Chronic Endothelin-1 Treatment Leads to Insulin Resistance In Vivo

Jason J. Wilkes,1 Andrea Hevener,2 and Jerrold Olefsky3

We determined whether chronic endothelin-1 (ET-1) treatment could lead to in vivo insulin resistance. Like insulin, ET-1 acutely stimulated glucose transport in isolated soleus muscle strips of WKY rats. ET-1 pretreatment (1 h) decreased insulin-stimulated glucose transport in muscle strips (~23%). Both ET-1–mediated effects were generated through ETA receptors, because a specific ET_A receptor antagonist (BQ6610) blocked these effects of ET-1. Osmotic minipumps were used to treat normal rats with ET-1 for 5 days. Subsequent hyperinsulinemic-euglycemic clamps showed that ET-1 treatment led to an ~30% decrease in insulin-stimulated glucose disposal rates in male and female rats. In addition, ex vivo study of soleus muscle strips showed decreased glucose transport into muscle from ET-1–treated animals. With respect to insulin signaling, chronic in vivo ET-1 treatment led to a 30–40% decrease in IRS-I protein content, IRS-I–associated p110α, and AKT activation. In summary, 1) in vitro ET-1 pretreatment leads to decreased insulin-stimulated glucose transport in skeletal muscle strips; 2) chronic ET-1 administration in vivo leads to whole-body insulin resistance, with decreased skeletal muscle glucose transport and impaired insulin signaling; and 3) elevated ET-1 levels may be a cause of insulin resistance in certain pathophysiologic states. Diabetes 52:1904–1909, 2003

Endothelin-1 (ET-1) is a 21–amino acid polypeptide produced by endothelial cells (1). ET-1 belongs to a family of endothelin polypeptides along with ET-2 and ET-3 (2). Although encoded by different genes, endothelin peptides are believed to be structurally and functionally similar in nature. Endothelin peptides bind to two known heptahelical transmembrane G protein–coupled receptors, namely ET-A and ET-B, and ET-1 binds most strongly to ET-A (3). ET-1 has potent vasoactive effects in vascular smooth muscle important for blood pressure regulation. However, ET-A and ET-B receptors are expressed in a variety of tissues, besides vascular tissue, suggesting that ET-1 has additional biological effects. We (4–6) and others (7,8) have previously demonstrated that ET-1 has an insulin-like effect that is mediated through ET_A in 3T3-L1 adipocytes in vitro. Our past work showed that ET-1, like insulin, can promote GLUT4 translocation and stimulate glucose transport through a signaling pathway involving G_9q/11 and phosphatidylinositol 3-kinase (P3K) (6).

In addition to acute effects of ET-1, we have previously shown that chronic ET-1 treatment of 3T3-L1 cells induces cellular insulin resistance. This heterologous desensitization of insulin action is associated with downregulation of IRS-I and G_9q/11 with loss of insulin’s ability to stimulate GLUT4 translocation (5). As with 3T3-L1 cells in vitro, it seems that ET-1 may be detrimental to insulin action pathways in physiologic tissues in vivo as well. Thus, when administered acutely in high amounts, ET-1 seems to attenuate insulin action in humans (9). Acute ET-1 treatment can also impair insulin action in conscious male Sprague-Dawley rats (10). Because elevated ET-1 levels have been reported in various insulin-resistant and diabetic states in both humans (11–14) and animals (15), it is possible that chronic increases in ET-1 concentrations can promote insulin resistance.

Skeletal muscle is the most important tissue for whole-body glucose disposal (16), and it is not known whether chronically elevated levels of ET-1 in vitro or in vivo can negatively affect insulin action in this tissue. In the current studies, we show that in vitro ET-1 pretreatment leads to decreased insulin-stimulated skeletal muscle glucose transport. Furthermore, chronic in vivo ET-1 administration leads to in vivo insulin resistance with a decrease in skeletal muscle glucose uptake and inhibition of the insulin signaling pathway.

RESEARCH DESIGN AND METHODS

Materials. Anti–IRS-1 was purchased from Upstate Biotechnology (Lake Placid, NY). Anti–phospho-specific AKT was purchased from Cell Signaling (Beverly, MA). Anti-p110α, protein A/G plus agarose, and horseradish peroxidase–linked anti-rabbit anti-mouse antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). BQ610 was purchased from Peninsula Laboratories (San Carlos, CA). Radioisotopes were purchased from ICN Radiochemicals (Costa Mesa, CA). ET-1 and general reagents were purchased from Sigma Chemical Company (St. Louis, MO).

Animal use. For in vivo experiments, animals used were normotensive WKY rats (Charles River Laboratories, Wilmington, MA) weighing 175–200 g. For in vitro experiments, smaller male rats weighing 100–110 g were used. Animals were provided with a normal rodent diet and tap water and were individually housed. The local committee on animal care at the University of California, San Diego approved the animal experiments.

Determination of 2-deoxyglucose uptake in isolated soleus muscle strips. Soleus muscles with tendons still attached were isolated in the animal’s hindquarter and removed rapidly. Isolated muscles were stripped lengthwise. Stripping soleus muscles is known to make these muscles more suitable for glucose uptake measurements (17). Strips were placed in a...
Krebs-Henseleit buffer (KHB) solution containing 32 mmol/l mannitol, 8 mmol/l D-glucose, and 0.1% BSA. Strips were incubated without additions (basal) or with 140 nmol/l ET-1 alone or with 14 nmol/l insulin (Novo Nordisk) alone or with ET-1 and insulin together, muscle strips were pretreated (for 1 h) with ET-1 or ET-1 plus 1 μmol/l BQ610 before the inclusion of insulin. Before glucose transport measurements, 14C was removed by washing strips two times for 5 min each in a glucose-free KHB with 38 mmol/l mannitol and 2 mmol/l pyruvate. For determining 2-deoxyglucose (2-DG) uptake, strips were incubated with (1.5 Ci) 2-deoxy-3-[3H]glucose (1 mmol/l) and (0.1 Ci) 14C-mannitol (37 mmol/l) for 20 min. Strips were removed rapidly, rinsed, blotted, and snap-frozen in liquid N2. Muscles were analyzed for 14C and 3H in digested muscle extract.

Surgery and hyperinsulinemic-euglycemic clamp procedure. Rats were placed under single-dose anesthesia (42 mg/kg ketamine HCl, 5 mg/kg xylazine, and 0.75 mg/kg acepromazine maleate) and cannulated with carotid artery cannulae for blood sampling and dual jugular vein cannulae for glucose and insulin infusions. Cannulae were tunneled underneath the skin, sutured to the outside, and encased in silastic tubing (0.2-cm ID) for protection. In addition, miniosmotic pumps (Alzet model #2002) containing 0.2 mg/ml ET-1 were chronically implanted in the animal’s intra-abdominal cavity for the total duration of the treatment period. ET-1 was delivered in a saline vehicle at 8.2 ng · kg−1 · min−1. Control rats received saline pumps. Immediately after surgery, rats were provided with light warmth and permitted to recover fully for 5 days. Six hours before the euglycemic-hyperinsulinemic clamp procedure was performed, food was withdrawn from cages. All rats were subjected to the same general insulin-clamp procedure as we have previously described in detail (18). A terminal dose of Nembutal (100 mg/kg I.V.) was administered after clamping to dissect red tibialis anterior (RTA) muscles from killed rats. For obtaining basal muscle, one set of rats were rested, provided with food and water, and then allowed to recover for 2 days. Recovered rats were killed by CO2 asphyxiation, and muscles were collected for ex vivo glucose transport (soleus) and Western blotting measurements (RTA).

Immunoprecipitation of IRS-1 protein. RTA muscles were homogenized in ice-cold homogenization buffer containing 150 mmol/l NaCl, 50 mmol/l Tris (pH 7.5), 30 mmol/l sodium pyrophosphate, 10 mmol/l sodium fluoride, 1 mmol/l DTT, 10% [vol/vol] glycerol, 1% Triton-X-100, plus complete protease inhibitor cocktail (1 tablet/50 ml). Muscle homogenate was centrifuged at 4°C (15,000 × g for 10 min) to remove unwanted insoluble material. Homogenates at this stage were cleared further by gentle rotation with protein A/G agarose (15,000 × g for 10 min) to remove unwanted insoluble material. Homogenates at this stage were cleared further by gentle rotation with protein A/G agarose (15,000 × g for 10 min) to remove unwanted insoluble material. Homogenates at this stage were cleared further by gentle rotation with protein A/G agarose (15,000 × g for 10 min) to remove unwanted insoluble material. Homogenates at this stage were cleared further by gentle rotation with protein A/G agarose (15,000 × g for 10 min) to remove unwanted insoluble material.

Detection of IRS-1, IRS-1–associated PDK3 (p110c), and AKT phosphorylation by Western blotting. Samples were separated by SDS–polyacrylamide electrophoresis and transferred to an Immobilon membrane by electromembrane transfer. Membranes were blocked overnight in 5% nonfat dry milk (NFDM) made in Tris-buffered saline (pH 7.6). Proteins were detected by incubating blocked membranes with primary antibody at a dilution recommended by the manufacturer in nonfat dry milk/Tris-buffered saline (pH 7.6) followed by incubating with horseradish peroxidase–linked secondary antibodies diluted (1:2,000). Visualization of Western blots was done using an enhanced chemiluminescence system. Bands were quantified using a Macintosh connected with an Arcus scanner and NIH-Image 1.6 software.

Blood chemistry analysis. Plasma glucose was assayed by the glucose oxidase method (YSI). Plasma insulin was measured via a radioimmunoassay kit (Linco Research, St. Charles, MO). Plasma free fatty acids (FFAs) were measured enzymatically using a commercially available kit (NEFA C; Wako Chemicals USA, Richmond, VA).

Calculations and statistical analysis. Hepatic glucose output (HGO) and glucose disposal rate (GDR) were calculated using Steele’s equation (19). Data obtained in vitro in glucose transport experiments were analyzed using two-way ANOVA. One- and two-tailed t tests were used as appropriate for all other assays. All data are reported as means ± SE.

RESULTS

Table 1 shows some of the general characteristics of the chronically ET-1– and saline-treated WKY rats in the basal state. Body weights of WKY rats treated with ET-1 were no different from weights of saline-treated controls. Basal plasma insulin concentrations were increased in rats that were chronically treated with ET-1 twofold compared with controls. Basal plasma FFA concentrations and HGO levels both were increased modestly in ET-1–treated rats compared with controls (P < 0.05).

To examine the effects of ET-1 on glucose uptake by skeletal muscle, we incubated isolated skeletal muscle strips in vitro with ET-1 and/or insulin under various conditions. As seen in Fig. 1, ET-1 (140 nmol/l) led to a significant increase in muscle 2-DG uptake after 30 min of incubation (P < 0.05), consistent with our earlier data showing that ET-1 stimulated GLUT4 translocation and stimulated glucose transport in 3T3-L1 adipocytes (6).

When soleus muscles were incubated with the specific ETA receptor antagonist BQ610 (20), the effects of ET-1 on glucose transport were completely blocked, showing that the glucose transport stimulatory effects of ET-1 were mediated through the ETA receptor. The effects of ET-1 alone on glucose transport were the same at 30 and 60 min (data not shown). Insulin led to the expected increase in levels both were increased modestly in ET-1–treated rats compared with controls (P < 0.05).

FIG. 1. ET-1 stimulates 2-DG uptake and induces insulin resistance in split soleus muscle strips in vitro. Muscle strips were incubated with additions (basal) or with 140 nmol/l ET-1 alone or with 14 nmol/l insulin alone at 29°C for 30 min. In experiments in which ET-1 and insulin are together, muscle strips were pretreated (for 1 h) with ET-1 or ET-1 plus BQ610 before inclusion of insulin. Glucose uptake in stimulated muscle strips was assessed with 3H-2DG thereafter, as outlined in research design and methods. Bars represent means ± SE of nine separate experiments. Muscles were harvested from three animals for BQ610 alone and BQ610 plus insulin (last two bars). *P < 0.05 vs. basal uptake; †P < 0.05 vs. uptake determined in muscle strips stimulated with insulin alone.

**TABLE 1**

<table>
<thead>
<tr>
<th>Controls</th>
<th>ET-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>172 ± 8.2</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>129.5 ± 4.5</td>
</tr>
<tr>
<td>Insulin (nmol/l)</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>FFA (nmol/l)</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>HGO (mg · kg−1 · min−1)</td>
<td>10.4 ± 1.0</td>
</tr>
</tbody>
</table>

Values reported are means ± SE. *P < 0.05.
glucose transport, but pretreatment of muscle strips with ET-1 (140 nmol/l) for 60 min inhibited the subsequent effect of insulin to stimulate glucose transport by 23%. The effect of ET-1 to induce skeletal muscle insulin resistance was prevented by coincubation of the muscle strips with the ETA receptor antagonist (BQ610). Thus, acute stimulation with ET-1 produces insulin mimetic effects to stimulate glucose transport, but more prolonged exposure to ET-1 desensitizes the system, producing an apparent state of insulin resistance.

To determine the acute and prolonged in vitro effects of ET-1 on insulin signaling molecules in isolated muscle strips, we performed Western blots with anti–IRS-1 and anti-phosphoSER473 AKT antibodies after treating strips with ET-1 alone or for 1 h followed by insulin stimulation. IRS-1 protein levels were the same with all in vitro treatments (densitometry values /[basal] 1,589 ± 331; [ET-1 for 60 min] 1,796 ± 310; [insulin] 1,308 ± 250; [ET-1 + insulin] 1,364 ± 128). As was expected, insulin stimulated AKT phosphorylation on SER473 by approximately three- to fourfold. ET-1 induced AKT phosphorylation (twofold), whereas pretreatment of strips with ET-1 (1 h) significantly attenuated insulin-stimulated AKT phosphorylation on SER473 by ~20% (P < 0.05) (densitometry values /[basal] 2,209 ± 494; [30 min ET-1] 4,307 ± 891; [60 min ET-1] 4,462 ± 654; [insulin] 6,775 ± 439; [ET-1 + insulin] 5,631 ± 641). Thus, as with ET-1 effects on muscle glucose uptake, the acute effects of ET-1 alone lead to modest AKT activation, whereas in combination with insulin, the long-term effects of ET-1 inhibit insulin actions.

To extend the results of the in vitro experiments to the in vivo situation, we performed hyperinsulinemic-euglycemic clamp studies to assess insulin-stimulated GDRs in male and female WKY rats that had been chronically treated with ET-1 (8.2 ng·kg⁻¹·min⁻¹) for 5 days using miniosmotic pumps. These results are summarized in Fig. 2, which shows that both male and female ET-1–treated animals demonstrate a 30–40% decrease in insulin-stimulated GDR compared with saline controls after chronic in vivo ET-1 administration. In addition, we assessed the ability of insulin to suppress HGO. As seen in Fig. 2C, insulin was able to lower HGO by 60% in control animals (P < 0.05), whereas HGO suppression was substantially less in animals that were chronically treated with ET-1 (25%).

To determine whether this in vivo insulin resistance was expressed in skeletal muscle, we removed soleus muscle strips from the in vivo–treated animals for measurement of ex vivo glucose transport. As seen in Fig. 3, muscles from
the ET-1–treated animals demonstrated normal basal rates of glucose transport with a marked inhibition of insulin-stimulated glucose uptake.

We next assessed the status of several molecules in the insulin-signaling cascade in skeletal muscle samples from the chronically ET-1–treated animals compared with controls. Because our earlier in vitro studies in 3T3-L1 adipocytes demonstrated that chronic ET-1 treatment leads to a decrease in IRS-1 content (5), we measured IRS-1 content by performing Western blots for IRS-1 protein in muscle lysates prepared from control and ET-1–treated animals. Consistent with the earlier in vitro data in adipocytes (5), chronic ET-1 treatment led to a significant reduction in skeletal muscle IRS-1 content, as seen in Fig. 4A.

Upon insulin stimulation, phosphorylated IRS-1 associates with PI3K. Therefore, we measured the amount of IRS-1–associated PI3K by Western blots of the p110 subunit of PI3K in IRS-1 precipitates prepared from insulin-stimulated muscle obtained at the end of the hyperinsulinemic clamp experiment. The results demonstrated a decrease in IRS-1–associated PI3K comparable to the decrease in total cellular IRS-1 (Fig. 4B).

PI3K is an upstream activator of AKT, and recent evidence indicates an important role for AKT in insulin-stimulated glucose transport (21). Consequently, we measured phospho AKT levels in muscle lysates obtained from animals in the basal state and at the end of the hyperinsulinemic-euglycemic clamp. As seen in Fig. 5, insulin leads to a marked stimulation of AKT phosphorylation in the control animals, and this effect is blunted in the muscles from the chronic ET-1–treated group.

DISCUSSION

ET-1 is a vasoactive peptide that exerts its biologic effects by binding to the heptahelical G protein–coupled ETA receptor. The ETA receptor is expressed in a variety of cell types, including adipocytes and skeletal muscle, and can utilize the heterotrimeric G protein, Gαq/11, to mediate downstream signaling events (22,23). In previous studies, we have shown that the insulin receptor can also couple into Gαq/11, and this event can mediate GLUT4 translocation and stimulation of glucose transport (6). Along these lines, we have demonstrated that ET-1 can stimulate GLUT4 translocation and glucose transport in 3T3-L1 adipocytes and that this effect is mediated through Gαq/11 activation and stimulation of PI3K (6). Thus, ET-1 exerts insulin mimetic effects through signaling pathways similar to those engaged by insulin. It is well known that chronic ligand stimulation often leads to desensitization of that ligand’s downstream signaling pathway. When two ligands use common signaling pathways, this phenomenon can lead to heterologous desensitization such that chronic stimulation with one ligand can inactivate the signaling pathway used by the second ligand. Indeed, we have recently confirmed this concept by demonstrating that chronic stimulation of 3T3-L1 adipocytes with ET-1 leads to heterologous desensitization of the insulin action pathway, producing a state of cellular insulin resistance (5).

FIG. 4. Total IRS-1 protein and IRS-1 association with PI3K are decreased in muscle after chronic ET-1 treatment in vivo. In vivo insulin-stimulated muscle was obtained at the end of the glucose clamp study and homogenized under conditions needed for the immunoprecipitation of interacting proteins. Resulting homogenates were blotted for IRS-1 or immunoprecipitated with anti–IRS-1 antibody and analyzed by blotting with anti-p110α antibody. Levels of insulin signaling proteins are indicated in graph as means ± SE of scanner values. IRS-1 protein expression (A) and IRS-1–associated p110α (B). *P < 0.05 vs. controls.

FIG. 5. Insulin-stimulated AKT activation in muscle is reduced with chronic ET-1 treatment in vivo. Insulin-stimulated muscles were dissected from ET-1–treated or control rats at the end of the clamp study or obtained from rats recovered from clamps (basal). Muscles were homogenized as described above, and whole-muscle homogenates were blotted with anti–phospho-specific AKT (serine 473) antibody. Data are shown as bars of means ± SE of four separate experiments. AKT activation (insulin–basal) is shown as a separate bar (delta). *P < 0.05 vs. insulin-induced AKT activation in controls.
levels are increased in various rodent models of insulin resistance and diabetes (15). In light of the in vitro evidence for heterologous desensitization of insulin action by chronic ET-1 stimulation (5), the elevated levels of ET-1 in obesity and diabetes raise the possibility that chronically increased circulating levels of ET-1 may contribute to the development of insulin resistance in pathophysiologic states. In the current studies, we tested this hypothesis by chronically treating normal animals with ET-1 by use of implanted osmotic minipumps. Our results show that 5 days of ET-1 treatment leads to an overall state of insulin resistance, as measured by a 25% decrease in insulin-stimulated GDR during hyperinsulinemic-euglycemic glucose clamps. Because skeletal muscle accounts for the majority of in vivo glucose uptake, these results are consistent with in vivo ET-1–induced skeletal muscle insulin resistance. To demonstrate this directly, we isolated soleus muscle strips for ex vivo measurements of insulin action. We found that in vitro treatment of muscle strips with ET-1 leads to a subsequent decrease in insulin-stimulated glucose uptake. More important, when muscle strips were isolated from saline- versus ET-1–treated animals and studied ex vivo, a striking decrease in insulin-stimulated glucose transport was observed in the muscle strips obtained from the ET-1 group. Taken together, these data provide strong evidence that chronic ET-1 treatment, either in vitro or in vivo, can lead to a state of skeletal muscle insulin resistance.

It is interesting that the ET-1–induced state of in vivo insulin resistance was not confined to skeletal muscle. Another important effect of insulin in vivo is to inhibit hepatic glucose production, and this effect of insulin was blunted during the glucose clamp studies in the ET-1–treated animals. Because ET-1 receptors can mediate calcium activation of glycogenolysis in hepatocytes (26), chronic stimulation of the hepatic ETA receptor is a likely mechanism of the hepatic insulin resistance.

On the basis of the in vitro effects of ET-1 to cause insulin resistance in muscle, shown in the current studies, and in adipocytes, as shown in previous studies (5), it seems most likely that the ET-1–associated insulin-resistance state in vivo reflects a direct effect of ET-1 on insulin target tissues. In our preliminary work, we noted that concentrations of ET-1 (10 nmol/l) that induce insulin resistance in vitro in adipocytes in 24 h were not effective at causing insulin resistance in muscle strips in 1 h (data not shown). Because 3T3-L1 adipocytes can be studied for several days, whereas muscle strips begin to lose viability by 2–3 h, we used higher doses of ET-1 to achieve effects by 1 h of incubation. It is of interest that in muscle strips studied ex vivo from ET-1–treated animals, we found decreased IRS-1 levels, decreased IRS-1–associated P38 kinase, and decreased insulin-stimulated AKT phosphorylation. When ET-1 was added directly in vitro to muscle strips for 1 h, muscle insulin resistance to glucose transport stimulation was clearly produced, but total IRS-1 levels were unchanged, whereas decreased AKT activation was still observed. This suggests that in vivo, the decreased IRS-1 levels may not be the sole cause of the insulin resistance. Furthermore, the ET-1–treated animals became hyperinsulinemic, and because elevated insulin levels can cause a decrease in IRS-1, it is not clear whether the in vivo decreases in IRS-1 content are a direct effect of ET-1 or secondary to hyperinsulinemia.

ET-1 is a vasoactive peptide that, at least when given acutely, can decrease capillary blood flow (9). Because blood flow to skeletal muscle tissues is an additional determinant of skeletal muscle glucose uptake, the possibility can be raised that chronic ET-1 administration reduced skeletal muscle blood flow and that this could contribute to the insulin resistance that we have observed. Although we cannot completely rule out this possibility, we think that it is unlikely or, at best, only a contributory factor for several reasons. First, the insulin-resistant state induced by chronic ET-1 treatment in our studies was not confined to skeletal muscle, because we observed decreased insulin-induced suppression of HGO as well as elevated circulating FFA levels, indicating both hepatic and adipose tissue insulin resistance. Second, our previous studies demonstrated direct in vitro effects of ET-1 to cause insulin resistance in adipocytes, and the current studies show that ET-1 administration in vitro also causes insulin resistance in skeletal muscle strips. Third, when studied ex vivo, skeletal muscle strips from the chronic ET-1–treated animals maintained the insulin-resistant state, a finding that would be incompatible with effects simply because of altered in vivo blood flow. Fourth, the insulin signaling defects that we demonstrated in skeletal muscle from the ET-1–treated animals were comparable to the earlier in vitro results in adipocytes. Furthermore, one would not expect these kinds of signaling defects in insulin action to persist ex vivo if reduced blood flow was the main cause of ET-1–induced insulin resistance.

It has been shown that insulin can promote ET-1 gene expression (27) and release (28). Thus, one possibility is that insulin resistance leads to hyperinsulinemia, which causes increased circulating ET-1 levels, which then further exacerbates the insulin-resistant state. In this way, a positive feedback system may exist in vivo, in which insulin resistance begets more insulin resistance through the ET-1 system. At this point in our knowledge, this sequence of events is speculative, and future experiments will be needed to determine whether this feed-forward concept can occur.

In summary, we have shown that acute in vitro treatment of skeletal muscle with ET-1 can mimic insulin action on glucose transport, whereas a longer pretreatment period leads to a state of heterologous desensitization of insulin action with decreased insulin-stimulated glucose transport. Consistent with this, chronic in vivo treatment of normal rats with ET-1 leads to overall in vivo insulin resistance at the level of skeletal muscle, as well as the liver, with decreased insulin-stimulated glucose transport and insulin signaling in ex vivo studied soleus muscle strips. These results suggest that chronically elevated levels of ET-1 may contribute to or exacerbate insulin resistance in pathophysiologic states.

ACKNOWLEDGMENTS

This work was supported by a research grant from the National Institutes of Health (DK33651) and the Veterans Administration San Diego Health Care System, Research Service.
REFERENCES


