Leptin receptors are expressed in coronary arteries, and hyperleptinemia causes significant coronary endothelial dysfunction

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Am J Physiol Heart Circ Physiol 289: H48–H56, 2005. First published March 4, 2005; doi:10.1152/ajpheart.01159.2004.—Obesity is associated with marked increases in plasma leptin concentration, and hyperleptinemia is an independent risk factor for coronary artery disease. As a result, the purpose of this investigation was to test the following hypotheses: 1) leptin receptors are expressed in coronary endothelial cells; and 2) hyperleptinemia induces coronary endothelial dysfunction. RT-PCR analysis revealed that the leptin receptor gene is expressed in canine coronary arteries and human coronary endothelium. Furthermore, immunocytochemistry demonstrated that the long-form leptin receptor protein (ObRb) is present in human coronary endothelium. The functional effects of leptin were determined using pressurized coronary arterioles and anesthetized, open-chest dogs, primarily at high, pharmacological concentrations; and 2) hyperleptinemia induces significant coronary endothelial dysfunction.

Plasma leptin concentration positively correlates with adiposity (8), and hyperleptinemia is an independent risk factor for coronary artery disease (37) and a strong predictor of acute myocardial infarction (43). Additionally, leptin has been implicated in many atherogenic processes common to the pathogenesis of cardiovascular disease, including promotion of platelet aggregation and thrombosis (5, 22, 23); production of inflammatory cytokines, e.g., TNF-α, IL-6, and IL-12 (27); promotion of neointimal growth in mice (39); stimulation of mitochondrial superoxide production in aortic endothelial cells (52); and calcification of vascular smooth muscle cells (35). Interestingly, recent reports have demonstrated that leptin possesses cytokine-like properties and that elevated plasma leptin levels occur concomitantly with elevated IL-6 and C-reactive protein in human obesity, the metabolic syndrome, and non-insulin-dependent diabetes mellitus (25, 28, 32). As a result, hyperleptinemia could contribute to obesity-related coronary endothelial dysfunction; however, little is known about the effects of leptin on the coronary circulation. In fact, only one study has addressed the direct effects of leptin on the coronary circulation. This study, by Matsuda et al. (30), suggests that leptin exerts nitric oxide-independent coronary vasodilation in humans; however, the subjects studied were undergoing cardiac catheterization for angina, and no data are available for normal human or animal subjects.

Because obesity is pandemic in Western society (12) and hyperleptinemia is essentially universal in the obese population, it is important that the mechanisms by which leptin affects the coronary vasculature be delineated. As a result, the aim of this investigation was to test the following hypotheses: 1) the leptin receptor gene (db) is expressed in coronary endothelial cells, and signaling-competent receptors are present; 2) leptin induces nitric oxide-mediated vasodilation in isolated coronary arterioles and anesthetized, open-chest dogs, primarily at high, pharmacological concentrations; and 3) hyperleptinemia (plasma concentrations similar to those observed in obese

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LEPTIN AND CORONARY VASCULAR FUNCTION

Committee in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1996).

Mongrel dogs (n = 7), Wistar rats (n = 6), lean Zucker rats (n = 3), and obese Zucker rats (n = 4) were anesthetized with pentobarbital sodium (dogs, 30 mg/kg iv; rats, 65 mg/kg ip; Abbott Laboratories).

Hearts were excised and immediately immersed in cold (4°C) lactated Ringer solution. Epicardial coronary arterioles (60–130 μm in situ internal diameter and 0.6–1.0 mm in length without branches) were dissected out as previously described (18, 24). Arterioles were cannulated with glass micropipettes, pressurized to 60 cm H2O, and bathed in physiological salt solution (PSS) containing 1% bovine serum albumin (pH 7.4, 37°C). Arterioles developed spontaneous (myogenic) basal tone, and concentration-diameter relationships were assessed for leptin (10 pmol/l–10 nmol/l, Sigma). Endothelin-1 (1 nmol/l, Sigma) was administered to assess the viability of selected dog arterioles (n = 2) that failed to develop spontaneous, myogenic tone. These microvessels developed and maintained tone similar to that developed spontaneously in other microvessels. Furthermore, after washing (30 min) was completed, relaxation to leptin was unchanged by endothelin-1 administration. Arterioles from dogs (n = 3) were denuded with intraluminally administered 3-(β-cholamidopropyl)dimethylammonio)-1-propanesulfonate (0.4%, zwiterionic detergent, Bio-Rad) dissolved in albumin-PSS. Microvessels were then washed with intraluminally administered albumin-PSS for 5 min, and the leptin concentration-response was repeated. Also, arterioles (dogs, n = 4; Wistar rats, n = 4) were treated with nitric oxide synthase (NOS) inhibitor Nω-nitro-L-arginine methyl ester (l-NNAME) (10 nmol/l for 30 min, Sigma), and the leptin concentration-response protocol was repeated. This concentration and duration of l-NNAME treatment have previously been shown to be efficacious and specific for NOS inhibition (16–18, 44). At the end of each experiment, the vessel was relaxed with 100 μmol/l sodium nitroprusside to obtain its maximum diameter. All diameter changes in response to agonists were normalized to the dilation response to 100 μmol/l sodium nitroprusside and expressed as a percentage of maximal dilation.

In vivo coronary dose-response experiments. Mongrel dogs (n = 24) were sedated with morphine (Baxter, 3 mg/kg sc), anesthetized with α-chloralose (100 mg/kg iv, Sigma), and ventilated with room air that was supplemented with oxygen. The left anterior descending coronary artery (LAD) was cannulated and perfused with an extra-corporeal circuit as previously described (48). Briefly, the left femoral artery was catheterized to feed a servo-controlled peristaltic-pump circuit used to artificially perfuse the LAD. Coronary perfusion pressure was held constant (100 mmHg) throughout the experimental protocol, and coronary blood flow was measured with an inline Doppler transit-time flow probe (Transonic Systems). Consequently, changes in coronary blood flow reflect changes in coronary microvascular resistance.

After a 20-min recovery period, leptin (0.1–30.0 μg/min) was infused into the coronary perfusion line (n = 9 dogs). Each leptin dose was infused for ~3 min at constant coronary perfusion pressure of 100 mmHg, and data were recorded when coronary blood flow was stable. Arterial and coronary venous blood samples were drawn during steady-state coronary flow at each leptin dose to determine myocardial oxygen consumption (n = 5 dogs). Additional dogs (n = 3) were pretreated with the ganglionic blocker hexamethonium (5 mg/kg iv, Sigma), and the leptin dose-response protocol was performed as described above.

To determine the effects of obese hyperleptinemia on endothelium-dependent and -independent coronary vasodilation, acetylcholine (0.3–30.0 μg/min ic, Sigma) and sodium nitroprusside (1.0–100.0 μg/min ic, Sigma) dose-response experiments were conducted in open-chest dogs (acetylcholine, n = 5; sodium nitroprusside, n = 5) that were anesthetized and instrumented as described above. Each acetylcholine and sodium nitroprusside dose was infused for 2–3 min, and data were recorded when coronary blood flow was stable. After a 10-min recovery period, a continuous intracoronary infusion of leptin...
developed in response to precontraction with U-46619. Relaxation responses were expressed as percent maximum relaxation.

uct and ment revealed 96% homology between the 185-bp PCR prod-

expected 296-bp PCR product shown in Fig. 1

ANOVA was used to test the effect of leptin on changes in arteriolar

and changes in arteriolar diameter. Two-way ANOVA was used to

myocardial oxygen consumption, systemic hemodynamic variables,

ANOVA was used to test the effects of leptin on coronary blood flow,

testing detected overall treatment effects. One-way repeated-measures

was used to compare mean EC50 and mean maximum responses of

acetylcholine and sodium nitroprusside-mediated coronary vasodila-

for 30 min. Following washes, leptin (625 pmol/l or ~10 ng/ml) was

added to the baths in a cumulative manner. Rings were then washed

for 30 min. Following washes, leptin (625 pmol/l or ~10 ng/ml) was

added to the baths. Ten minutes later, rings were precontracted, and

the acetylcholine concentration-response protocol was repeated. Ad-

tional experiments, using the protocol delineated above, were per-

formed using physiological concentrations of leptin (250 pmol/l or ~4

ng/ml, n = 8 rings from 3 dogs). Also, time-control experiments were

conducted without leptin administration (n = 8 rings from 2 dogs).

Maximum relaxation responses were expressed as percent maximum relaxation. Maximum relaxation is equivalent to the loss of all active tension developed in response to precontraction with U-46619.

Statistical analyses. Data are expressed as means ± SE. Statistical testing detected overall treatment effects. One-way repeated-measures ANOVA was used to test the effects of leptin on coronary blood flow, myocardial oxygen consumption, systemic hemodynamic variables, and changes in arteriolar diameter. Two-way ANOVA was used to compare coronary arteriolar responses to leptin in lean versus obese Zucker rats as well as coronary flow and hemodynamic responses to leptin with and without hexamethonium. Two-way repeated-measures ANOVA was used to test the effect of leptin on changes in arteriolar diameter before and after l-NAME treatment. Two-way repeated-measures ANOVA was also used to test the effect of leptin on acetylcholine- and sodium nitroprusside-mediated coronary vasodilation in vivo and acetylcholine-mediated relaxation of coronary rings in vitro. When significance was found with ANOVA (P < 0.05), a Student-Newman-Keuls post hoc multiple-comparison test was performed to detect differences between treatment levels. Paired t-test was used to compare mean EC50 and mean maximum responses of coronary rings to acetylcholine before and after leptin treatment. Mean arteriolar diameters before and after l-NAME administration or denudation were also compared using a paired t-test. P < 0.05 was taken to be significant (i.e., α = 0.05).

RESULTS

ObRb is expressed in HCAEC. We performed RT-PCR using RNA isolated from confluent cultures of HCAEC and canine left circumflex coronary arteries. The presence of the expected 185-bp PCR product demonstrates that the ObRb gene is expressed in canine left circumflex coronary arteries. Immunocytochemistry analysis demonstrated the presence of signaling-competent ObRb on HCAEC (Fig. 1B). No immunoreactivity was found in blocked cells incubated only with FITC-conjugated goat anti-rabbit IgG Fc (data not shown).

Leptin induces nitric oxide-dependent vasorelaxation in isolated coronary arterioles. Baseline characteristics of isolated coronary arterioles are given in Table 1. The level of basal tone was similar in all treatment groups described below. The vertical lines in Fig. 2 and Fig. 3 denote the range of leptin concentrations that have been reported in normal (3–5 ng/ml) and morbidly obese (90–95 ng/ml) humans (41). Leptin (10 pmol/l–10 µmol/l) produced a concentration-dependent vasodilation in coronary arterioles isolated from Wistar rats (Fig. 2A) and mongrel dogs (Fig. 2C). Furthermore, the dilation was abolished by denudation (canine arterioles, Fig. 2D) and pretreatment with l-NAME (10 µmol/l for 30 min, Wistar rat and canine arterioles), demonstrating that leptin-mediated coronary arteriolar dilation requires the endothelium and is nitric
Administration (from 75/110 mmHg) arteriolar tone (decrease in diameter) following L-NAME administration. There was a significant increase in resting tone throughout the course of the experimental protocol (pH 7.37 ± 0.02; arterial PCO2, 42.3 ± 0.6 mmHg; arterial PO2, 110.7 ± 2.5 mmHg). Leptin (0.1–30.0 μg/min ic) did not significantly change coronary blood flow, myocardial oxygen consumption, or aortic pressure (Fig.3). However, there was an uptrend in mean aortic pressure (89 ± 9–98 ± 8 mmHg) and heart rate (118 ± 12–131 ± 13 beats/min; P < 0.05) with increasing doses of leptin.

Because the coronary vasomotor effects of leptin could be masked by increased sympathetic nervous activity, we performed intracoronary leptin dose-response experiments in dogs (n = 3), pretreated with the autonomic ganglionic blocker hexamethonium (5 mg/kg iv). Although autonomic ganglionic blockade reduced myocardial oxygen consumption, coronary blood flow, heart rate, and blood pressure, the leptin dose response was unaltered in the animals treated with hexamethionum (Fig. 3). Taken together, these findings demonstrate that leptin has little effect on coronary blood flow and myocardial oxygen consumption in vivo in open-chest, anesthetized dogs. This provides further evidence that leptin-mediated coronary vasodilation is predominantly pharmacological (evident at concentrations that were not reached in the in vivo experiments).

Effects of intracoronarily administered leptin on open-chest anesthetized dogs. To further examine the coronary vascular effects of leptin, we tested the effects of normal and obese concentrations of leptin on coronary blood flow and hemodynamic parameters in open-chest, anesthetized dogs. Blood gas parameters were maintained within normal physiological limits throughout the course of the experimental protocol (pH 7.37 ± 0.02; arterial PCO2, 42.3 ± 0.6 mmHg; arterial PO2, 110.7 ± 2.5 mmHg). Leptin (0.1–30.0 μg/min ic) did not significantly change coronary blood flow, myocardial oxygen consumption, or aortic pressure (Fig. 3). However, there was an uptrend in mean aortic pressure (89 ± 9–98 ± 8 mmHg) and heart rate (118 ± 12–131 ± 13 beats/min; P < 0.05) with increasing doses of leptin.

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Effects of obese hyperleptinemia on coronary endothelial function. To test the hypothesis that obese hyperleptinemia induces coronary endothelial dysfunction, we determined the effects of leptin on coronary responses to graded doses of acetylcholine (0.3–30.0 μg/min ic) and sodium nitroprusside (1.0–100.0 μg/min ic) in anesthetized, open-chest dogs (Fig. 4A and acetylcholine (10 mol/l–10 μmol/l) in left circumflex canine coronary rings (Fig. 4B). The fact that coronary artery disease occurs predominantly in large coronary arteries pro-

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<th>Baseline Diameter, μm</th>
<th>Passive Diameter, μm</th>
<th>Tone Developed, %</th>
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<tr>
<td>Wistar rats 6</td>
<td>72±5</td>
<td>27±3</td>
</tr>
<tr>
<td>Lean Zucker rats 3</td>
<td>82±8</td>
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<td>89±10</td>
<td>26±4</td>
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<tr>
<td>Dogs 7</td>
<td>88±14</td>
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Values are means ± SE; n, no. of animals.
vides powerful rationale for studying the effects of hyperleptinemia on vasoreactivity in epicardial rings.

In vivo, obese concentrations of leptin (81 ± 7 ng/ml, calculated coronary plasma concentration) significantly attenuated coronary dilator responses to acetylcholine (10 and 30 μg/min, Fig. 4A) but did not significantly affect coronary dilation to graded doses of sodium nitroprusside (Fig. 5). Hence, endothelium-independent coronary vasodilation was unaffected by leptin, whereas endothelium-dependent coronary vasodilation was significantly impaired. Leptin exerted no direct effect on coronary blood flow (1.19 ± 0.3 ml·min⁻¹·g⁻¹ before leptin infusion and 1.23 ± 0.2 ml·min⁻¹·g⁻¹ following 10 min of leptin infusion). Time-control experiments, in which intracoronary acetylcholine dose-response experiments were performed without leptin infusion (n = 2 dogs), revealed no changes in the coronary flow response to acetylcholine, i.e., no tachyphylaxis (data not shown).

In left circumflex coronary rings, obese concentrations of leptin (625 pmol/l or 10 ng/ml) markedly attenuated relaxation responses to acetylcholine (6.25 nmol/l–6.25 μmol/l; Fig. 4B). Leptin (625 pmol/l) shifted the EC₅₀ from 221 ± 56 to 507 ± 178 nmol/l (P = 0.067) and reduced the maximum effect of acetylcholine from 89.4 ± 4.8 to 69.5 ± 7.3% of maximum relaxation (P = 0.001). Physiological concentrations of leptin (250 pmol/l or ~4 ng/ml) had no effect on relaxation responses to acetylcholine (6.25 nmol/l–6.25 μmol/l; Fig. 4C). Leptin (250 pmol/l) did not significantly shift the EC₅₀ (145 ± 80–155 ± 82 nmol/l; P = 0.251) or change the maximum effect (93.4 ± 4.5–97.6 ± 3.3% of maximum relaxation; P = 0.094) of acetylcholine (Fig. 4C). Thus obese concentrations of leptin were required to significantly attenuate relaxation responses to acetylcholine in coronary rings. Time-control experiments were also performed (n = 9 rings from 3 dogs), and there was no change in the maximal effect of acetylcholine in the absence of leptin (P = 0.34; data not shown). In summary, obese hyperleptinemia caused significant coronary endothelial dysfunction in anesthetized, open-chest dogs and in isolated, epicardial coronary rings (Fig. 4); however, leptin did not significantly alter endothelium-independent coronary vasodilation in vivo (Fig. 5).

DISCUSSION

A major new finding of this investigation is that ObRb is expressed in human coronary endothelium and canine left circumflex coronary arteries (Fig. 1). Furthermore, hyperleptinemia significantly impairs coronary endothelial function in vivo and in vitro, as assessed by response to muscarinic agonism (acetylcholine dose response). Additionally, we have demonstrated that pharmacological concentrations of leptin induce NO-mediated vasodilation in coronary arterioles isolated from Wistar rats, lean Zucker rats, and mongrel dogs. Notably, coronary vascular effects of leptin are consistent across species lines, i.e., leptin induces comparable vasodilator responses in arterioles from rats and dogs.

It is important to note that arteriolar vasodilation to leptin occurred at high, pharmacological concentrations of leptin (10 nmol/l–10 mmol/l, Fig. 2), i.e., little to no effect of leptin at low concentrations. Consistent with this finding, normal and obese concentrations of leptin have little, if any, effect on coronary blood flow or myocardial oxygen consumption in open-chest, anesthetized dogs (Fig. 3). Taken together, these
findings may seem paradoxical in that leptin mediates endothelium-dependent vasodilation and attenuates endothelium-dependent vasodilation under the same conditions. However, careful examination of the data reveals that the leptin-induced nitric oxide-mediated dilation occurs only in vitro at concentrations well above those typically observed in morbidly obese humans. Concentrations of leptin consistent with those measured in obese humans have little to no direct effect on coronary blood flow or coronary arteriolar tone, yet leptin significantly attenuates acetylcholine-mediated vasodilation. In summary, physiological and pathophysiological (obese) concentrations of leptin have little direct effect on coronary vasomotor tone; however, obese concentrations of leptin produce significant coronary endothelial dysfunction both in vitro and in vivo.

Recent reports indicate that obese hyperleptinemia is an independent, positive risk factor for cardiovascular disease (37) and coronary artery disease (43). Moreover, leptin has been implicated in the chronic systemic inflammation associated with obesity (4). As a result, one of the major objectives of the present investigation was to test the hypothesis that hyperleptinemia causes coronary endothelial dysfunction. The findings of the present investigation demonstrate that obese concentrations of leptin cause coronary endothelial dysfunction in vivo (Figs. 4A and 5).

Coronary artery disease is chiefly a disease of conduit arteries; therefore, this hypothesis was further tested in epicardial coronary rings. Consistent with our in vivo findings, obese concentrations of leptin also caused significant coronary endothelial dysfunction in isolated left circumflex coronary rings (Fig. 4B); however, physiological concentrations of leptin did not alter coronary endothelial function (Fig. 4C). Thus obese concentrations of leptin were required to induce coronary endothelial dysfunction.

These findings are intriguing given that endothelial dysfunction is the inciting event in the pathogenesis of atherosclerosis (38). Hyperleptinemia is universal in the obese population, with plasma concentrations directly correlating with the degree of adiposity and ranging from 8 to 90 ng/ml (21, 40, 41). Additionally, obese humans are at much greater risk for cardiovascular disease (4, 11). Our findings clearly implicate leptin as a potential mediator of early endothelial dysfunction in the coronary circulation.

The precise mechanisms by which leptin induces coronary endothelial dysfunction remain unknown. Leptin has been
shown to increase endothelin-1 mRNA levels and protein secretion in human umbilical vein endothelial cells (36). Thus leptin-mediated endothelin-1 production could be responsible, in part, for the observed endothelial dysfunction. However, in the present investigation, raising coronary plasma leptin concentration to levels comparable to those observed in obese humans had no significant effect on coronary blood flow (see RESULTS). Given the constrictor potency of endothelin-1, it is unlikely that increased endothelin-1 release is playing a major role in mediating the observed endothelial dysfunction. Another plausible explanation for the endothelial dysfunction is the production of reactive oxygen species. Leptin has also been shown to induce mitochondrial superoxide anion production in aortic endothelial cells via augmented fatty acid oxidation (52). Conceivably, increased superoxide production in the coronary circulation may quench nitric oxide (favor the formation of peroxynitrite) and thus reduce local nitric oxide bioavailability. The result could be attenuated dilator responses to nitric oxide-mediated agonists, e.g., acetylcholine. Also, leptin may induce proinflammatory changes in the coronary endothelium, specifically, via the production of acute phase reactants (e.g., IL-6, IL-12, and TNF-α) (27). Future studies are needed to address these issues.

**Leptin receptors and coronary endothelium.** Previous studies have shown that the leptin receptor is expressed in umbilical artery and vein endothelial cells (1). However, whether the leptin receptor is expressed in coronary endothelial cells has not been previously investigated. Findings from the present study demonstrate that ObRb is present in human coronary endothelium (Fig. 1). The presence of ObRb in human coronary endothelium is important in that it may provide a link by which leptin could contribute to coronary vascular tone and altered coronary vascular function in disease states, e.g., obesity and coronary artery disease (37, 43, 50). Furthermore, we recently published a partial cDNA sequence for the canine leptin receptor (accession number: AY823396). As a result, molecular studies were extended to the canine coronary circulation. The signaling-competent ObRb gene is expressed in canine left circumflex coronary arteries, thus providing a potential thoroughfare by which leptin induces coronary endothelial dysfunction. Determining the precise coronary cell type(s) that expresses the receptor requires further study. However, given that all the effects of leptin observed in the present investigation are mediated via the endothelium, we speculate that canine coronary endothelial cells express the ObRb gene. Studying ObRb protein levels in a dog is difficult. A current limitation is the lack of commercially available anti-canine leptin receptor antibodies. Antibodies raised against leptin receptors in other species (e.g., humans and mice) exhibit poor cross-reactivity with the canine receptor.

To test the hypothesis that leptin-mediated coronary arteriolar vasodilation requires the leptin receptor, we chose to use the obese Zucker rat. The obese Zucker rat carries a missense mutation in the leptin receptor gene (46), thus serving as a functional leptin receptor knockout. The lean Zucker rat has functional leptin receptors superimposed on the same genetic background as the obese Zucker rat and was used as a control. Our results suggest that leptin-mediated coronary arteriolar dilation requires the leptin receptor, because leptin caused a dose-dependent dilation in coronary arterioles isolated from lean Zucker rats but had very little effect on coronary arterioles isolated from obese Zucker rats (Fig. 2B). However, the lack of a dilator response to leptin in coronary arterioles isolated from obese Zucker rats may be due in part to endothelial dysfunction, because several earlier studies have demonstrated that obese Zucker rats show marked endothelial dysfunction in various vascular beds (7, 9, 19). In contrast, another study demonstrates that large- and intermediate-diameter cerebral arteriolar responses to endothelium-dependent vasodilator agonists are similar in lean and obese Zucker rats (2). Therefore, whether leptin mediates dilation through the leptin receptor merits future study. However, at present, there are no commercially available leptin receptor antagonists, so directly addressing this hypothesis in dogs is difficult. Because leptin acutely induces coronary endothelial dysfunction, the presence of the leptin receptor in human coronary endothelium may prove important in further elucidation of the pathophysiological changes in the coronary circulation associated with obesity.

**Leptin and peripheral vascular function.** Many studies have implicated leptin as a vasodilator in the peripheral vasculature in vitro (20, 26, 33, 51); however, there is controversy in the literature over the mechanisms by which leptin induces vasodilation. For example, Kimura et al. (20) found that leptin mediates nitric oxide-dependent vasodilation in rat superior mesenteric artery rings. Additionally, leptin has been shown to activate endothelial NOS in rat aortic rings (49). In contrast, Lembo et al. (26) indicate that leptin-mediated dilation of rat superior mesenteric artery rings is completely nitric oxide independent. To our knowledge, the direct effects of leptin on microvascular vasomotor responses have not been previously studied.

**Leptin and coronary vascular function.** Earlier studies have demonstrated that leptin is vasoactive in noncoronary vascular beds (29, 53), but the coronary vascular effects of leptin are largely uncharacterized. Again, several clinical, epidemiologic studies have shown a strong association between hyperleptinemia and risk for coronary artery disease (37, 43, 50); however, few studies to date have examined the effects of leptin on the coronary circulation. A recent study by Sundell et al. (45) found that adenosine-mediated increases in myocardial blood flow are inversely related to serum leptin concentrations in humans. The investigators concluded that leptin might play a role in the regulation of the coronary circulation in obese subjects; however, this hypothesis was not directly tested in their study. Matsuda et al. (30) examined the direct effects of subphysiological concentrations of leptin on coronary vasomotor responses in human subjects undergoing cardiac catheterization for angina (30). In their investigation, leptin induced a dose-dependent, nitric oxide-independent increase in both coronary artery diameter and coronary blood flow. However, myocardial oxygen consumption (the major determinant of coronary blood flow control) or a clinically acceptable index thereof (e.g., pressure-rate product or tension-time index) was not reported. Thus whether the leptin-mediated changes in coronary blood flow were due to alterations in myocardial metabolism was not addressed. Additionally, Matsuda et al. did not assess coronary endothelial function in their patients. It is likely that many of the subjects had coronary endothelial dysfunction, because they were being catheterized for angina. These findings are at odds with the present investigation in that leptin induced nitric oxide-dependent dilation only at high, pharmacological doses, whereas normal and obese concentra-
tions of leptin had no effect on coronary blood flow in vivo in open-chest, anesthetized dogs (Fig. 3). It is also important to point out that leptin had little effect on myocardial oxygen consumption; i.e., leptin does not mediate metabolic coronary vasodilation (Fig. 3B). These disparate findings between the present study and Matsuda et al. (30) may be due to differences in concentration of administered leptin or species differences or both. Additionally, the subjects used in the Matsuda et al. study suffered from symptomatic coronary artery disease, whereas healthy animal subjects were used in our study.

To our knowledge, no animal studies have examined the direct effects of leptin on coronary vascular function. Although leptin did not significantly alter coronary blood flow in vivo, our data demonstrate that leptin does mediate coronary vasodilation via a nitric oxide-dependent mechanism in isolated coronary arterioles from rats and dogs, primarily at high, pharmacological leptin concentrations (>10 nmol/l) in vitro. It is crucial to recognize that the vasomotor effects of normal and obese concentrations of leptin on isolated coronary arterioles are modest, and thus it is not surprising that leptin does not alter coronary blood flow at these concentrations in vivo in open-chest, anesthetized dogs. In summary, obese concentrations of leptin do not significantly affect coronary blood flow, but they do impair coronary endothelial function.

Leptin and sympathetic nervous activity. Numerous studies have shown that leptin (administered intracerebrally or systemically) increases sympathetic nervous activity (6, 10, 13–15, 31, 34, 41, 42). It is possible that leptin-mediated activation of the sympathetic nervous system could mask leptin-dependent coronary vasodilation by way of increasing α-adrenoceptor-mediated coronary vasoconstriction (3, 47). To address this hypothesis, we performed additional intracoronary leptin dose-response experiments on open-chest, anesthetized dogs pre-treated with the autonomic ganglionic blocker hexamethonium. Administration of hexamethonium decreased baseline myocardial oxygen consumption and coronary blood flow, but the leptin dose response was unaltered. Because leptin did not increase coronary blood flow in the presence of autonomic ganglionic blockade, we deduce that leptin-mediated activation of the sympathetic nervous system does not mask an endothelium-dependent vasodilator effect in vivo. As a result, we conclude that the effects of physiological and obese concentrations of leptin do not have direct coronary vasomotor effects in vivo in open-chest, anesthetized dogs.

In conclusion, our data demonstrate that signaling-competent ObRb is present in human coronary endothelium and canine coronary arteries. Furthermore, leptin induces nitric oxide-dependent vasodilation in coronary arterioles isolated from Wistar rats, lean Zucker rats, and mongrel dogs primarily at high pharmacological concentrations. In addition, normal and obese concentrations of leptin have little effect on coronary blood flow and myocardial oxygen consumption in vivo in open-chest, anesthetized dogs; therefore, physiological and pathophysiological concentrations of leptin have little direct effect on coronary-vasomotor tone. However, obese concentrations of leptin induce significant coronary endothelial dysfunction both in vivo and in vitro. This finding implicates leptin as an important mechanistic player in the initiation of atherosclerosis and accelerated progression of cardiovascular disease in obese humans.

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GRANTS

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