly augment coronary O\textsubscript{2}\textsuperscript{-} production (Fig. 4). This finding is in odds with the recent study of Kougias et al. (9) who reported that 10 and 40 ng/ml of resistin increased O\textsubscript{2}\textsuperscript{-} production by 40% and 88%, respectively, using the lucigenin-enhanced chemiluminescence method. Our experiments with bradykinin demonstrate that DHE is sensitive enough to detect agonist-induced changes (Fig. 4E); however, we did not observe any effect of resistin to increased DHE fluorescence. Another argument against a major role for O\textsubscript{2}\textsuperscript{-} comes from our functional studies, as the effect of resistin to impair bradykinin vasodilation persisted in the presence of Tempol (Fig. 5). O\textsubscript{2}\textsuperscript{-} reacts with endothelium-derived NO to produce peroxynitrite (12), and this reaction quenches NO, thereby diminishing its
bioavailability and endothelium-dependent vasodilation (2). Such a mechanism appears unlikely, as the inhibitory effect of resistin persisted in the presence of l-NAME or Tempol.

Implications of resistin-induced coronary endothelial dysfunction. The present findings indicate that resistin specifically attenuates coronary endothelium-dependent vasodilation to bradykinin. However, it must be pointed out that this effect was modest (one-half of a log order shift in the EC50), and whether resistin-induced coronary endothelial dysfunction significantly contributes to the development of coronary atherosclerosis is unknown. Recent investigations do support a proatherogenic role of resistin in that this adipokine is associated with increased levels of proinflammatory molecules (tumor necrosis factor-α, interleukin-6, lipoprotein phospholipase A2) (19), smooth muscle cell proliferation (3), augmented expression of VCAM-1 and monocyte chemotactic chemokine-1, and a downregulation of tumor necrosis factor receptor-associated factor-3, an inhibitor of CD40 ligand-mediated endothelial cell activation (21). Thus resistin is associated with several primary mediators of vascular dysfunction and atherosclerosis; however, none of these findings directly implicates resistin as a major catalyst for coronary vascular disease. To fully elucidate the role of resistin in the pathogenesis of coronary atherosclerosis, additional studies are needed in which resistin levels are elevated chronically in an in vivo setting, independent of other disease processes.

In conclusion, this study is the first to examine the effects of the novel adipokine resistin on coronary vasomotor tone and endothelial function in vivo. We found that pathophysiologically relevant concentrations of resistin have little or no effect on coronary blood flow but do impair endothelial function, specifically to bradykinin. This attenuation of endothelial function is not associated with coronary O2 production and is not prevented by Tempol. The effect of resistin to impair bradykinin-induced vasodilation is not likely due to changes in NO/PGI2 signaling. Additional studies are needed to more accurately define the role of chronic hyperresistinemia in obesity-related coronary vascular disease.

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