Reduced constrictor reactivity balances impaired vasodilation in the mesenteric circulation of the obese Zucker rat

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Romanko, Olga P., and David W. Stepp. Reduced constrictor reactivity balances impaired vasodilation in the mesenteric circulation of the obese Zucker rat. *Am J Physiol Heart Circ Physiol* 289: H2097–H2102, 2005. First published June 10, 2005; doi:10.1152/ajpheart.00213.2005.—Obesity causes whole body insulin resistance and impaired vasodilation to nitric oxide (NO). Because NO is a major contributor to the regulation of mesenteric blood flow, the mesenteric circulation of obese animals is faced with reduced capacity to increase flow and increased demand for flow associated with elevated consumption of food. This study hypothesized that insulin resistance impairs NO-mediated dilation but that constrictor reactivity would be reduced to compensate in obese animals. We further hypothesized that elevated superoxide levels caused impaired responses to NO in insulin resistance. Vasodilator reactivity and vasoconstrictor reactivity of mesenteric resistance arteries from lean (LZR) and obese (OZR) Zucker rats were examined in vitro using videomicroscopy. Insulin resistance independent of obesity was induced via fructose feeding in LZR (FF-LZR), endothelium-dependent NO-mediated dilation was reduced in OZR and FF-LZR compared with LZR. Impairments in NO-mediated dilation were reversed with 1 mM tempol, a SOD mimetic. Constrictor reactivity to norepinephrine was reduced in OZR but not in FF-LZR relative to LZR. Basal mesenteric vascular resistance was similar in LZR and OZR despite impaired NO-dependent dilation in OZR. Mesenteric vascular resistance was increased in FF-LZR relative to LZR. These data indicate that there is reduced constrictor reactivity in OZR that may offset the impaired NO-mediated dilation and preserve mesenteric blood flow in hyperphagic, obese animals.

OBESITY IS AN EMERGING EPIDEMIC in Western cultures, especially in the United States, where an estimated 180 million people are overweight. The causes of obesity vary but include metabolic impairment, a high-fat diet, and overeating (hyperphagia). Functional hyperemia of the gut is required for food absorption and thus weight gain, but the effects of obesity and chronic hyperphagia on mesenteric perfusion are incompletely understood.

Obesity also induces whole body insulin resistance, resulting in impaired control of plasma glucose, hyperinsulinemia, and chronic triglyceride dyslipidemia. Insulin resistance is considered an emerging risk factor for vascular disease and has been documented to impair nitric oxide (NO)-dependent mesenteric vasodilation in animal models of insulin resistance (20, 22–24). Given that NO is a major contributor to absorptive functional hyperemia (1, 9, 12, 17) and flow-mediated regulation (2, 12, 19), it raises the question of how the mesenteric circulation compensates for the impaired dilator response caused by insulin resistance against the elevated metabolic demands associated with increased ingestion of food in obese or hyperphagic individuals. Potential mechanisms include reduced responses to vasoconstriction, augmented responses to alternative vasodilator pathways, and increases in the vascularity of the gut.

Perfusion of the gut is a balance between the constrictor tone that maintains tonic resistance and the metabolic hyperemia that accounts for elevated flow during absorption. In the current study, we tested the hypothesis that NO-mediated dilation is impaired in an animal model of obesity and constrictor reactivity is altered to compensate for this impaired NO-mediated dilation. We used the obese Zucker rat (OZR), a well-established model of obesity and insulin resistance, as our model (26). Relative to lean controls (LZR), the OZR display intestinal organomagely, elevated total mesenteric blood flow, and similar blood flow normalized to tissue mass (14). In vitro vasodilation to the NO-dependent agonist acetylcholine was examined in mesenteric resistance arteries (<175 μm inner diameter) to determine the effects of obesity on NO-mediated dilation. Because superoxide has been implicated in vascular dysfunction in other beds, the effects of the SOD mimetic tempol was used to examine a potential role for superoxide. Because metabolic dilation is balanced against extrinsic sympathetic tone, reactivity to the primary sympathetic neurotransmitter norepinephrine was examined. Specificity of constrictor reactivity changes was assessed by determining reactivity to endothelin and potassium chloride. To determine the potential contribution of insulin resistance independent of obesity, vasodilator reactivity and constrictor reactivity were examined in fructose-fed lean Zucker rats (FF-LZR), a model of insulin resistance without obesity.

MATERIALS AND METHODS

Animals. All experiments used 15- to 18-wk-old male LZR and OZR (Harlan), which were fed standard rat chow and tap water ad libitum. Rats were housed in the American Association for Accreditation of Laboratory Animal Care-approved animal care facility of the Medical College of Georgia, and the Institutional Animal Care and Use Committee approved all protocols.

A moderate insulin resistance independent of obesity was induced by feeding a diet in which 66% of the calories were derived from fructose. The fructose diet was purchased from Harlan Teklad (89247 fructose diet formula TD pellets), and the diet regimen was derived from that described by Miller et al. (10). LZR were used in the fructose-feeding protocol to avoid variance in genetic background between groups. Fructose feeding was begun ad libitum at 8–9 wk of age and continued for 8 wk.

In vivo hemodynamics. Hemodynamic parameters were assessed in anesthetized rats under isoflurane anesthesia as described previously (14). Arterial pressure was measured by the advancement of a fluid-
filled catheter into the thoracic aorta, and the heart rate was derived from the pressure pulse with the use of a cardiotachometer (CSI). The mesenteric blood flow was measured with a Transonic 1R flow probe around the superior mesenteric artery. The total perfused territory was determined by the infusion of crystal violet into the mesenteric circulation, and the dyed mass was dissected, cleaned of intestinal contents, and weighed. The blood flows were normalized to mesenteric tissue mass, and the vascular resistance was calculated as pressure divided by flow (in mmHg·ml⁻¹·min⁻¹·g⁻¹). To assess whether the increased blood flow and mesenteric mass were simply due to fat accumulation, the omental mass and vascular mass were dissected free of the intestine and the change in the intestinal mass was quantified. As shown in Table 1, the intestinal mass increased in parallel to the mesenteric mass in the obese rats.

Preparation of isolated vessels. The mesenteric resistance arteries (<175 μm in passive diameter) were surgically dissected and placed in a heated chamber (37°C) that allowed the lumen and exterior of the vessel to be perfused and superfused, respectively, with physiological salt solution (PSS) from separate reservoirs. The PSS used in these experiments was equilibrated with 21% O₂-5% CO₂-74% N₂ and had the following composition (in mM): 119 NaCl, 4.7 KCl, 1.17 MgSO₄, 1.6 CaCl₂, 1.18 NaH₂PO₄, 24 NaHCO₃, 0.026 EDTA, and 5.5 glucose. The vessels were cannulated at both ends with glass micropipettes (~100 μm tip diameter) and secured to the inflow and outflow pipettes with the use of a 10-0 nylon suture. Any side branches were ligated with a single strand teased from 6-0 silk sutures. The inflow and outflow pipettes were connected to a reservoir system that allowed the intraluminal pressure to be controlled. Arteries were extended to their in situ length and equilibrated at 60 mmHg. After initial pressurization, the inflow and outflow pressures were equal and the vessel experienced no flow. The vessel diameter was measured with television microscopy and an on-screen video micrometer. Serial dose-response curves to vasoactive agents were generated over the following dose ranges: acetylcholine (1 × 10⁻⁸ to 3 × 10⁻⁶), norepinephrine (1 × 10⁻⁸ to 3 × 10⁻⁵), endothelin (1 × 10⁻¹¹ to 1 × 10⁻⁸), and KCl (0–20 mM). Vasodilation was expressed as the percentage of the passive diameter in a Ca²⁺-free PSS. Because myogenic tone in extraparenchymal resistance arteries tends to be limited (~10% in these studies), the tone was supplemented with norepinephrine (~1 × 10⁻⁷ to 3 × 10⁻⁷) until 50% constricted in each group. No differences in myogenic activity were observed among groups. Vasoconstriction was expressed as the percentage of baseline diameter. One vessel for each dilator or constrictor curve was selected from the literature values (7, 16, 20) and indicate that fructose-fed lean Zucker rats tended to be

Table 1. Baseline hemodynamic and metabolic characteristics of lean, obese, and fructose-fed lean Zucker rats

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Lean</th>
<th>Obese</th>
<th>Fructose-Fed Lean</th>
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<tr>
<td>Age, wk</td>
<td>15–18</td>
<td>15–18</td>
<td>15–18</td>
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<tr>
<td>Body weight, g</td>
<td>346±16</td>
<td>583±26*</td>
<td>366±9*</td>
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<td>Food intake, g/day</td>
<td>24±1</td>
<td>31±1*</td>
<td>22±1*</td>
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<td><strong>Hemodynamics</strong></td>
<td></td>
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<tr>
<td>MAP, mmHg</td>
<td>93±4</td>
<td>110±3*</td>
<td>102±3*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>318±16</td>
<td>338±11</td>
<td>324±5</td>
</tr>
<tr>
<td>Total MBF, ml/min</td>
<td>18±1</td>
<td>27±1*</td>
<td>15±1*†</td>
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<tr>
<td>Mesenteric mass, g</td>
<td>14±1</td>
<td>26±1*</td>
<td>15±1*</td>
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<td>Intestinal mass, g</td>
<td>9±2</td>
<td>15±3*</td>
<td>10±2†</td>
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<tr>
<td>Normalized MBF, ml⁻¹·g⁻¹</td>
<td>1.1±0.06</td>
<td>1.0±0.08</td>
<td>0.9±0.06*</td>
</tr>
<tr>
<td>MVR, mmHg·ml⁻¹·min⁻¹·g⁻¹</td>
<td>88±10</td>
<td>99±10</td>
<td>112±7*</td>
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<tr>
<td><strong>Metabolic</strong></td>
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<tr>
<td>Fasting blood glucose, mg/dl</td>
<td>110±12</td>
<td>120±18</td>
<td>138±25</td>
</tr>
<tr>
<td>Plasma cholesterol, mg/dl</td>
<td>51±5</td>
<td>95±5*</td>
<td>65±10†</td>
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<td>Plasma triglyceride, mg/dl</td>
<td>54±4</td>
<td>443±80*</td>
<td>185±21†</td>
</tr>
<tr>
<td>Plasma insulin, μU/ml</td>
<td>1.21±0.6</td>
<td>9.85±1.1*</td>
<td>2.48±0.9*†</td>
</tr>
<tr>
<td>Plasma lipid peroxides, μM</td>
<td>8±1</td>
<td>18±1*</td>
<td>13±1*</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; MBF, mesenteric blood flow; MVR, mesenteric vascular resistance. *P < 0.05 vs. lean; †P < 0.05 vs. obese.

RESULTS

Baseline parameters. Baseline hemodynamics and metabolic data from LZR, OZR, and FF-LZR are shown in Table 1. OZR were heavier and consumed more food and water than did either LZR or FF-LZR. A modest (~10 mmHg) increase in arterial pressure was evident under isoflurane anesthesia in both insulin-resistant rat groups. Heart rates were similar among all groups. Mesenteric blood flow was significantly elevated in OZR compared with LZR. OZR displayed splanchnomegaly, explaining the increased blood flow because blood flow was similar in LZR and OZR when normalized to tissue mass. Normalized mesenteric blood flow was reduced in FF-LZR. Mesenteric vascular resistance was also significantly higher in FF-LZR compared with LZR. Resistance was similar in LZR and OZR.

In the rats that were fasted overnight, blood glucose levels were similar in LZR, OZR, and FF-LZR, indicating no fasting hyperglycemia in any of the strains. Plasma insulin levels were markedly elevated in OZR and more moderately increased in FF-LZR compared with LZR. These data are consistent with the literature values (7, 16, 20) and indicate that fructose feeding induced a state of moderate insulin resistance, whereas obesity induces a more severe form. Plasma cholesterol and triglycerides were significantly elevated in both OZR and FF-LZR, consistent with insulin resistance. Lipid changes in OZR rats were more pronounced than those in FF-LZR, consistent with the greater severity of insulin resistance in OZR.
Plasma lipid peroxides were assessed as an index of whole body oxidant stress. Lipid peroxides reflect the combined concentration of malondialdehydes and hydroxynonenals. Lipid peroxide levels were elevated in fructose-fed rats relative to controls and further elevated in OZR. These data indicate that oxidant stress is a component of vascular disease in both moderate and severe states of insulin resistance.

Baseline characteristics of mesenteric resistance arteries from LZR, OZR, and FF-LZR are shown in Table 2. Passive diameter at 60 mmHg was similar in all groups. The WT and wall-to-lumen ratio were unaltered by obesity or insulin resistance, indicating a lack of vascular hypertrophy in this segment of the mesenteric circulation. The β-coefficient of the stress-strain curve was also similar among all groups, ruling out alteration in vascular distensibility or compliance as a contribution to altered vascular function.

Endothelium-dependent vasodilation. Acetylcholine was used to elicit endothelium-dependent vasodilation. In mesenteric resistance arteries <175 μm in diameter, responses to acetylcholine were abolished by treatment with 500 μM L-NAME in LZR (92 ± 2 vs. 4 ± 3% relaxation, control vs. L-NAME at 3 × 10−6 M acetylcholine, n = 5 experiments, P < 0.05) or OZR (61 ± 8 vs. 2 ± 3% relaxation, control vs. L-NAME, n = 5 experiments, P < 0.05). Thus we conclude that in this segment of the mesenteric circulation, responses to acetylcholine are almost exclusively mediated by NO. Treatment with L-NAME had no baseline effects on preconstricted mesenteric diameter, suggesting minimal NO production under conditions in which flow is absent.

Dose-response curves to acetylcholine in control and insulin-resistant rats are shown in Fig. 1. In both OZR and FF-LZR rats, vasodilation induced by acetylcholine was significantly reduced. No statistical difference in reactivity was resolved between OZR and FF-LZR, suggesting that both moderate and severe insulin resistance impairs NO-mediated vasodilation to a similar degree.

The role of superoxide in impaired endothelium-dependent vasodilation was tested by examining acetylcholine-induced vasodilation in the presence and absence of the SOD mimetic Tempol (1 mM). Results are shown in Fig. 2 for LZR (Fig. 2A), OZR (Fig. 2B), and FF-LZR (Fig. 2C). Tempol had no baseline effects on the vascular diameter in any of the three groups. In LZR, no significant effect of tempol on reactivity to acetylcholine was observed. However, in mesenteric resistance arteries from either OZR or FF-LZR, Tempol significantly improved endothelium-dependent dilation to the extent that maximum dilation in all three Tempol-treated groups was similar (70 ± 4% in LZR, 68 ± 9% in OZR, and 85 ± 5% in FF-LZR, at 3 × 10−6 M acetylcholine; P = not significant). These data suggest that superoxide production is a major detriment to NO-mediated vasodilation in insulin-resistant states.

Vasoconstrictor reactivity. No differences in baseline myogenic activity to pressurization at 60 mmHg were observed among groups. Reactivity to norepinephrine in LZR, OZR, and FF-LZR is shown in Fig. 3. Norepinephrine elicited dose-dependent decreases in vascular diameter. The decreases in diameter were significantly reduced in mesenteric resistance arteries from OZR. The constriction to norepinephrine in FF-LZR was not different from that in control rats, indicating that obesity but not moderate insulin resistance is required to reduce reactivity to norepinephrine.

To address specificity and potential mechanisms of the observed reduction in constrictor reactivity in OZR, reactivity to endothelin and potassium chloride was also compared between LZR and OZR rats. Data from these experiments are shown in Fig. 4. Reactivity to depolarization with KCl (Fig. 4A) was similar in mesenteric resistance arteries from LZR and OZR, suggesting that the general constrictor reactivity to changes in calcium is unaffected by obesity. In contrast, reactivity to endothelin (Fig. 4B) was reduced in mesenteric resistance arteries from OZR relative to LZR.

DISCUSSION

This study examined the effects of obesity on mesenteric microvascular function in LZR, insulin-resistant OZR, and insulin-resistant nonobese Zucker rats. The key findings of this study are that 1) impairment of NO-dependent mesenteric vasodilation in obese rats can be associated with moderate insulin resistance, 2) the mechanism of impaired NO-mediated vasodilation in insulin-resistant states involves oxygen-free radicals, and 3) there is a reduction in reactivity to vasoconstrictors in obesity but not insulin resistance.

NO is a key mediator of tissue perfusion in the mesenteric circulation, in both the response to flow (2) and the absorption of nutrients (1, 17, 25). The effect of vascular risk factors on NO-mediated dilation has been the subject of intensive study, and recent evidence has documented that obesity also impairs NO-dependent dilation in both clinical (6, 21) and experimental animal populations (3, 5, 24). The aspects of obesity that

Table 2. Baseline characteristics of mesenteric resistance arteries from lean, obese, and fructose-fed lean Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>Fructose-Fed Lean</th>
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<tbody>
<tr>
<td>Inner diameter, μm</td>
<td>173±11</td>
<td>171±13</td>
<td>170±12</td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>11±1</td>
<td>12±2</td>
<td>11±2</td>
</tr>
<tr>
<td>Wall-to-lumen ratio</td>
<td>0.13±0.1</td>
<td>0.15±0.1</td>
<td>0.14±0.1</td>
</tr>
<tr>
<td>β-Coefficient of stress-strain relationship</td>
<td>4.1±0.5</td>
<td>3.8±0.4</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are means ± SE. ND, not determined.
contribute to impaired NO-mediated vasodilation remain incompletely understood, but the associated insulin resistance common in obesity has been implicated as causal. Indeed, even moderate insulin resistance induced by fructose feeding is sufficient to induce impaired NO-dependent dilation in the aorta (15), the coronary circulation (10), and the cerebral microcirculation (4). Our results (Fig. 1) are in good agreement with these previous results in that we also find impairment of NO-dependent vasodilation in the mesenteric microcirculation in both OZR and FF-LZR. Furthermore, with moderate insulin resistance induced by fructose feeding, similar impairment in NO-dependent dilation occurs in FF-LZR as observed in more profoundly insulin-resistant OZR. We interpret this result to indicate that moderate reductions in insulin sensitivity may be sufficient to induce the reduced endothelium-dependent relaxation observed in obese animals.

The mechanisms of impaired NO-dependent responses in obese and insulin-resistant animals remain controversial. An emerging concept is that elevated oxidant stress is a major mechanism impeding normal endothelium-dependent dilation. Laight et al. (8) found that exacerbation of oxidant stress impaired depressor responses to NO-mediated vasodilator in OZR, and previous studies from our laboratory have correlated...
superoxide production with impaired microvascular dilation in the skeletal muscle of OZR (5). Shinozaki et al. (15) reported that superoxide limited NO-dependent vasodilation in the aorta of fructose-fed rats, and Onuma et al. (11) have recently reported that scavenging of superoxide with tempol improves renal medullary blood flow in fructose-fed rats in vivo. Our results (Fig. 2) provide new evidence from isolated microvessels that vascular superoxide production limits NO-mediated vasodilation in insulin-resistant animals. In the mesenteric microcirculation, treatment with the SOD mimetic tempol improves NO-mediated dilation to acetylcholine in both OZR and FF-LZR. Furthermore, increases in lipid peroxidation (Table 1) were observed in both groups of insulin-resistant rats. These findings provide new support for the hypothesis that insulin resistance provokes a prooxidant state that impairs NO-mediated dilation. The extension of this observation into the mesenteric circulation, when combined with previous results, argues the pervasive nature of this defect. This ubiquity suggests that chronic exposure to altered plasma composition may be causal because plasma is the one component of the body that touches all vessels. The identity of the causal factor in the effect of insulin resistance on oxidant tone remains to be elucidated.

OZR face a conundrum in which mesenteric NO-mediated vasodilation is blunted, thus compromising the regulation of mesenteric blood flow and chronic hyperphagia in which the demand for absorptive hyperemia is increased. This study hypothesized that one resolution of this dilemma may involve reducing the degree of sensitivity to vasoconstrictor stimulation to help preserve perfusion of the gut when active vasodilator tone is impaired. To test this hypothesis, constrictor reactivity was assessed in vitro to remove the potential confounding variables of parenchymal factors, innervation, or differences in plasma composition. In the current studies, we found that reactivity to norepinephrine and endothelin (Figs. 3 and 4) was reduced in mesenteric resistance arteries from OZR relative to those from LZR. In nonobese FF-LZR, where NO-mediated dilation is impaired to the same degree as observed in OZR, reactivity to norepinephrine was unaffected. When one examines basal mesenteric vascular resistance (Table 1), one finds that mesenteric resistance is comparable between LZR and OZR, despite the impairment of NO-mediated vasodilation in OZR. Thus compensation may exist to normalize perfusion when an essential vasodilator is lost. In FF-LZR, where NO-mediated vasodilation is impaired but reactivity to norepinephrine is not altered, mesenteric resistance is significantly elevated. Thus our findings suggest that the reduced reactivity to vasoconstrictors may help preserve perfusion in the face of obesity and hyperphagia when NO-mediated dilation is compromised.

The precise mechanism by which vasoconstrictor reactivity is reduced in obese animals remains to be elucidated. Nevertheless, three findings in the current studies offer insight into potential mechanisms. First, the reduction in reactivity is relatively specific for G protein-coupled receptors. Reactivity to endothelin and norepinephrine is blunted but not to depolarization with potassium chloride (Fig. 4). This finding suggests that receptor expression or receptor signaling is the mechanistic site of reduced sensitivity to constrictors, rather than alterations in reactivity to calcium. These changes in receptor function may be generalized or may reflect desensitization secondary to changes in concentrations of these ligands in the mesenteric perivascular interstitium. Second, the reduction in constrictor reactivity does not reflect a simple reaction to impaired dilation to NO. Both OZR and FF-LZR show similar levels of impaired dilation to NO, yet only OZR show reduced reactivity to vasoconstrictors. This comparison also rules out oxidant stress as a factor in reduced reactivity to norepinephrine because oxidant stress (as assessed by lipid peroxides and impairment of NO-mediated vasodilation) was elevated in fructose-fed rats without changes in reactivity to norepinephrine. Finally, the reduced reactivity is specific to obesity or severely insulin-resistant states. In FF-LZR, a model of moderate insulin resistance, constrictor reactivity to norepinephrine was not different from LZR. This suggests that other aspects of obesity, including increased sympathetic nerve activity (3, 13), hyperphagia, or more severe insulin resistance (Table 1), may be causal in reducing vascular reactivity to noradrenergic stimulation. Because reactivity to norepinephrine is actually increased in the skeletal muscle circulation (18) of OZR, we speculate that hyperphagia superimposed on impaired vasodilation is a key mechanism for reduced vasoconstriction in the mesenteric circulation.

In summary, we find that metabolic dysfunction induces impairments of NO-mediated vasodilation secondarily to oxidant stress. We find that when obesity and hyperphagia occur concurrently with impaired vasodilation, vasoconstrictor reactivity is reduced. This reduced reactivity to constriction may provide an important compensation to maintain adequate perfusion in the mesenteric circulation of obese individuals. Moreover, because diversion of mesenteric blood flow by adrenergic constriction is a hemodynamic requirement of exercise or compensation for hemorrhage, the reduced vasoconstrictor reactivity present in the mesenteric circulation may represent a source of hemodynamic dysfunction when obese animals are subjected to physiological challenge.

ACKNOWLEDGMENTS

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GRANTS

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REFERENCES


