Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in obese (Lep\textsuperscript{ob}) mice

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WINTERS, Bradford, Zhiping Mo, Esther Brooks-Asplund, Soonyul Kim, Artin Shoukas, Dechun Li, Daniel Nyhan, and Dan E. Berkowitz. Reduction of obesity, as induced by leptin, reverses endothelial dysfunction in obese (Lep\textsuperscript{ob}) mice. J Appl Physiol 89: 2382–2390, 2000.—Obesity is a major health care problem and is associated with significant cardiovascular morbidity. Leptin, a neuroendocrine hormone released by adipose tissue, is important in modulating obesity by signaling satiety and increasing metabolism. Moreover, leptin receptors are expressed on vascular endothelial cells (ECs) and mediate angiogenesis. We hypothesized that leptin may also play an important role in vasoregulation. We investigated vasoregulatory mechanisms in the leptin-deficient obese (ob/ob) mouse model and determined the influence of leptin replacement on endothelial-dependent vasorelaxant responses. The direct effect of leptin on EC nitric oxide (NO) production was also tested by using 4,5-diaminofluorescein-2 diacetate staining and measurement of nitrate and nitrite concentrations. Vasoconstrictor responses to phenylephrine, norepinephrine, and U-46619 were markedly enhanced in aortic rings from ob/ob mice and were modulated by NO synthase inhibition. Vasorelaxant responses to ACh were markedly attenuated in mesenteric microvessels from ob/ob mice. Leptin replacement resulted in significant weight loss and reversal of the impaired endothelial-dependent vasorelaxant responses observed in ob/ob mice. Preincubation of ECs with leptin enhanced the release of NO production. Thus leptin-deficient ob/ob mice demonstrate marked abnormalities in vasoregulation, including impaired endothelial-dependent vasodilation, which is reversed by leptin replacement. These findings may be partially explained by the direct effect of leptin on endothelial NO production. These vascular abnormalities are similar to those observed in obese, diabetic, leptin-resistant humans. The ob/ob mouse may, therefore, be an excellent new model for the study of the cardiovascular effects of obesity.

Nitric oxide; diabetes

Obesity, a major health care problem in the US, is associated with significant cardiovascular morbidity, including hypertension (28), atherosclerotic, and coronary artery disease (34), as well as the obesity hypoventilation syndrome and associated pulmonary hy-
complex mechanisms underlying vascular dysfunction in obesity and related disorders.

METHODS

Animals

Twenty obese male C57BL/6J-Lepob mice and their weight (WT) controls, between 3 and 6 mo of age (Jackson laboratory, Bar Harbor, ME), were studied. The study was approved by the Institutional Animal Care and Use Committee and complies with the American Physiological Society guidelines.

Protocols

Protocol 1. To determine the effects of the ob/ob phenotype on vascular reactivity, we studied aortic rings and mesenteric microvessels from 10 ob/ob and 10 WT control mice.

Protocol 2. To determine the effect of leptin replacement on vascular function, 100-ml-capacity osmotic minipumps (Alza, Palo Alto, CA), filled with mouse recombinant leptin (Amgen), were used for continuous (14-day) administration of leptin ($n = 6$). Pumps were filled with leptin (0.3 mg·kg$^{-1}$·day$^{-1}$) or PBS (control; $n = 6$) and implanted subcutaneously in the interscapular region by using a brief halothane anesthetic. Three silk sutures were used to close the skin, and bacitracin ointment was placed on the wound. Animals were inspected daily to confirm satisfactory wound healing and general health status. This method of leptin administration has been previously used in our laboratory and results in a mean plasma leptin concentration of 6 ng/ml (3). Animal weight and food intake were monitored regularly.

Aortic Vascular Reactivity

Mice were anesthetized using ketamine-acepromazine (100 and 10 mg/kg ip, respectively). The thoracic aorta was carefully dissected, rapidly removed, and placed in ice-cold oxygenated Krebs-Ringer bicarbonate solution [composition (in mM): 118.3 NaCl, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.6 CaCl2, 25 NaHCO3, and 11.1 glucose]. The vessel was carefully freed of loose connective tissue. The mouse aorta was cut into 1.5-mm rings and suspended between two wire stirrups (150 mm) in a myograph (Mangus) in 5 ml Krebs-Ringer (95% O2-5% CO2, pH 7.4, 37°C). One stirrup was connected to a three-dimensional micromanipulator, and the other to a force transducer (FT03, Grass Instruments). All concentration-effect curves were performed on arterial rings beginning at their optimum resting tone. This was deter-

Table 1. Vasoconstrictor response in aorta from ob/ob and WT control mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>log EC50</th>
<th>E$_{\text{max}}$ (%KCl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WT</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>U-46619</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Norepinephrine + L-NAME</td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of mice. WT, weight; L-NAME, N$\text{G}$-nitro-L-arginine methyl ether; E$_{\text{max}}$, maximal response. *$P < 0.05$, ob/ob vs. WT groups.
mice (ob/ob) and WT control mice. Vasodilator response in aortic rings from ob/ob and WT control mice.

Table 2. Vasodilator response in aortic rings from ob/ob and WT control mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>log EC50</th>
<th>Emax (% relax)</th>
<th>log EC50</th>
<th>Emax (% relax)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>ob/ob</td>
<td>WT</td>
<td>ob/ob</td>
</tr>
<tr>
<td>ACh</td>
<td>–6.8 ± 0.1</td>
<td>–6.9 ± 0.2</td>
<td>70 ± 5</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>–7.3 ± 0.1</td>
<td>–8.1 ± 0.3</td>
<td>93 ± 3</td>
<td>93 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n no. of mice. Relax, relaxation. *P < 0.05, ob/ob vs. WT groups.

Table 3. Vessels characteristics in mesenteric microvessels from ob/ob and WT control mice

<table>
<thead>
<tr>
<th></th>
<th>Internal Diameter, µm</th>
<th>Wall Thickness, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>166 ± 19</td>
<td>35 ± 3</td>
</tr>
<tr>
<td>ob/ob</td>
<td>224 ± 23*</td>
<td>36 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, ob/ob vs. WT groups.

Fig. 2. Vasodilator responses to ACh (A) and sodium nitroprusside (SNP; B) in aortic rings from ob/ob and WT mice. Vessels were preconstricted to 50–75% of contractile Emax with U-46619 (10-8 M), and cumulative dose responses to ACh or SNP were performed. ACh induced a dose-dependent relaxation in WT and ob/ob rings. The vasorelaxant Emax to ACh was attenuated in aortic rings from ob/ob mice (P < 0.05, n = 10), whereas the Emax remained unchanged. In contrast, the efficacy of the vasorelaxant response to SNP was enhanced in rings from ob/ob mice, whereas the Emax values were not significantly different.

Table 3. Vascular response in mesenteric microvessels from ob/ob and WT control mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>log EC50</th>
<th>Emax (% relax)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>ob/ob</td>
<td>WT</td>
<td>ob/ob</td>
</tr>
<tr>
<td>ACh</td>
<td>–8.0 ± 0.2</td>
<td>–6.5 ± 0.3</td>
<td>79 ± 4</td>
<td>41 ± 5*</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>–8.2 ± 0.2</td>
<td>–7.4 ± 0.1</td>
<td>88 ± 4</td>
<td>86 ± 4</td>
</tr>
<tr>
<td>Phenylephrine</td>
<td>–5.3 ± 0.1</td>
<td>–5.6 ± 1</td>
<td>120 ± 11</td>
<td>118 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n no. of mice. Relax, relaxation. *P < 0.05, ob/ob vs. WT groups.

Mouse Microvessel Preparations

To determine microvessel reactivity, small arterioles were isolated from branches of the mesenteric artery under the dissecting microscope (100- to 200-µm diameter, 1-mm length). The vessels were cannulated at both ends with glass micropipettes, secured with 12-0 nylon monofilament, and placed in a microvascular chamber (Living Systems Instruments, Burlington, VT). The arteries were maintained in a no-flow state and held at a constant transmural pressure of 40 mmHg. The chamber was superfused with control solution, maintained at 37°C with a pH of 7.4, and gassed with 95% O2-5% CO2. The chamber was then placed on the stage of an inverted microscope (×20, Nikon TMS-F). The vessel image was projected onto a video monitor, and intraluminal and wall diameter were determined continuously by a video-dimension analyzer (Living Systems Instrumentation). Cumulative dose-response curves to PE were performed. The vessels were then preconstricted to ~50% maximal contractile response (~10-6 M PE). Vasodilator response curves were then generated to the endothelial-dependent and -independent vasodilators ACh and SNP, respectively. Data were recorded using a MacLab system and the software CHART (AD Instruments, Mountain View, CA).

Cell Culture

Bovine pulmonary endothelial cells (BPAEC) were grown in MEM (Life Technology) supplemented with 10% fetal calf serum and 2.4 µg thymidine and were characterized as previously described (23). Cells were maintained in a humidified 37°C, 5% CO2 incubator and used between passage 14 and 16.

4,5-Diaminofluorescein Staining

NO detection was performed by fluorometric examination of cells loaded with 4,5-diaminofluorescein (DAF-2) diacetate, a membrane-permeable fluorescent indicator for NO. DAF-2 staining was then performed after preconstriction with U-46619 (approximately EC50).

Table 4. Vessels characteristics in mesenteric microvessels from ob/ob and WT control mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>log EC50</th>
<th>Emax (% relax)</th>
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<tr>
<td></td>
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Values are means ± SE; n no. of mice. Relax, relaxation. *P < 0.05, ob/ob vs. WT groups.
BPAEC were grown in the 24-well plates in the medium until confluent. Before each experiment, the medium was switched to serum-free MEM, and the cells were incubated for 24 h. Leptin \(10^{-7} - 10^{-6}\) M was then added and incubated for 16 h. After being washed with Krebs-Ringer phosphate (KRP) buffer (in mM: 120 NaCl, 4.8 KCl, 0.54 CaCl\(_2\), 1.2 MgSO\(_4\), 11 glucose, and 15.9 sodium phosphate; pH 7.2), the cells were incubated for 1 h at 37°C in KRP containing 10 \(\mu\)M DAF-2 diacetate (CalBiochem), and then the buffer was changed into KRP containing l-arginine (1 mM) for 15 min. The cells were observed under an inverted fluorescent microscope equipped with 495-nm excitation, 515-nm emission filters.

**Measurement of Nitrite and Nitrate Concentration**

BPAEC were stimulated with leptin \(10^{-7} - 10^{-6}\) M for 3 h. The nitrate and nitrite concentration in the medium was measured by using a nitrite-nitrate colorimetric method kit (Roche), according to the manufacturer’s instructions.

**Data Analysis**

Aortic vasoconstrictor responses are expressed as a percentage of KCl maximum response. Vasodilator responses are expressed as a percentage of preconstricted tension. All microvessel vasoconstrictor responses are expressed as a percentage of intraluminal diameter. Vasodilator responses are expressed as a percentage of the preconstricted diameter. EC\(_{50}\) and maximal response (E\(_{\text{max}}\)) were calculated by using nonlinear logistic regression analysis with the software PRIZM (Graphpad). Statistical differences were determined by ANOVA or unpaired Student’s t-test, as determined by the experimental design.

**RESULTS**

The body weight of the ob/ob mice was significantly greater than that of the WT littermate controls \((57 \pm 1\) vs. \(34 \pm 1\) g; \(n = 20, P < 0.0001\)).

**Vascular Responses in Mouse Aorta**

**Vasoconstrictor responses.** Vasoconstrictor responses to the agonists NE, PE, and U-46619 were markedly enhanced in ob/ob mice as manifest by a leftward shift in the dose-response curves and an increase in E\(_{\text{max}}\) (Fig. 1, Table 1). To determine whether impaired NO production may contribute to the enhanced vasoconstrictor responses, NE dose responses were performed in the presence of l-NAME \((10^{-5}\) M). L-NAME significantly enhanced the vasoconstrictor responses to NE in both WT and ob/ob mice. In the presence of l-NAME \((10^{-5}\) M) (Fig. 1, Table 1), the E\(_{\text{max}}\) to NE was identical in WT and ob/ob mice. However, the EC\(_{50}\) remained shifted to the left in the ob/ob mice compared with WT. In fact the EC\(_{50}\) to NE in ob/ob mice was identical to that observed with NE in the presence of l-NAME in WT mice.

**Vasodilator responses.** To determine the effects of leptin deficiency on endothelial-dependent vasorelaxation, mouse aortas were preconstricted with U-46619 \((\sim 10^{-8}\) M), and dose-response curves were constructed to the endothelial-dependent vasodilator ACh and the endothelial-independent NO donor SNP. ACh resulted in significant dose-dependent relaxation in mouse
The vasorelaxant responses in the aortic rings from ob/ob mice were significantly attenuated compared with those from WT controls. The $E_{\text{max}}$ was $70 \pm 5$ vs. $91 \pm 2\%$ ($n = 8$, $P < 0.05$; Fig. 2, Table 2). Surprisingly, vasorelaxant responses to SNP were enhanced in aortic rings from ob/ob mice compared with those from WT controls ($\log EC_{50}$: $-8.1 \pm 0.3$ vs. $-7.3 \pm 0.1$; $n = 6$, $P < 0.05$; Fig. 2, Table 2).

**Microvascular Responses**

**Vessel characteristics.** Arterial segments from identical divisions of the mesenteric tree were obtained for study. The vessels obtained from the ob/ob animals were of greater diameter than those from the WT controls (Table 3). Despite the differences in vessel diameter, there were no differences in the vessel wall thickness between groups.

**Contractile responses.** PE elicited a similar response in microvessels from both control and ob/ob animals (Table 4).

**ACh and SNP-induced relaxation.** ACh induced concentration-dependent relaxation of the PE-preconstricted WT mouse mesenteric microvessel. In contrast to aorta, both the $EC_{50}$ and $E_{\text{max}}$ to ACh were markedly decreased in mesenteric microvessels from ob/ob mice (Fig. 3, Table 4). SNP induced a maximal relaxant response in ob/ob microvessels that was similar to that observed in WT controls. However, there was a significant rightward shift in the dose-response curve (Fig. 4, Table 4).

**Vascular Reactivity After Leptin Replacement**

Continuous leptin infusion resulted in marked weight loss in ob/ob mice ($17.9 \pm 1.3$ g, $\sim 35\%$ of body mass), whereas control (PBS) infused mice continued to gain weight ($3.4 \pm 1.1$ g). In leptin-replacement mice, the vasorelaxant response to ACh in mesenteric microvessels demonstrated significant attenuation of the impaired endothelial-dependent vasodilatory responses observed in PBS controls ($E_{\text{max}}$: $68 \pm 8$ vs. $20 \pm 4\%$; $P < 0.01$, $n = 5$; Fig. 5).

**DAF-2 Staining and Nitrite and Nitrate Release**

Incubation of BPEC with leptin ($10^{-7}$ M) resulted in significantly enhanced fluorescence with the addition of L-arginine substrate that was not observed in control cells (Fig. 6). Nitrite and nitrate measurement in the media of cells preincubated with leptin ($10^{-7}$ and $10^{-6}$ M) demonstrated significant release compared with controls (Fig. 7). The release was $\sim 50\%$ of that observed with the Ca$^{2+}$ ionophore A-23187.

**DISCUSSION**

The recent observation that leptin receptors are expressed on endothelial cells (and are important mediators of angiogenesis) led us to hypothesize that leptin could be important in modulating vasoregulatory function. We examined the vasoreactivity of vessels from control and leptin-deficient mice, as well as the direct...
effect of leptin on endothelial cell function, to address this hypothesis. We have demonstrated 1) enhanced vasoconstrictor responses to agonists in ob/ob mice, 2) impaired vasorelaxant responses to ACh in ob/ob mice, 3) reversal of the observed vasorelaxant responses after leptin administration, and 4) enhanced NO release from endothelial cells exposed to leptin. Our data support the concept that leptin is important in modulating vasodilator function, both directly through enhancing the release of NO from endothelial cells or indirectly by altering the hormonal milieu through reduction of obesity.

Leptin plays a central role in the regulation of energy homeostasis. There is now increasing evidence that leptin may have multiple effects potentially relevant to the cardiovascular system (28, 31), including sympathetic activation (19) and sodium excretion, as well as an increase in insulin sensitivity. Thus leptin may mediate its cardiovascular effects indirectly through the autonomic, endocrine, or renal systems. In addition, leptin may modulate cardiovascular function through receptors expressed directly on cardiovascular tissues. Our data represent the first evidence of impaired endothelial function and enhanced vasoconstrictor responses in a leptin-deficient obese mouse model. More importantly, this vascular dysfunction can be reversed with leptin replacement. These results in this specific model illustrate the importance of leptin in regulating vascular function.

The in vivo physiological effects of leptin on the cardiovascular system have been studied. For example, leptin infusions produce an increase in sympathetic nerve activity to the kidney, adrenals, and hindlimb (18, 20). This effect is not observed in obese Zucker rats with a mutation in the leptin receptor gene. This suggests that this is a direct receptor-mediated phenomenon. Despite the effects of leptin on renal sympathetic nervous system activity, acute infusions of leptin have little net effect on blood pressure, thus implying that leptin has additional counterregulatory cardiovascular effects. For example, there is preliminary evidence to suggest that leptin may induce NO-dependent vasorelaxation. Moreover, mean arterial pressure is reduced in ob/ob mice compared with their nonobese, lean littermate controls (28). This is also true in other rodent models of leptin resistance and obesity (9).

It is now established that leptin receptors are present on endothelial cells, which, when stimulated, activate specific signal transduction pathways. The OB-Rb isoform, the predominant isoform expressed on endothelial cells, stimulates tyrosine autophosphorylation (38). In addition, leptin stimulation of endothelial cells induces tyrosine phosphorylation of the transcription factor STAT-3 (signal transducer and activator of transcription-3) (38). Moreover leptin also activates the mitogen-activating protein kinase pathway (1). The role of these signaling pathways in mediating the angiogenic effects of leptin has been demonstrated. Our results demonstrating morphological differences in the microvessels from ob/ob mice could be due to these effects of leptin. In contrast, the modulating effects of
leptin on pathways involved in vasoregulation have not been investigated. It is well established that cytokines acting through cytokine receptors and the janus kinase/STAT signaling pathways induce the expression of NOS (inducible NOS, NOS II) (25, 30). Moreover, leptin receptors on endothelial cells have significant homology with cytokine receptors and are considered members of the cytokine receptor superfamily. It is unknown what the effects of leptin-receptor signaling are on the expression of vasoregulatory peptides (e.g., endothelin) or enzyme systems (e.g., NOS III) that modulate vascular function. The demonstration that leptin induces the release of NO in endothelial cells in culture suggests that leptin may be coupled to NOS through an as-yet-undefined signaling pathway. Evidence for the direct effect of leptin on NO release is further strengthened by the work of Fruhbeck (11). He demonstrated that leptin induced hypotension in rats with autonomic blockade that was reversed with L-NAME. Leptin infusion was also associated with an increase in serum nitrite and nitrate concentrations. Thus leptin may modulate endothelial NO production directly.

Our results demonstrate vasoregulatory dysfunction characterized by enhanced responses to vasoconstrictors and impaired responses to endothelial-dependent vasodilators. There is a qualitative difference in the impaired vasorelaxant responses to ACh in aorta vs. mesenteric microvessels in ob/ob mice. This is characterized by an altered $E_{\text{max}}$ but unchanged EC$_{50}$ in aortic rings. In contrast, both $E_{\text{max}}$ and EC$_{50}$ were profoundly altered in mesenteric microvessels. This could be explained on the basis of the differential regulation of endothelial-derived vasoactive compounds in the microvasculature. Whereas NO is the primary endothelial-derived vasodilator in the aorta (21), other endothelial-derived factors, such as endothelium-derived hyperpolarizing factor, have been shown to be involved in the regulation of small artery tone (2) and are likely to contribute to the vasorelaxant properties of ACh (12). It is, therefore, possible that leptin deficiency may have a greater effect on the modulation of endothelium-derived hyperpolarizing factor and its associated signaling pathway in this model. The enhanced vasorelaxant responses to the direct NO donor SNP in aortic rings contrasted with the attenuated response in the mesenteric microvessels. This could be due to differential endogenous NO production in these vessel beds. Enhanced vasorelaxation to NO has been demonstrated in models in which endogenous NO production is impaired. For example, vasorelaxant response of carotid artery to SNP is augmented in endothelial NOS-deficient mice (10). An absence of NO or relative deficiency of NO could result in compensatory upregulation of downstream pathways and thus explain the enhanced response observed.

Aortic rings from wild-type mice did not demonstrate robust responses to NE or PE. This is likely due to endogenous NO release. This potential explanation is further substantiated by the robust response observed in the presence of L-NAME. Indeed, the profound response to PE and NE in rings from ob/ob mice in the absence of L-NAME suggests impaired endogenous NO production. The response of aortic rings to ACh in ob/ob mice, while significantly impaired, was not as profoundly altered as one may have predicted based on the differential contractile responses to PE. One might have predicted profound impairment of the ACh responses if there had been a significant decrement in endothelial cell NO release. This could have at least two explanations: 1) basal and stimulated NO release from endothelial cells involves different mechanisms of activation and release, and 2) NO has been demon-
stratified to modulate (inhibit) the sensitivity of the contractile apparatus (myosin light chain phosphorylation) to prevailing intracellular Ca$^{2+}$. This is thought to be mediated by cGMP-dependent kinase regulation of myosin phosphatase (41). Thus, in our ob/ob aortic rings, the contractile apparatus might have been sensitized as a result of impaired NO release, and the response to ACh may have been facilitated.

Leptin is well known to have several important endocrine effects. These effects include increased insulin sensitivity in muscle and adipose tissue (15, 33, 39, 43), as well as complex direct and indirect interactive effects on lipid metabolism. The observed vascular effects need to be interpreted within the context of these changes. Glucose and lipid homeostasis as well as insulin have complex, interdependent effects on the vasculature. Our understanding of these phenomena has been gleaned from studies of diabetic humans as well as studies in animal models. The effects of leptin or altering these biomolecules have been thoroughly studied by Harris et al. (17). They demonstrated that 10 mg/day of leptin for 7 days could normalize insulin and glucose levels in ob/ob mice. The same doses had only transient effects on appetite and weight in lean control mice, suggesting enhanced leptin sensitivity in ob/ob mice. Thus the improvement in endothelial function with leptin replacement could be partially explained on the basis of changes in insulin sensitivity and glucose concentrations.

The deleterious effects of hyperglycemia and insulin resistance on the vasculature may be separate but additive. A number of proposed mechanisms explain the effect of hyperglycemia on endothelial function (for review, see Ref. 14). There is mounting evidence that the generation of reactive oxygen species (ROS) may play an important role in the etiology of diabetic vascular complications. This hypothesis is supported by evidence that pathways strictly associated with hyperglycemia can increase ROS production. Furthermore, exposure of endothelial cells to high glucose leads to augmented production of ROS, which may then quench NO and decrease its bioavailability. These effects may be reversed by free-radical scavengers. The effect of ROS in the ob/ob mouse is yet to be explored, but this mechanism may be involved in this model of vascular dysfunction.

Mechanisms to explain the association between insulin resistance and cardiovascular disease have been attributed to insulin’s effects on vascular smooth muscle and endothelial function. Insulin enhances the release of NO (45) and increases the expression of endothelial NOS (NOS III) in the vasculature. Physiologically, insulin stimulates vasodilation by enhancing NO release (36, 40). Recently, Jiang et al. (22) have characterized selective resistance to insulin signaling in the vasculature of obese Zucker (fa/fa) rats, which have leptin receptors that are mutated. They demonstrated a selective resistance to phosphatidylinositol 3-kinase (PI3-kinase) activation. Biologically, the activation of PI3-kinase has been linked to NO production. Thus, in our model, endothelial dysfunction may be the result of impaired PI3-kinase activation with resultant impaired NO signaling.

Obesity and insulin resistance are associated with abnormalities in lipid metabolism and hypercholesterolemia (8). The mechanisms underlying the vascular dysfunction in the setting of elevated low-density lipoproteins have been extensively investigated. These include, but are not limited to, abnormalities in the NO signaling pathway, receptor-G protein coupling, and NOS and NO bioactivity, with evidence for enhanced superoxide formation and the consequent production of the less potent dilator peroxynitrite. The effects of lipids on endothelium-dependent vasodilation can be reversed not only by reducing the elevated lipids levels, but also by provision of the NOS substrate L-arginine and by the provision of antioxidants, although the mechanism for these effects are not fully elucidated. Thus perturbations in lipid metabolism may contribute to the vascular effects observed with leptin deficiency.

Thus leptin may modulate vascular function, both directly through its interaction with endothelial leptin receptor and indirectly by its actions on sympathetic nerve activity, insulin sensitivity, and perhaps other neurohumoral metabolic pathways. We have demonstrated significant abnormalities in vascular endothelial and contractile function in vessels from the ob/ob mouse that are reversed by leptin replacement. In addition, we have demonstrated the direct effect of leptin on NO modulation in endothelial cells in culture. These pathophysiological changes may be the result of the absence of leptin or the neurohumoral milieu that is associated with leptin deficiency. The direct effects of leptin on vascular function remain to be explored in vitro. The ob/ob mouse, however, remains an excellent model for understanding the interaction among obesity, diabetes, and vascular dysfunction, because the phenotype can be readily reversed.

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REFERENCES