Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (falfa) rats

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Selective resistance to vasoactive effects of insulin in muscle resistance arteries of obese Zucker (falfa) rats. Am J Physiol Endocrinol Metab 293: E1134–E1139, 2007. First published July 10, 2007; doi:10.1152/ajpendo.00516.2006.—Obesity is related to insulin resistance and hypertension, but the underlying mechanisms are unclear. Insulin exerts both vasodilator and vasoconstrictor effects on muscle resistance arteries, which may be differentially impaired in obesity.

Objectives: To investigate whether vasodilator and vasoconstrictor effects of insulin are impaired in muscle resistance arteries of obese rats and the roles of Akt and endothelial NO synthase (eNOS).

Methods/Results: Effects of insulin were studied in resistance arteries isolated from cremaster muscles of lean and obese Zucker rats. In arteries of lean rats, insulin increased activity of both NO and endothelin (ET-1), resulting in a neutral effect under basal conditions. In arteries of obese rats, insulin induced endothelin-mediated vasconstriction (25 ± 1% at 1 nM, P < 0.05 vs. lean). Insulin induced vasodilatation during endothelin receptor blockade in arteries of lean rats (20 ± 5% at 1 nM) but not in those of obese rats. Inhibition of NO synthesis increased vascular tone (by 12 ± 2%) and shifted insulin-mediated vasoactivity to vasocstriction (25 ± 1% at 1 nM) in lean rats but had no effect in arteries of obese rats, indicating reduced NO activity. Protein analysis of resistance arteries revealed that insulin-mediated activation of Akt was preserved in obese rats, whereas expression of eNOS was markedly decreased.

Conclusions: Vasodilator but not vasoconstrictor effects of insulin are impaired in muscle resistance arteries of obese rats, and this selective impairment is associated with decreased protein levels of eNOS. These findings provide a new mechanism linking obesity to insulin resistance and hypertension.

Obesity is associated with impairment of insulin-mediated insulin resistance, i.e., glucose disposal (19) and hypertension (29), but the mechanisms behind these relationships have not been elucidated.

We and others have hypothesized that impaired endothelium-dependent vasodilatation in resistance arteries plays an important role in both insulin resistance and hypertension (32). Impaired endothelium-dependent vasodilatation in these arteries increases vascular resistance, which, in muscle, may contribute to decreased insulin-mediated glucose disposal (7).

A specific type of endothelium-dependent vasodilatation that may be relevant in this regard is insulin-mediated vasodilatation. Both vasodilator and vasoconstrictor effects of insulin have been described, the normal response to insulin being vasodilator or neutral (8, 36). We and others have previously shown that, in muscle resistance arteries, NO-dependent vasoconstrictor effects of insulin are antagonized by endothelin-mediated vasoconstrictor effects. It was previously shown that insulin’s vasodilator effects in muscle resistance arteries are mediated by phosphatidylinositol 3-kinase (PI3-kinase), Akt, and endothelial NO synthase (eNOS) (8, 10), whereas insulin’s vasoconstrictor effects on these arteries are mediated by endothelin (ET-1) and are independent of PI3-kinase. Selective impairment of insulin’s vasodilator effects in these arteries results in insulin-mediated vasoconstriction (8, 30).

In obese subjects, impaired insulin-mediated vasodilatation and even insulin-mediated vasoconstriction (15) have been demonstrated. Furthermore, insulin-stimulated production of ET-1 is normal in type 2 diabetic subjects (11), and ET-1 activity is increased in obese subjects (4). However, the relationships between hyperinsulinemia, impairment of insulin signaling, enhanced ET-1 activity, and narrowing of muscle resistance arteries in obesity are unclear.

The obese Zucker rat, which lacks functional leptin receptors, is a rat model of obesity-related insulin resistance and late-onset hypertension (16, 20, 39). The muscle microcirculation of these rats is characterized by increased vascular resistance (12) and impairment of insulin-mediated blood flow (37). The mechanisms behind these impairments have not been elucidated.

Several mechanisms for altered vasoreactivity to insulin in microvessels of obese Zucker rats have been suggested, including impaired activation of PI3-kinase/Akt signaling (18), decreased expression of eNOS (23), increased production of reactive oxygen species (ROS) (13, 21), and enhanced insulin-mediated ET-1 synthesis (38). We hypothesized that 1) selective impairment of insulin’s acute vasodilator actions results in insulin-mediated, endothelium-dependent vasoconstriction of skeletal muscle resistance arteries of obese Zucker rats, and 2) impairment of insulin-mediated vasoreactivity is caused by impaired insulin-mediated activation of Akt and/or downregulated expression of eNOS. To investigate these hypotheses, we used resistance arteries (diameter ~90 μm) isolated from the cremaster muscle as a model.

Methods

This investigation conformed to the National Research Council’s Guide for the Care and Use of Laboratory Animals (NIH publication...
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no. 85-23, revised 1996), and the local ethics committee for animal experiments approved the procedures.

Measurement of blood pressure, nonfasting blood glucose, and plasma insulin. Systolic blood pressure, diastolic blood pressure, mean arterial pressure, and pulse pressure were determined in rats anesthetized with pentobarbital sodium (Nembutal; 70 mg/kg ip), using a pressure transducer system with an intravascular fluid-filled catheter inserted into the carotid artery. Blood glucose was determined in blood taken from the tail tip with a Haemo-Gluco test (Accutrend Alpha, Boehringer, Mannheim, Germany). Plasma insulin levels were analyzed by radioimmunoassay (Diasorin, Saluggia, Italy).

Smooth muscle tone, endothelial integrity, vasoreactivity to insulin, and ET-1 sensitivity. Vasoreactivity experiments were conducted on resistance arteries (≈90 μm) isolated from cremaster muscles of 14- to 16-wk-old lean (fa−) and obese (fa/fa) Zucker rats (n = 32). Cremaster resistance arteries were isolated and studied in the pressure myograph as described previously (8). When multiple segments were isolated from the same artery, these segments were subjected to different study protocols.

Smooth muscle tone, expressed as a percentage of the maximal arterial diameter, was compared between cremaster resistance arteries from lean and obese rats. Endothelial integrity was determined by measuring the vasodilator response to acetylcholine (ACh; 0.1 μmol/l) and compared between lean and obese rats.

Acute effects of insulin (Actrapid, Novo Nordisk) on the diameter of cremaster resistance arteries were studied by exposing arterial segments to four concentrations of insulin (0.01, 0.1, 1, and 10 nM). Insulin-mediated diameter changes were measured during 30 min after each concentration step.

Vasoreactivity to insulin was compared between lean and obese Zucker rats (n = 5/group). To control for diameter changes in time, separate groups of vessel segments from lean and obese rats were treated with solvent (MOPS buffer + 0.1% BSA) instead of insulin.

To study the role of the endothelium in responses of resistance arteries from obese rats to insulin, the endothelium was removed by air bubble treatment as described previously (9), and responses to insulin were studied (n = 4). To study the role of NO in the responses of resistance arteries to insulin, resistance arteries of lean and obese rats were pretreated with the NO inhibitor Nω-nitro-L-arginine (L-NA; 0.1 mM), and responses to insulin were studied as described above (n = 5–6/group).

To study the role of ET-1 in responses of resistance arteries to insulin, resistance arteries of lean and obese rats were pretreated with the nonselective endothelin receptor antagonist PD-142893 (3 μmol/l), and responses to insulin were studied (n = 5/group).

To determine whether smooth muscle sensitivity to ET-1 was altered in obese Zucker rats, vasoconstrictor responses of resistance arteries of lean and obese rats to ET-1 (10−11 to 10−8 M) were studied (n = 4–5/group).

Measurement of insulin-mediated activation of Akt and protein levels of eNOS. Insulin-mediated activation of Akt and protein levels of eNOS in cremaster resistance arteries were determined by Western blotting. Western blotting of cremaster resistance arteries was performed as previously described (9).

Insulin-mediated activation of Akt. Insulin-mediated activation of Akt was determined by measuring phosphorylation of Akt at Ser473 as described previously (10). Two segments of resistance arteries (≈2-mm long, n = 6 rats/group) were exposed to solvent (controls) or insulin (1 nm) for 15 min in MOPS buffer, and phosphorylated Akt (pAkt) was measured with a specific primary antibody against pAkt (1:1,000; New England Biolabs). In a separate set of experiments, protein levels of Akt were determined in cremaster resistance arteries of lean and obese rats (n = 3/group). To control for protein loading, the same blots were stained for extracellular signal-regulated kinase-1 (ERK1; 1:1,000, New England Biolabs).

Protein expression of eNOS. The abundance of eNOS was analyzed by Western blotting of lysates of resistance arteries of lean and obese Zucker rats (n = 4–5/group). Equal amounts of arterial protein (3 μg/lane) were loaded on gel, and eNOS was determined using a specific primary antibody (1:1,000; Santa Cruz Biotechnology).

Statistics. Steady-state responses are reported as mean changes in diameter from baseline (in %) ± SE. The baseline diameter was defined as the arterial diameter just before addition of the first insulin concentration. Differences between diameter changes at each concentration were assessed by a one-way ANOVA with Bonferroni post hoc tests. Differences in pAkt staining between insulin-treated arteries were tested with a paired one-way ANOVA. Differences in eNOS staining in resistance arteries of lean and obese rats were tested by a two-sided t-test. Differences were considered statistically significant when P < 0.05.

RESULTS

Metabolic characteristics and blood pressure characteristics of lean and obese Zucker rats. Obese Zucker rats were obese compared with their lean counterparts and showed profound hyperinsulinemia but not hyperglycemia (Table 1). Obese Zucker rats showed higher systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and pulse pressure compared with lean Zucker rats (Table 1).

Vascular diameter, vascular tone, and general endothelium-dependent vasodilatation of resistance arteries of lean and obese rats. Smooth muscle tone did not differ between cremaster resistance arteries of lean and obese rats (46 ± 1 vs. 49 ± 2% of maximal diameter, P = 0.38). Both baseline diameters (97 ± 4 vs. 83 ± 4 μm, P < 0.01) and maximal diameters of skeletal muscle resistance arteries from obese rats (180 ± 5 vs. 162 ± 3 μm, P = 0.02) were reduced compared with those of lean rats. This inward remodeling of muscle resistance arteries was not accompanied by hypertrophy of the vessel wall (Supplemental Table 1; supplemental data are available at the online version of this article). Vasodilator responses to the endothelium-dependent vasodilator ACh (0.1 μM) did not differ between lean and obese rats (31 ± 3 vs. 30 ± 5% of baseline, P = 1.0). Dose-response relationships to ACh did not show differences in sensitivity or maximal response (Supplemental Fig. 2; see Fig. 3).

Vasodilator but not vasoconstrictor effects of insulin are abolished in endothelium of muscle resistance arteries of obese Zucker rats. In muscle resistance arteries of lean rats, insulin alone did not significantly change diameters; this neutral response was caused by a balance of NO-dependent vasodilator effects and ET-1-dependent vasoconstrictor effects (Fig. 1A). Blockade of endothelin activity with the endothelin receptor antagonist PD-142893 uncovered insulin-mediated, NO-dependent vasodilatation in muscle resistance arteries of lean rats.

Table 1. Metabolic and blood pressure characteristics of lean and obese Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>P Value</th>
</tr>
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<tbody>
<tr>
<td>Weight, g</td>
<td>367±11</td>
<td>529±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Blood glucose, mM</td>
<td>8.4±0.8</td>
<td>8.4±0.4</td>
<td>0.93</td>
</tr>
<tr>
<td>Plasma insulin, nM</td>
<td>0.26±0.05</td>
<td>1.36±0.25</td>
<td>0.003</td>
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<tr>
<td>SBP, mmHg</td>
<td>124±10</td>
<td>166±9</td>
<td>0.015</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>99±7</td>
<td>123±5</td>
<td>0.030</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>111±9</td>
<td>142±6</td>
<td>0.023</td>
</tr>
<tr>
<td>PP, mmHg</td>
<td>25±4</td>
<td>43±4</td>
<td>0.017</td>
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Values are means ± SE. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.
Impairment of insulin’s vasodilator effects in muscle resistance arteries of obese rats is associated with reduced basal and insulin-stimulated NO activity. Since insulin’s vasodilator actions in muscle resistance arteries depend on NO synthesis (8, 30), we tested whether basal and insulin-stimulated NO activity is reduced in muscle resistance arteries of obese rats.

In resistance arteries of lean rats, inhibition of NO synthesis with L-NA (see METHODS) induced vasoconstriction, indicating basal NO synthesis (Fig. 2A). Moreover, inhibition of NO synthesis in these arteries uncovered a marked vasoconstrictor effect of insulin (Fig. 2B). Insulin-mediated vasoconstriction started at 0.01 nM (~18 ± 5% from baseline vs. 2 ± 1% without L-NA) and reached a plateau at 0.1 nM (~24 ± 4% of baseline diameter).

In muscle resistance arteries of obese rats, inhibition of NO synthesis did not induce significant vasoconstriction (Fig. 2A) and did not alter insulin-mediated vasoreactivity (Fig. 2B),
indicating a reduction of basal and insulin-stimulated NO activity in resistance arteries of obese rats.

Reduced basal PI3-kinase/Akt activity but normal insulin-mediated activation of PI3-kinase/Akt signaling in resistance arteries of obese rats. To study whether insulin-stimulated NO activity in resistance arteries of obese Zucker rats is caused by reduced activation of Akt, insulin-mediated phosphorylation of Akt at Ser\(^473\) was studied in resistance arteries of lean and obese rats. In lean rats, insulin (1 nM) induced a twofold increase in Akt phosphorylation at Ser\(^473\) (Fig. 3A). In obese rats, insulin increased Akt phosphorylation by 1.5-fold compared with lean controls (insulin obese vs. insulin lean, \(P = 0.20\)). Basal levels of Ser\(^473\) pAkt were reduced to 37 ± 6% of lean controls in muscle resistance arteries of obese rats (\(P < 0.001\) vs. lean). A representative blot is depicted in Fig. 3A, top. Differences in Ser\(^473\) pAkt were not caused by differences in Akt protein expression, as protein expression of Akt was not different between lean and obese Zucker rats (Fig. 3B).

Reduced protein expression of eNOS in resistance arteries of obese rats. To examine whether reduced expression of eNOS contributes to the reduction of basal and insulin-stimulated NO activity, expression of eNOS protein in skeletal muscle resistance arteries from lean and obese rats was compared. Muscle resistance arteries of obese rats showed a marked decrease in eNOS protein compared with muscle resistance arteries of lean rats (Fig. 4).

**DISCUSSION**

The main novel findings of this study are that 1) insulin is a physiological vasoconstrictor of muscle resistance arteries of obese Zucker rats, 2) insulin-mediated vasoconstriction results from impairment of insulin’s NO-mediated vasodilator effects in combination with preservation of insulin-mediated ET-1 release, and 3) impairment of insulin’s vasodilator effects is associated with decreased protein levels of eNOS but not with impaired activation of Akt.

We and others have previously shown that insulin exerts antagonistic vasodilator and vasoconstrictor effects on muscle resistance arteries that are mediated by NO and ET-1, respectively (5, 8, 10, 36). In our system, the sum of these effects results in an apparent lack of effect of insulin alone on muscle resistance arteries of lean rats (Fig. 1). It was previously shown that insulin induces vasoconstriction of these arteries when NO synthesis is impaired (8).

Selective resistance to insulin’s vasoactive effects in obese Zucker rats. The fact that insulin-induced NO activity is strongly reduced in skeletal muscle resistance arteries of obese Zucker rats is demonstrated by a lack of insulin-mediated vasodilatation during endothelin receptor blockade (Fig. 1A) and a lack of effect of NOS inhibition on insulin-mediated vasoreactivity (Fig. 1B). This impairment of vasodilator effects of insulin in resistance arteries of obese rats is in agreement with the reported impairment of insulin-mediated increases in muscle blood flow observed in Zucker rats in vivo (37) and impairment of insulin-mediated vasodilatation in obese subjects (33).

In contrast to insulin’s vasodilator effects, insulin’s ET-1-mediated vasoconstrictor effects in resistance arteries of obese Zucker rats are preserved (Fig. 2). These vasoconstrictor effects are mediated by endothelium-derived ET-1. Although an earlier study in mesenteric arteries of fructose-fed rats showed increased insulin-mediated ET-1 activity (26), we did not find this in our study. Blocking endothelin activity induces similar shifts in insulin-mediated vasoreactivity in resistance arteries of lean and obese Zucker rats (Fig. 1A), and insulin-mediated vasoconstriction during NOS inhibition is not increased in resistance arteries of obese rats (Fig. 2B). Because smooth muscle sensitivity to ET-1 was similar in lean and obese rats, the preserved vasoconstrictor effects of insulin were not caused by enhanced ET-1 sensitivity. Therefore, preservation of insulin-mediated ET-1 activity is caused by preservation of ET-1 release from the endothelium. These data are consistent with earlier studies showing that insulin-stimulated ET-1 release is normal in type 2 diabetics (11) and that insulin increases vascular resistance in the forearm of obese insulin-resistant hypertensives (15). In conclusion, insulin-stimulated ET-1 release is preserved in endothelium of muscle resistance arteries of obese Zucker rats, resulting in insulin-induced, ET-1-mediated vasoconstriction at physiological insulin concentrations.

Mechanisms underlying reduced NO activity in resistance arteries of obese rats. Impairment of NO-dependent vasodilatation in obesity has been proposed to result from reduced insulin-mediated activation of Akt (18), decreased expression of eNOS (23), uncoupling of eNOS by decreased availability of the eNOS co-factor tetrahydrobiopterin (BH\(_4\)) (24), or increased production ofROS (14).

We have shown that protein levels of eNOS are decreased in muscle resistance arteries of obese Zucker rats (Fig. 4). Our finding agrees with earlier reports of reduced eNOS mRNA in adipose tissue of obese Zucker rats (23) and reduced eNOS protein expression in internal mammary arteries of type 2 diabetics (27). These data indicate that reduced expression of eNOS impairs basal and insulin-stimulated NO activity in resistance arteries in obesity.
Although decreased eNOS expression in resistance arteries of obese Zucker rats is likely a major cause of reduced NO activity in these rats, our results do not exclude involvement of increased ROS production and/or decreased BH4 synthesis. The roles of these factors in microvascular dysfunction in obesity warrant further investigation.

Interestingly, impairment of insulin’s NO-dependent vasodilator effects is not accompanied by impairment of ACh-mediated vasodilatation in muscle resistance arteries of obese rats. Our findings agree with earlier reports on ACh-mediated vasodilatation in the hindlimb and coronary microcirculation of obese Zucker rats (1, 21) but contrast with an in vivo study on muscle resistance arteries of obese Zucker rats (14). The variability in these results may result from two causes. First, insulin-induced vasodilatation in muscle resistance arteries is entirely NO dependent (6, 8), while ACh-induced vasodilatation in these arteries is also mediated by endothelium-derived hyperpolarizing factors (2). The latter component has been shown to be increased in obese Zucker rats and may mask a decrease in ACh-mediated NO synthesis (1). Second, different levels of blood glucose may determine ACh responses in blood vessels of obese Zucker rats (28, 34), as impairment of ACh-mediated vasodilatation has been demonstrated in diabetic Zucker rats (14) but not in normoglycemic Zucker rats (1, 21).

**Perspectives.** Microvascular dysfunction has been proposed to contribute to insulin resistance, through impairment of muscle blood flow, and to hypertension, by increasing vascular resistance (31), and is characterized by impaired NO activity, increased ET-1 activity, and vasoconstriction (25). We have shown that insulin’s NO-dependent vasodilator actions are impaired in a rat model of obesity, insulin resistance, and hypertension, resulting in a direct vasoconstrictor effect of insulin on muscle resistance arteries. In rats, reduction of NO production and obesity have been shown to impair insulin-mediated muscle blood flow and muscle glucose uptake (7). Furthermore, hyperinsulinemia, in combination with impairment of NO synthesis, induces hypertension in rats (3). Our data may therefore provide a link between obesity, microvascular dysfunction, insulin resistance, and hypertension.

In summary, we have shown that insulin-mediated vasoreactivity is shifted toward endothelium-dependent, endothelium-independent, and other paracrine and/or endocrine signals.

We found insulin-mediated activation of Akt not to be decreased in muscle resistance arteries of obese Zucker rats (Fig. 3A). In adipose tissue microvessels of these rats, Jiang et al. (18) have shown that insulin-mediated activation of PI3-kinase and Akt is impaired in obese Zucker rats. The apparent discrepancy between those results and ours may be caused by adipose tissue-derived cytokines, such as tumor necrosis factor-α (TNFα), that impair insulin signaling to Akt (17, 22, 35). Indeed, it was recently found that TNFα impairs insulin-mediated activation of Akt in muscle resistance arteries (10). Since TNFα and other paracrine and/or endocrine signals are present in vivo, our results do not rule out the possibility that insulin-mediated activation of PI3-kinase/Akt is impaired in vivo.

**Fig. 3.** A: lower basal phosphorylation of Akt at Ser473 but intact stimulation of Akt phosphorylation by insulin in resistance arteries of obese Zucker rats. Segments of equal length of 1 resistance artery (~3 mm/segment, n = 4) were isolated as described in METHODS, followed by incubation with insulin (1 nM, 15 min). Arterial proteins were separated by SDS-PAGE and immunoblotted with anti-pAkt (Ser473) antibody (top). ERK1 was used as a loading control. AU, arbitrary units. *P* = 0.02 vs. lean control. B: total Akt levels do not differ between muscle resistance arteries of lean and obese rats (n = 3/group).

Fig. 4. Reduced protein levels of endothelial NOS (eNOS) in cremaster resistance arteries of obese Zucker rats. Equal amounts of arterial protein (3 μg/lane, n = 4–5/group) were separated by SDS-PAGE and immunoblotted with anti-eNOS antibody. *P* < 0.01 vs. lean.
mediated vasoconstriction in skeletal muscle resistance arteries of obese Zucker rats. This shift is caused by decreased basal and insulin-stimulated NO activity and normal insulin-stimulated ET-1 activity. The decrease in NO activity is associated with decreased protein levels of eNOS but not with impaired activation of Akt by insulin. These findings may provide a mechanistic explanation for the impairment of muscle blood flow and muscle glucose disposal in obesity.

REFERENCES


