Original Article

Leptin Causes Nitric-Oxide Independent Coronary Artery Vasodilation in Humans

Keiji MATSUDA, Hiroki TERAGAWA, Yukihiro FUKUDA, Keigo NAKAGAWA, Yukihito HIGASHI * , and Kazuaki CHAYAMA

Recent studies have shown that leptin causes vasodilation. However, it is unclear whether leptin causes coronary vasodilation in humans. To determine how leptin affects human coronary arteries and whether endothelium-derived nitric oxide (EDNO) is involved in the coronary arterial response to leptin, we infused leptin (0.3, 3 and 30 ng/kg/min) for 2 min into the left coronary ostium before and after an infusion of nitric oxide synthase inhibitor, N<sup>ω</sup>-monomethyl-L-arginine (L-NMMA), in 11 men with angiographically normal coronary arteries. The diameter of the epicardial coronary arteries was quantitatively measured, and coronary blood flow (CBF) was calculated by quantitative angiography and Doppler flow velocity measurements. The changes in these parameters in response to leptin are expressed as the % change from the baseline values. Leptin caused coronary dilation (0.3 ng/kg/min: 2.0 ± 0.5%; 3 ng/kg/min: 4.9 ± 0.7%; 30 ng/kg/min: 3.8 ± 0.9%) and increased CBF (13.6 ± 3.3%, 36.8 ± 5.6%, and 39.2 ± 7.4%, respectively). After the infusion of L-NMMA, leptin also caused coronary dilation (2.0 ± 0.4%, 4.5 ± 0.7%, and 4.6 ± 0.8%, respectively) and increased CBF (14.6 ± 2.8%, 39.2 ± 5.7%, 40.3 ± 6.2%, respectively). Leptin-induced coronary vasodilation was not affected by the infusion of L-NMMA. These results suggest that leptin dilates coronary arteries in humans. Furthermore, EDNO may not contribute to leptin-induced coronary dilation. (Hypertens Res 2003; 26: 147–152)

Key Words: leptin, nitric oxide, coronary artery, coronary blood flow

Introduction

Leptin is produced by the obesity gene, which was cloned from the hereditary obesity ob/ob mouse in 1994, and is synthesized in adipose tissue (1). It has been reported that leptin plays an important role in the control of obesity and weight gain, primarily by suppressing feeding suppression. In addition, leptin increases energy expenditure through increasing activity, body temperature, sympathetic activity, and oxygen consumption through its effects on the hypothalamus (2, 3).

Recently, much interest has focused on the effect of leptin on the vasculature. It has been reported that leptin has a strong dose-dependent sympathoexcitatory effect, mediated by leptin receptors in the hypothalamus (4). Furthermore, chronic leptin infusion increases arterial pressure and heart rate (5). However, several studies have shown that leptin also has a vasodilatory effect (6, 7). Lembo et al. have demonstrated that leptin has a vasodilatory effect at the level of both conduit and resistance vessels in an experimental setting (6). In humans, our previous study demonstrated that leptin causes vasodilation in the forearm resistance vessels in healthy men (7). However, there have been no studies investigating the coronary responses to leptin infusion in vivo.

In addition, endothelium-derived nitric oxide (EDNO) has been suggested to be responsible for leptin-induced vasodilation (6, 8). Lembo et al. reported that leptin dilates the conduit vessels in an EDNO-dependent manner, whereas it dilates resistance vessels via endothelium-dependent hyperpolarizing factor (6). Our previous study has shown that leptin-
induced vasodilation in the forearm microcirculation is independent of EDNO (7). Therefore, in the present study we investigated the coronary vascular responses to intracoronary infusion of leptin in humans. In addition, we established that EDNO contributes to the coronary vascular response to leptin using Nω-monomethyl-L-arginine (L-NMMA), a nitric oxide (NO) synthase inhibitor.

Methods

Study Population
We studied 11 Japanese men (mean age, 60 years; range, 48–78 years) undergoing coronary angiography for the evaluation of atypical chest pain. All of the patients had angiographically normal epicardial coronary arteries, normal left ventricular function (contrast ventriculographic ejection fraction > 50%) and a normal coronary flow reserve. Patients with angiographically documented coronary spasm (> 50% luminal narrowing) after intracoronary injection of acetylcholine were excluded from the study. No patients had a history of prior myocardial infarction, heart failure, or other serious disease. Written informed consent was obtained from all patients enrolled in the study. The protocol was approved by the human investigation ethics committee of the Graduate School of Biomedical Sciences, Hiroshima University.

Study Design
The study design has been described in detail previously (9, 10). In brief, cardiac medications were withheld for at least 48 h prior to catheterization, except for the unrestricted use of sublingual nitroglycerin, which was withheld 1 h prior to catheterization. A 6 Fr guide catheter was introduced into the left main coronary artery. A 0.014-inch Doppler flow guide wire (FloWire; EndoSonics, Cordova, USA) was then advanced through the guide catheter into the proximal segment of the left anterior descending coronary artery and positioned in a straight segment of the vessel to acquire an adequate flow velocity signal. A 6 Fr Goodale-Lubin catheter (Bard Access System, Murray Hill, USA) was inserted via the right internal jugular vein into the coronary sinus to obtain blood samples.

Study Protocol
After baseline control conditions were established, increasing doses of leptin (0.3, 3 and 30 ng/kg/min) were infused into the left main coronary artery for 2 min using a constant rate infusion pump (Terufusion Pump; Terumo, Tokyo, Japan). These doses were based on the doses used in human forearm blood flow studies (7). In preliminary study, leptin at these doses had no effects on systemic hemodynamics, but caused significant increases in the plasma leptin concentration (baseline: 2.6 ± 0.6 ng/ml; 30 ng/kg/min: 6.9 ± 0.9 ng/ml) in the coronary sinus. After 5 min, L-NMMA was infused into the left coronary artery at a rate of 40 µmol/min for 5 min. After steady state conditions had been reestablished, serial infusions of leptin (0.3, 3 and 30 ng/kg/min) were again performed. Finally, nitroglycerin (200 µg) was given as an intracoronary injection.

Coronary angiography was performed at baseline and just after the end of each drug infusion. The coronary blood flow (CBF) velocity was monitored continuously using a 12-MHz pulsed Doppler velocimeter (FloMap; EndoSonics). Arterial pressure, heart rate, and the electrocardiogram were monitored continuously and recorded using a multichannel recorder (Nihon Kohden Polygraph System; Nihon Kohden, Tokyo, Japan). We waited at least 5 min after each drug infusion to obtain stable values for blood flow velocity and coronary diameter.

Quantitative Coronary Angiography and Determination of Coronary Blood Flow
Coronary angiograms were acquired and analyzed using digital image acquisition (HICOR X-ray system; Siemens, Forchheim, Germany) and analysis systems (CAAS II QCA system; Pie Medical, Maastricht, Netherlands). In each patient, the luminal diameter of the left anterior descending coronary artery at the segment 2 mm distal to the Doppler wire tip was measured to determine the effects of various drugs on epicardial coronary diameter at end diastole. The measurements were performed without knowledge of the clinical characteristics of the patient. The average of three measurements of the luminal diameter was used for analysis. A strong correlation for intraobserver measurements was noted (r = 0.987, p < 0.0001). Analysis of interobserver variability also showed a high reproducibility (r = 0.982, p < 0.0001). CBF velocity was measured at baseline and under steady state conditions for each drug infusion. CBF was calculated as the product of CBF velocity and vessel diameter using the following formula: \( \pi \cdot \text{CBF velocity} \cdot \text{diameter}^2 \). Changes in the coronary diameter and CBF are expressed as the percent change from the control values.

Biochemical Parameters
Serum glucose and insulin were assayed by an enzymatic method and radioimmunoassay, respectively. Plasma epinephrine and norepinephrine were assayed by high-performance liquid chromatography. Plasma leptin was assayed by radioimmunoassay (Human Leptin RIA Kit; Linco Research Inc., St. Charles, USA). The limit of sensitivity for the human leptin assay was 0.5 ng/ml, and the inter- and intraassay coefficients of variation for the human leptin assay were 5.0 ± 0.9% and 4.5 ± 0.6%, respectively. All measurements were performed in duplicate.
serum cholesterol concentration of left ventricular hypertrophy. Hypercholesterolemia (total patients had hypertension but no echocardiographic evidence of left ventricular hypertrophy). Current smokers (270 mg/dl during the period when they were not receiving antihypercholesterolemic drug treatment. One patient had diet-controlled diabetes mellitus.

**Drug Preparations**

Human leptin and L-NMMA were obtained from Sigma Chemical Co. (St Louis, USA). All other drugs were obtained from commercially available sources and were dissolved in saline (0.9% NaCl; Ohtsuka Pharmaceutical Co., Tokyo, Japan) immediately before use. All drugs were sterilized by specialists in the Department of Pharmacology, Hiroshima University Hospital.

**Statistical Analysis**

Results are presented as the mean \( \pm \) SEM. Serial changes in hemodynamic variables, epicardial coronary diameter and CBF in response to leptin were compared by one-way analysis of variance. Serial changes in the epicardial coronary diameter and coronary blood flow before and after the administration of L-NMMA were compared by two-way analysis of variance. Paired data were compared using the Wilcoxon signed-ranks test. Values of \( p < 0.05 \) were considered to indicate statistical significance.

**Results**

**Clinical Characteristics**

The mean body mass index for our patients was 24.5 \( \pm \) 0.8 kg/m\(^2\), ranging from 20.6 kg/m\(^2\) to 28.8 kg/m\(^2\). Four patients were current smokers (\( > 10 \) cigarettes/day), but were instructed not to smoke for at least 48 h before the study. Two patients had hypertension but no echocardiographic evidence of left ventricular hypertrophy. Hypercholesterolemia (total serum cholesterol concentration \( > 240 \) mg/dl) was present in 5 patients, but their total serum cholesterol concentration was \( < 270 \) mg/dl during the period when they were not receiving antihypercholesterolemic drug treatment. One patient had diet-controlled diabetes mellitus.

**Biochemical Parameters**

The baseline plasma leptin concentration was 3.6 \( \pm \) 0.6 ng/ml and did not correlate with the body mass index. The plasma leptin concentration in the coronary sinus increased after the first infusion of leptin compared with the baseline value (3.6 \( \pm \) 0.6 ng/ml to 9.2 \( \pm \) 1.0 ng/ml), and returned to the baseline level before the second infusion of leptin. Concentrations of plasma epinephrine and norepinephrine in the coronary sinus did not change significantly before or after the leptin infusions. In addition, there were no significant changes in serum glucose or insulin concentrations before or after the leptin infusions.

**Hemodynamic Variables**

Intracoronary administration of leptin or L-NMMA did not significantly alter the baseline mean arterial pressure or heart rate in either group. Nitroglycerin decreased the mean arterial pressure, but it increased the heart rate compared with control values (Table 1).

**Coronary Response to L-NMMA and Nitroglycerin**

After intracoronary infusion of L-NMMA, the coronary diameter at baseline decreased from 2.74 \( \pm \) 0.21 mm to 2.66 \( \pm \) 0.21 mm (\( p < 0.05 \), Table 1), and CBF at baseline decreased from 56.3 \( \pm \) 5.6 ml/min to 48.7 \( \pm \) 4.5 ml/min (\( p < 0.01 \), Table 1). After the intracoronary infusion of nitroglycerin, the coronary diameter at baseline increased from 2.74 \( \pm \) 0.21 mm to 2.66 \( \pm \) 0.21 mm (\( p < 0.01 \), Table 1).

**Coronary Response to Leptin Infusion**

Leptin infusion caused significant increases in the coronary diameter (0.3 ng/kg/min: 2.0 \( \pm \) 0.5%; 3 ng/kg/min: 4.9 \( \pm \) 0.7%; 30 ng/kg/min: 3.8 \( \pm \) 0.9%; Fig. 1) and in CBF (0.3 ng/kg/min: 13.6 \( \pm \) 3.3%; 3 ng/kg/min: 36.8 \( \pm \) 5.6%)

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**Table 1. Systemic Hemodynamic and Coronary Diameter and Blood Flow Variables**

<table>
<thead>
<tr>
<th></th>
<th>HR (beats/min)</th>
<th>MAP (mmHg)</th>
<th>Coronary diameter (mm)</th>
<th>Coronary blood flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline before L-NMMA</td>
<td>62 ( \pm ) 3</td>
<td>108 ( \pm ) 5</td>
<td>2.74 ( \pm ) 0.21</td>
<td>56.3 ( \pm ) 5.6</td>
</tr>
<tr>
<td>Leptin (ng/kg/min) 0.3</td>
<td>62 ( \pm ) 3</td>
<td>107 ( \pm ) 4</td>
<td>2.79 ( \div ) 0.21 **</td>
<td>63.5 ( \pm ) 6.0</td>
</tr>
<tr>
<td>3</td>
<td>63 ( \pm ) 3</td>
<td>107 ( \pm ) 5</td>
<td>2.87 ( \div ) 0.21 **</td>
<td>76.5 ( \div ) 7.7 **</td>
</tr>
<tr>
<td>30</td>
<td>63 ( \pm ) 3</td>
<td>109 ( \pm ) 6</td>
<td>2.84 ( \div ) 0.21 **</td>
<td>76.6 ( \div ) 7.1 **</td>
</tr>
<tr>
<td>Baseline after L-NMMA</td>
<td>61 ( \pm ) 2</td>
<td>110 ( \pm ) 6</td>
<td>2.66 ( \div ) 0.21 #</td>
<td>48.7 ( \div ) 4.5 **</td>
</tr>
<tr>
<td>Leptin (ng/kg/min) 0.3</td>
<td>61 ( \pm ) 2</td>
<td>111 ( \pm ) 6</td>
<td>2.71 ( \div ) 0.21 **</td>
<td>55.5 ( \div ) 4.9</td>
</tr>
<tr>
<td>3</td>
<td>63 ( \pm ) 3</td>
<td>110 ( \pm ) 6</td>
<td>2.77 ( \div ) 0.21 **</td>
<td>67.4 ( \div ) 6.3 **</td>
</tr>
<tr>
<td>30</td>
<td>62 ( \pm ) 3</td>
<td>111 ( \pm ) 5</td>
<td>2.78 ( \div ) 0.20 **</td>
<td>67.9 ( \div ) 6.6 **</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>67 ( \pm ) 2</td>
<td>102 ( \pm ) 7#</td>
<td>3.20 ( \div ) 0.20 **</td>
<td>65.9 ( \pm ) 6.1</td>
</tr>
</tbody>
</table>

Values are mean \( \pm \) SEM. HR, heart rate; MAP, mean arterial pressure; L-NMMA, \( N^0 \)-monomethyl-L-arginine. \( \# p < 0.05 \) vs. others; \( \# p < 0.05 \) vs. baseline; \( * p < 0.01 \) vs. most recent baseline; \( ** p < 0.01 \) vs. most recent baseline.

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"Matsuda et al: Leptin and Coronary Dilation 149"
ng/kg/min: 39.2 ± 7.4%; Fig. 1). Leptin-induced increases in coronary diameter and CBF were not associated with either the body mass index or baseline plasma leptin concentration. The serial changes in coronary diameter and CBF from baseline to an infusion of leptin at a dose of 3 ng/kg/min increased in a dose-dependent manner. However, the changes in those vessels in response to an infusion of leptin at a dose of 30 ng/kg/min were not different from those at a dose of 3 ng/kg/min.

**Effect of L-NMMA on Leptin-Induced Coronary Response**

After the intracoronary infusion of L-NMMA, leptin infusion induced a significant increase in the coronary diameter (0.3 ng/kg/min: 2.0 ± 0.5%; 3 ng/kg/min: 4.5 ± 0.7%; 30 ng/kg/min: 4.6 ± 0.8%; Fig. 1) and in CBF (0.3 ng/kg/min: 14.6 ± 2.8%; 3 ng/kg/min: 39.2 ± 5.7%; 30 ng/kg/min: 40.3 ± 6.2%; Fig. 1). Leptin-induced increases in coronary diameter and CBF did not change after an infusion of L-NMMA (Fig. 1).

**Discussion**

The results of the present study suggest that leptin causes significant dilation of coronary arteries, including the epicardial and resistance coronary arteries in male patients with a typical chest pain but angiographically normal coronary arteries. Furthermore, this study demonstrates that EDNO does not mediate leptin-induced coronary dilation within a subphysiologic leptin concentration. To our knowledge, this is the first report of leptin-induced coronary dilation in humans.

Several studies have investigated the effect of leptin on the vasculature. Jalali *et al.* (11) and Mitchell *et al.* (12) have shown that leptin infusion for 3 h did not increase blood flow. Furthermore, Shek *et al.* (5) demonstrated that chronic infusion of leptin for several days increases arterial pressure, suggesting chronic infusion of leptin causes arterial constriction. In contrast, Lembo *et al.* (6) reported that acute leptin infusion causes hypotension, suggesting a vasodilatory effect of leptin. The duration of leptin infusion may account for the differences in the vascular response. However, it is also possible that the acute effects of leptin cause vasodilation.

With respect to human studies *in vivo*, we first demonstrated that intraarterial infusion of leptin for 5 min increases forearm blood flow in healthy male individuals (7). However, there have been no reports concerning changes in coronary arteries in response to leptin infusion *in vivo*. The present study demonstrated that intracoronary infusion of leptin for 2 min caused dilation of the coronary arteries and increased CBF, suggesting that leptin dilates the coronary arteries at the level of both conduit and resistance vessels.

Various mechanisms have been hypothesized to account for the induction of vasodilation by leptin. First, the leptin-induced vasodilatory response may be mediated by EDNO. It has been reported that the vascular endothelium is one of the targets of leptin (13). In addition, Fruhbeck has shown that the administration of leptin increases the serum nitrite/nitrate concentration in Wister rats (14). Vecchione *et al.* (15) reported that leptin induces NO production by activating the Akt-endothelial NO synthase phosphorylation pathway in aortic rings. These findings may support the hypothesis that leptin-induced vasodilation is due to EDNO. However, we have recently shown that leptin-induced vasodilation in humans is independent of EDNO in forearm circulation (7). Furthermore, in the present study, we have demonstrated that leptin caused EDNO-independent vasodilation even in the coronary circulation. The plasma leptin concentrations in these studies were subphysiologic, and the differences in the dose of leptin or the concentration of leptin may have contributed to the differing results.

Endothelium-derived hyperpolarizing factor may be involved in leptin-induced vasodilation. Lembo *et al.* (6) have
shown that leptin-induced vasodilation at the level of the resistance vessels is mainly mediated by endothelium-derived hyperpolarizing factor. In contrast, EDNO plays an important role in leptin-induced vasodilation at the conduit vessel level in experimental studies. In the human coronary circulation, it is possible that endothelium-derived hyperpolarizing factor plays a role in leptin-induced vasodilation at both the conduit vessel level and the resistance vessel level. Furthermore, prostacyclin may be involved in leptin-induced vasodilation. Brunetti et al. have reported that leptin stimulates prostaglandin E2 and F2α production rather than NO production in the rat hypothalamus (16). The same may be true for the vascular endothelium, and we could not exclude the possibility that the effect of leptin in the vasculature is mediated by prostacyclin, because we did not use aspirin to block the effects of prostacyclin in the present study. Additionally, leptin-induced vasodilation may be mediated through improved insulin sensitivity. It has been reported that leptin administration increases insulin sensitivity in normal rats (17) as well as mice with congenital lypodystrophy (18). However, because there were no significant changes in serum glucose or plasma insulin concentrations after leptin infusion in our study, it is unlikely that leptin-induced vasodilation is mediated by improved insulin sensitivity. Finally, we cannot rule out the possibility that leptin may affect vascular smooth muscle cells directly.

Several epidemiologic studies have shown that hyperleptinemia is a risk factor for coronary artery disease (19, 20), suggesting that an increased leptin concentration may unfavorably influence the coronary arteries. However, we have demonstrated that acute infusion of leptin dilates the coronary arteries without any significant change in hemodynamic variables or metabolic parameters, suggesting a favorable effect of leptin on the coronary arteries. As we discussed above, the effects of leptin may be quite different according to whether leptin is infused acutely or chronically, and whether the plasma leptin concentration is increased transiently or persistently. In addition, it is possible that differences in leptin concentration may contribute to the differing responses of the coronary arteries. In the present study, the baseline leptin concentration was 3.6 ng/ml and did not differ from the values reported (>5 ng/ml) in the above studies (19, 20). Although we showed that leptin-induced coronary vasodilation is not associated with the baseline leptin concentration, the mean body mass index was 24.5 ± 0.8 kg/m², and severely obese patients were not included in the present study. Furthermore, the leptin-induced coronary responses increased in a dose-dependent manner up to a leptin dose of 3 ng/ml/kg. However, the degree of vasodilation induced by leptin at a dose of 30 ng/ml/kg was not different from the value obtained at a dose of 3 ng/ml/kg. These findings suggest that leptin causes coronary vasodilation at subphysiologic leptin concentrations, but the degree of coronary vasodilation induced by leptin may decrease at or above physiologic leptin concentrations, and this phenomenon may be associated with “leptin resistance” (21). These factors may contribute to the differences seen between epidemiologic studies (19, 20) and ours. It is likely that leptin may be used orally as an anti-obesity agent in the near future. At that time, further studies will be needed to confirm how chronic administration of leptin affects coronary arteries in obese patients.

There are several limitations to the present study. First, only a small number of individuals were included in our study. With this small number of patients, statistically significant differences could not be identified, especially with respect to the relationship between the baseline leptin concentration and body mass index, although it has been reported that leptin is increased in obese animals and humans (22, 23). Because plasma leptin concentrations are gender-dependent (24), we selected only male subjects in the present study. Therefore, it is unclear whether similar results would be obtained in a cohort of female subjects. The dose of L-NMMA may have been insufficient to inhibit EDNO production completely. The baseline coronary diameter and CBF decreased after the infusion of L-NMMA, indicating that the dose of L-NMMA was sufficient to inhibit basal EDNO production. However, the dose may have been insufficient to inhibit leptin-induced EDNO production. In a previous study, Quyyumi et al. (25) infused L-NMMA at a dose of 64 μmol/min for 5 min, and inhibited acetylcholine-induced dilation of coronary resistance vessels. However, we did not administer high dose L-NMMA because of the risk of increased systemic blood pressure and concomitant vasoconstriction in other vascular beds.

In conclusion, the present findings indicate that intracoronary leptin infusion causes dilation of human coronary arteries, including the conductance and resistance arteries. This leptin-induced human coronary dilation occurs through an NO-independent pathway.

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References


