Leptin is an endothelial-independent vasodilator in humans with coronary artery disease: evidence for tissue specificity of leptin resistance


Aims We sought to define the mechanisms and correlates of leptin’s vascular actions in humans with coronary artery disease.

Methods and results In 131 patients (age 65.7 ± 0.7 years mean ± SEM), ex vivo vascular reactivity to leptin (10^{-13} - 10^{-7} M) was assessed in saphenous vein (SV) rings. Leptin led to SV relaxation (maximal relaxation 24.5 ± 1.6%). In separate experiments, relaxation to leptin was unaffected by L-NMMA (17.4 ± 3.4 vs. 17.8 ± 3.3%, P = 0.9) or endothelial denudation (17.4 ± 4.4 vs. 22.5 ± 3.0%, P = 0.4). We explored the possibility that leptin’s vascular effects are mediated via smooth muscle hyperpolarization. In the presence of KCl (30 mmol/L) to inhibit hyperpolarization, the vasodilator effect of leptin was completely blocked (0.08 ± 4.1%, P < 0.001 vs. control). Similar results were demonstrated in internal mammary artery rings. The only independent correlate of leptin-mediated vasodilatation was plasma TNF-alpha (r = 0.25, P < 0.05). Neither body mass index nor waist circumference correlated with leptin-mediated vasorelaxation. This lack of a correlation with markers of total body fat/fat distribution suggests that leptin resistance may not extend to the vasculature.

Conclusion Leptin is a vasoactive peptide in human SV and internal mammary artery. Its action is not nitric oxide or endothelial-dependent. Markers of body fat did not correlate with leptin-mediated vasodilatation, raising the intriguing possibility of selective resistance to leptin’s actions.

Introduction

Leptin, the product of the ob gene secreted by adipocytes, is a multi-functional peptide hormone, the circulating concentration of which is proportional to fat mass. Leptin has a major influence on body weight and metabolism and is significantly increased in obesity leading to the concept of leptin resistance. Although the hypothalamus is considered to be the main target for leptin, it is becoming clear that leptin has actions on other tissues. A potential role for leptin in vascular physiology/pathophysiology is supported by studies demonstrating that leptin leads to sympathoactivation, superoxide production, vascular calcification, and angiogenesis. A number of studies have demonstrated that leptin is a vasodilator. The mechanisms underlying this effect are unclear and results of studies in different species contradictory. In rats, it has been shown that leptin leads to vasorelaxation, an effect, which may occur by activation of PI3K/Akt to stimulate endothelial nitric oxide (NO) synthase. Other studies have suggested that leptin leads to vasorelaxation by smooth muscle hyperpolarization, whereas a small study in humans did not support a role for NO in leptin’s vascular effects.

We sought to explore the effect of leptin on vascular tone in human saphenous vein (SV) and internal mammary artery ex vivo. The present series of studies demonstrate that: (i) leptin is an endothelial and NO-independent vasodilator in humans with coronary artery disease; (ii) the vasodilator action of leptin is at least in part due to smooth muscle hyperpolarization; and (iii) obesity does not lead to resistance to the vascular effects of leptin in patients with coronary artery disease.

Methods

A total of 187 unselected patients referred for first time elective coronary artery bypass surgery (CABG) at King’s College Hospital (London, UK) between January 2003 and December 2003 were
approached to take part in the study. One patient refused to take part in the study. Of the remaining 186 patients, 131 (91% male) had vascular function assessed ex vivo (Table 1), and these 131 patients make up the sample described in the present report. Patients were excluded if they had symptomatic heart failure or an acute coronary syndrome within the previous 6 months. Patients were recruited from a pre-admission clinic 1 month prior to surgery when smoking status (current or within last 6 months) was ascertained and anthropometric measurements taken. All patients were taking HMG-CoA reductase inhibitors and anti-platelet agents, 38% were taking angiotensin converting enzyme inhibitors, and 69% beta-adrenoceptor blockers. In total, 70% were current/recent smokers. Drugs were omitted for 24 h prior to surgery. On the morning of surgery, blood was obtained for measurement of lipids, C-reactive protein, TNF-alpha (TNF-α), leptin, interleukin-6 (IL-6), and fasting blood glucose. Segments of SV were obtained from 131 patients (11 female). The study protocol and collection of tissue specimens were approved by the local research Ethics Committee and all subjects provided written informed consent.

Vascular ring studies in humans

Vascular responses to human recombinant leptin \(10^{-13} - 10^{-7} \text{M} \) (Calbiochem, UK) were performed in SV and internal mammary artery rings ex vivo, using techniques as previously described. In more detail, SV or internal mammary artery was harvested without distension and minimal tissue handling and transferred in chilled pre-oxygenated buffer to the physiology laboratory. Veins and arteries were carefully cleared of adherent tissue and cut into 4 mm rings. SV and internal mammary artery rings were suspended between a fixed support and a force transducer in an organ bath containing 10 mL Krebs Henseleit solution (composition mmol/L: NaCl 119, KCl 4.7, KH2PO4 1.18, NaHCO3 25, MgSO4 1.19, CaCl2 2.5, and glucose 11.0) at 37 °C, bubbled with 95% O2/5% CO2 to maintain a pH of 7.4. After 45 min equilibration at a resting tension of 3 g, which we have found to be optimal, the maximal contractile response to SNP 80 μM was assessed. After washout and re-equilibration, a cumulative dose-response curve to phenylephrine pre-constricted tension. Paired data were compared using two-sided Student’s \( t \)-test, with application of a Bonferroni correction to adjust for multiple testing. A value of \( P < 0.05 \) to detect a 50% difference between control and treatment groups was considered significant. All analyses were performed using SPSS, Inc. version 9.0.

Exploring the mechanism of leptin-mediated vasodilatation

In a series of experiments, we assessed potential mechanisms of leptin-mediated vasodilatation. To do this, we compared the response with leptin in SV/internal mammary artery in a control ring and rings treated with the relevant intervention in the same patient. To assess the role of endothelial derived NO in leptin’s vascular actions, some studies were performed before and after exposure to the non-selective nitric oxide synthase inhibitor L-NMMA \(10^{-4} \text{M} \) (Calbiochem, UK) or after endothelial denudation (confirmed by response to Ach). To explore the possibility that leptin’s action is to hyperpolarize vascular smooth muscle, we added 30 mmol/L KCl prior to exposure to leptin in rings pre-constricted to 70% maximal tension. Gibenclamid 10 μm/L (Alexis Corp Nottingham, UK) was used to explore the possibility that leptin activated ATP-sensitive potassium channels. Some studies have suggested that \( \text{H}_{2}\text{O}_2 \) may be an endothelial derived hyperpolarizing factor and studies from our own laboratory have supported a role for \( \text{H}_{2}\text{O}_2 \) as a vasodilator in obesity. To explore the possibility that leptin’s effect was via production of superoxide and its dismutation product \( \text{H}_{2}\text{O}_2 \), we performed leptin relaxation curves after exposure of rings to catalase at 1250 U/L. Preliminary studies showed that none of the inhibitors effected SNP-mediated vasodilatation in SV rings (data not shown).

Plasma assays

Assays for serum total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and glucose were carried out in the hospital biochemistry laboratory. Serum samples for C-reactive protein, TNF-α, and IL-6 were stored at \(-80^\circ\text{C} \) until analysis. C-reactive protein was measured using a high-sensitivity turbidimetric immunosassay (WAKO Chemicals, Neuss, Germany) on the Cobas Mira Analyser (Roche Diagnostics, Lewes, UK). The lowest concentration of C-reactive protein that could be detected was 0.2 mg/L. Within batch variability was assessed and a coefficient of variation (CV) of 3.1% at 5.0 mg/L was achieved. TNF-α, IL-6, and leptin were measured using a commercially available enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, USA). The CV for TNF-α was 9%, for leptin <6%, and for IL-6 <9%.

Statistical methods

Continuous variables are presented as mean ± standard error of mean. Pearson’s correlation coefficients were used to examine relationships between variables. Non-normally distributed variables (C-reactive protein, HDL, TNF-α, triglycerides, and leptin) were log transformed. The independent association between leptin/leptin-mediated vasodilatation and other variables was analysed by using stepwise multiple regression analysis with leptin or leptin-mediated vasodilatation as the dependent variable, and age, gender, body mass index, waist circumference, mean arterial blood pressure, fasting glucose, LDL, HDL, triglycerides, TNF-α, IL-6, and C-reactive protein as covariates. To ensure a minimum of 10 patients per variable entered into the multivariable model, data from 130 patients were studied. In studies exploring the mechanisms of leptin’s vascular action, groups of 10 patients were studied. On the basis of variability of leptin-mediated vasodilatation, this sample provided 90% power at \( P < 0.05 \) to detect a 50% difference between control and treatment rings. Paired data were compared using two-sided Student’s \( t \)-tests with application of a Bonferroni correction to adjust for multiple testing. A value of \( P < 0.05 \) was considered significant. All analyses were performed using SPSS, Inc. version 9.0.

Table 1 Characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.9 ± 0.8</td>
<td>(40–84)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.8 ± 0.3</td>
<td>(16.5–39.1)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>99.1 ± 0.9</td>
<td>(73.0–131.0)</td>
</tr>
<tr>
<td>WHR</td>
<td>1.0 ± 0.08</td>
<td>(0.86–1.17)</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>138.6 ± 2.0</td>
<td>(80–200)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74.4 ± 1.0</td>
<td>(46–104)</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.4 ± 0.08</td>
<td>(1.4–6.8)</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>1.8 ± 0.06</td>
<td>(0.5–4.4)</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.0 ± 0.02</td>
<td>(0.6–1.9)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 ± 0.07</td>
<td>(0.3–4.0)</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.6 ± 0.2</td>
<td>(2.7–18.0)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.2 ± 0.7</td>
<td>(0.2–51.0)</td>
</tr>
<tr>
<td>TNF-α (µg/L)</td>
<td>3.3 ± 0.3</td>
<td>(0.5–15.7)</td>
</tr>
<tr>
<td>IL-6 (µg/L)</td>
<td>2.8 ± 0.3</td>
<td>(0.3–25.6)</td>
</tr>
<tr>
<td>Leptin (µg/L)</td>
<td>20.1 ± 2.5</td>
<td>(0.7–194.0)</td>
</tr>
</tbody>
</table>

Data expressed as mean (SEM) or as per cent of population. BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.
Results

Leptin-mediated vasodilatation and its correlates
This first part of the present study aimed to assess whether leptin is a vasodilator in humans and if so does obesity lead to resistance to leptin’s vascular actions. A large unselected group of patients undergoing CABG were studied (Table 1).

Leptin-mediated vasodilatation in human SV and internal mammary artery
Leptin led to significant vasodilatation of both SV and internal mammary artery at both physiological and supra-physiological concentrations (Figure 1). The extent of vasorelaxation to leptin was similar to Ach, whereas all rings relaxed ~100% to SNP.

Determinants of leptin-mediated vasodilatation in humans
In stepwise multiple regression analysis, body mass index, waist circumference, and circulating leptin concentration were not related to leptin-mediated vasodilatation. TNF-α positively correlated with leptin-mediated vasodilatation ($B = 12.0 \pm 5.4$, Beta = 0.25, 95% CI 1.2–22.8, $P < 0.05$).

Potential mechanisms underlying leptin-mediated vasodilatation in humans
In a subgroup of patients, we explored the potential mechanisms underlying leptin’s vascular effects. Each patient acted as their own control with an untreated SV ring exposed to leptin compared with treated rings. To explore the role of the endothelium and endothelial derived nitric oxide, we performed studies in rings denuded of endothelium and in the presence of L-NMMA ($n = 10$ in all groups). Maximal relaxation to leptin was $17.5 \pm 3.4\%$ in endothelial intact rings, $17.8 \pm 3.3\%$ in the presence of L-NMMA, and $22.5 \pm 3.0\%$ after endothelial denudation ($P$-value difference all $>0.6$).

To explore the possibility that leptin acts on blood vessels by hyperpolarizing vascular smooth muscle, we exposed rings to KCl 30 mmol/L ($n = 10$). Mean relaxation after KCl was $0.08 \pm 4.1\%$. To explore the possibility that hydrogen peroxide contributes to leptin-mediated vasodilatation, we performed relaxation before and after exposure of rings to catalase (1250 U/L, $n = 10$). Catalase had no effect on leptin-mediated relaxation of SV rings (Figure 1B).

To explore the possibility that the vascular effects of leptin are specific to veins, we repeated the experiments in a subset of internal mammary artery rings from similar patients, each patient having an untreated control internal mammary artery ring ($n = 10$ in all groups, Figure 2). In arterial rings, leptin led to vasorelaxation of $30.5 \pm 8.1\%$. This response was unaffected by L-NMMA ($48.4 \pm 10.6\%$) or endothelial denudation ($37.9 \pm 5.8\%$) (all $P > 0.4$ vs. control rings). Hyperpolarization with KCl blocked this effect, mean relaxation $-0.62 \pm 1.8\%$ ($P < 0.05$ vs. control).

Determinants of circulating leptin in humans with coronary artery disease
Significant correlates of plasma leptin are shown in Table 2. In stepwise multiple regression analysis, independent
Leptin's vascular actions in humans with coronary artery disease

The adipocyte as a vasoregulatory organ

In recent years, the view of adipose tissue as a simple fuel store has changed. Fat tissue has emerged as a complex endocrine organ. This concept has evolved further to include a role for fat tissue in inflammatory pathways, largely as adipocytes have been shown to secrete cytokines classically considered to be produced by macrophages. These cytokines include IL-6 and TNF-α, both of which are significantly increased in obese humans. Furthermore, recent studies have suggested that the adipocyte may secrete substances that regulate vascular tone. Solits and Cassis demonstrated that the presence of peri-adventitial fat blunts vascular responsiveness of rat aortic rings to norepinephrine. Verlohren et al. studied mesenteric rings from Sprague–Dawley rats, and demonstrated that the contractile response to a number of vasoconstrictors was attenuated by an adipocyte-derived substance. Similar to our findings with leptin, they demonstrated that this vasorelaxant effect was blunted by high K⁺ solutions.

Leptin-mediated vasorelaxation

In a series of experiments, Lembo and coworkers demonstrated that leptin has a vasorelaxant action in rat aortic rings. They also showed that this effect was blunted by endothelial denudation, L-NAME, and a selective guanylate cyclase inhibitor (consistent with a role for endothelium-derived NO). In mesenteric rings, however, the effect of leptin was unaffected by L-NAME, but blunted by Befeldin, an endothelial-derived hyperpolarizing factor inhibitor.

Our data in humans show that leptin is a vasodilator both in veins and arteries. This action is neither endothelium nor NO-dependent. Leptin is thought to exert its central actions by membrane hyperpolarization, an effect also demonstrated in pancreatic beta-cells. Consistent with a similar effect in vascular smooth muscle cells, we demonstrated that increasing local K⁺ concentration blocks leptin's vasorelaxant effect in both arteries and veins. We explored the possibility that glibenclamide (a commonly prescribed agent in obese diabetics) that blocks potassium channels blocks leptin's vasodilatory effect. In the presence of glibenclamide, leptin's effect was unchanged. The precise mechanism of leptin-mediated smooth muscle hyperpolarization warrants further study.

Determinants of leptin-mediated vasodilatation: potential implications for vascular regulation in inflammatory conditions

Recent studies have demonstrated that plasma leptin concentration rises in sepsis in humans. Moreover, there are compelling data that TNF-α can stimulate release of preformed adipocyte stores of leptin. Our data may thus have potential implications for vascular homeostasis in human conditions associated with inflammation such as sepsis, obesity, and diabetes. It is well established that sepsis is characterized by hypotension and peripheral vasodilatation; thus leptin and its interaction with cytokines may contribute to this. Our findings suggest that TNF-α may augment the vasodilator action of leptin on capacitance veins. Venous tone plays an important role in blood pressure homeostasis and is depressed in sepsis. This potentially important interaction between leptin and TNF-α on vascular tone warrants further study. It is also important to note that markers of body fat were not related to leptin-mediated vasodilatation. Although the presence of leptin resistance in obesity has been reported, our data set supports tissue selectivity of resistance to leptin with preservation of leptin's vasodilatory effects. This may reflect different mechanisms underlying leptin's vascular and metabolic/central effects—studies at the whole body level in humans may help answer this question.

What are the determinants of circulating leptin in patients with coronary artery disease?

In view of the fact that leptin seems to have potentially favourable (at least in terms of obesity-related hypertension) vascular effects, and that increased total and visceral predictors of plasma leptin concentration were body mass index ($B = 0.03 \pm 0.01$, Beta $= 0.30$, 95% CI 0.008–0.047, $P < 0.01$), waist circumference ($B = 0.014 \pm 0.004$, Beta $= 0.35$, 95% CI 0.005–0.022, $P < 0.01$), and fasting plasma glucose ($B = 0.58 \pm 0.17$, Beta $= 0.21$, 95% CI 0.23–0.93, $P < 0.01$).

Table 2 Pearson's correlation coefficients for circulating leptin concentrations in patients with severe coronary artery disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pearson's correlation coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.59</td>
<td>0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.58</td>
<td>0.0001</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>0.27</td>
<td>0.002</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.22</td>
<td>0.01</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>0.26</td>
<td>0.003</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure.
fat do not blunt this effect, the determinants of circulating leptin in this sample were explored. Shamsuzzaman et al. recently demonstrated an independent association between leptin and C-reactive protein in healthy subjects. This study, however, did not include variables such as blood glucose, blood pressure, or inflammatory cytokines. In a larger retrospective analysis of the West of Scotland Coronary Prevention database, Wallace et al. demonstrated in men with moderate hypercholesterolaemia without documented coronary artery disease, that leptin correlated with a number of major coronary risk factors including cholesterol, blood pressure, and C-reactive protein. Independent predictors of leptin were not reported in this study. In the present report exploring the correlates of leptin in patients with severe coronary disease, we found a significant relationship between leptin and body mass index, waist circumference, and fasting plasma glucose. The effect on plasma leptin of changing these variables in obese or diabetic patients with coronary artery disease warrants further study.

Study limitations

The present study explored leptin’s effects on vascular function in humans with coronary artery disease and the mechanisms underlying these effects in an ex vivo model. The advantages of this model are that it allows detailed pharmacological studies to be performed in large numbers of patients. The sample studied, however, are patients with severe coronary artery disease and extrapolation of these results to other groups and vascular territories should be performed with caution. It would therefore be useful to confirm our findings in subjects without coronary artery disease.

Clinical and in vivo relevance of present report

Previous studies exploring the effect of leptin on vascular function have found discordant results. The present report, in a large group of individuals, demonstrates for the first time in humans that leptin is an endothelium and NO-independent vasodilator. The effect is of potential clinical relevance to patients with coronary artery disease or those at risk of developing coronary artery disease, whereby leptin may have a favourable effect on vascular function. In contrast, our finding of a vasodepressor effect of leptin, which may be augmented by TNF-α, may be of relevance to sepsis when both leptin and TNF-α are elevated and may therefore contribute to the hypotension seen in humans with systemic sepsis. Our findings provide a starting point for the study of leptin’s vascular actions in different vascular pathologies.

The adipocyte is now recognized as being not only an important storage organ for triglyceride but also an active endocrine organ. In addition to leptin, adipocytes produce a number of potentially vasoactive substances including fatty acids, angiotensin II, and adiponectin. Moreover, leptin has been shown to stimulate sympathetic neural traffic. Hence, in obesity, these different factors may unite to influence the effect of leptin on vascular tone in vivo.

Studies of vascular function in humans are largely performed in vivo. In this situation, it is difficult to control for factors such as baseline vessel tone, neural, or humoral influences. The ex vivo system used in the present series of studies removes these influences. It should be, therefore, borne in mind that in the intact organism, multiple interactions may affect leptin’s vascular action. Despite this limitation, however, previous studies in patients, using an ex vivo system similar to ours have demonstrated data consistent and complimentary to in vivo studies.

The present series of studies demonstrate that leptin is a vasodilator in both human SV and internal mammary artery. Leptin-mediated vasodilatation is not dependent on an intact endothelium and is likely to be at least in part due to smooth muscle hyperpolarization. We have demonstrated that this effect is not blunted in obese humans consistent with the intriguing concept of tissue specificity of resistance to leptin.

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Conflict of interest: none declared.

References

Leptin’s vascular actions in humans with coronary artery disease


