Insulin-Mediated Hemodynamic Changes Are Impaired in Muscle of Zucker Obese Rats

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Insulin-mediated hemodynamic effects in muscle were assessed in relation to insulin resistance in obese and lean Zucker rats. Whole-body glucose infusion rate (GIR), femoral blood flow (FFB), hindleg glucose extraction (HGE), hindleg glucose uptake (HGU), 2-deoxyglucose (DG) uptake into muscles of the lower leg (Rg), and metabolism of infused 1-methylxanthine (1-MX) to measure capillary recruitment were determined for isoglycemic (4.8 ± 0.2 mmol/l, lean; 11.7 ± 0.6 mmol/l, obese) insulin-clamped (20 μU · min⁻¹ · kg⁻¹ × 2 h) and saline-infused control anesthetized age-matched (20 weeks) lean and obese animals. Obese rats (445 ± 5 g) were less responsive to insulin than lean animals (322 ± 4 g) for GIR (7.7 ± 1.4 vs. 22.2 ± 1.1 mg · min⁻¹ · kg⁻¹, respectively), and when compared with saline-infused controls there was no increase due to insulin by obese rats in FBF, HGE, HGU, and Rg of soleus, plantaris, red gastrocnemius, white gastrocnemius, extensor digitorum longus (EDL), or tibialis muscles. In contrast, lean animals showed marked increases due to insulin in FBF (5.3-fold), HGE (5-fold), HGU (8-fold), and Rg (~5.6-fold). Basal (saline) hindleg 1-MX metabolism was 1.5-fold higher in lean than in obese Zucker rats, and insulin increased in only that of the lean. Hindleg 1-MX metabolism in the obese decreased slightly in response to insulin, thus postinsulin lean was 2.6-fold that of the postinsulin obese. We conclude that muscle insulin resistance of obese Zucker rats is accompanied by impaired hemodynamic responses to insulin, including capillary recruitment and FFB. Diabetes 51:3492–3498, 2002

RESEARCH DESIGN AND METHODS

Animals. Male 20-week-old lean (Fa/−) and obese (fa/fa) Zucker rats were purchased from Monash University, Melbourne, Australia. Rats were then housed at a constant temperature of 21 ± 1°C in a 12-h light/dark cycle and allowed free access to water and a commercial diet as previously described (18). All procedures adopted and experiments undertaken were approved by the University of Tasmania Ethics Committee.

Surgery. Rats were anesthetized using Nembutal (50 mg/kg body wt) and had polyethylene cannulas (PE-50; Microtube Extrusions, North Rocks, Australia) surgically implanted into the carotid artery, for arterial sampling and measurement of blood pressure (pressure transducer Transpac IV; Abbott Critical Systems), and into both jugular veins for continuous administration of anesthetic and other intravenous infusions. A tracheotomy tube was inserted, and the animal was allowed to spontaneously breathe room air throughout the course of the experiment. Small incisions (1.5 cm) were made in the skin overlaying the femoral vessels of both legs, and the femoral artery was separated from the femoral vein and saphenous nerve. The epigastric vessels were then ligated, and an ultrasonic flow probe (Transonic Systems, V7 series 0.5 mm) was positioned around the femoral artery of the right leg just distal to the rectus abdominis muscle. The cavity in the leg surrounding the flow

The obese Zucker rat is a commonly used animal model of insulin resistance that exhibits many of the characteristics that coexist with type 2 diabetes in humans, including hyperinsulinemia (1), dyslipidemia (2), and hypertension (3). Obesity develops as a result of a recessive mutation in the gene for the leptin receptor (4). By 4–5 weeks of age, obese rats weigh significantly more than their lean littermates (5). The obese rats then go on to develop insulin resistance in the muscle and liver (6,7). This muscle insulin resistance involves reduced insulin sensitivity as well as a blunted maximal responsiveness to insulin (8). In addition, it is apparent in vivo (7,9), in the perfused hindlimb (2,10), and in individual muscles once they have been isolated and incubated (8). Furthermore, glycolysis, glucose oxidation, and glycogen synthesis are all impaired in obese Zucker rats (8). A number of alterations in the insulin signaling pathway that are likely to contribute to the insulin resistance have been identified in the obese rats. In the skeletal muscle, these include reduced expression of insulin receptor substrate (IRS)-1 and -2 (11), leading to decreased insulin-stimulated tyrosine phosphorylation and decreased phosphatidylinositol (PI) 3-kinase activity associated with IRS-1 (12). In addition, insulin-stimulated AKT1 (protein kinase B), AKT2 (12), and ERK2 (extracellular signal–regulated kinase) (13) activity were reduced in obese rats. However, there are no alterations in the levels of GLUT4 mRNA (14) and protein (15) in obese rats.

Along with the insulin resistance, obese Zucker rats also develop vascular alterations. Studies in isolated vessels have shown that the ability of insulin to attenuate vasoconstriction is impaired in obese Zucker rats (16,17). However, to our knowledge there have been no studies to directly investigate whether insulin’s hemodynamic actions in the obese Zucker are also impaired in vivo. Thus, the aim of this study was to examine the insulin-mediated increase in total flow and capillary recruitment, and whether defects in these actions may contribute to the insulin resistance in this animal model.
probe was filled with lubricating jelly (H-R; Mohawk Medical Supply, Utica, NY) to provide acoustic coupling to the probe. The probe was then connected to the flow meter (Model T106 ultrasonic volume flow meter; Transonic Systems). This was in turn interfaced with an IBM compatible PC computer that acquired the data (at a sampling frequency of 100 Hz) for femoral blood flow (FBF), heart rate, and blood pressure using WINDAQ data acquisition software (DATAQ Instruments). The surgical procedure generally lasted ~30 min and then the animals were maintained under anesthesia for the duration of the experiment using a variable infusion of Nembutal (0–0.6 mg · min⁻¹ · kg⁻¹) via the left jugular cannula. The femoral vein of the left leg was used for venous sampling, using an insulin syringe with an attached 29G needle (Becton Dickinson). A duplicate venous sample was taken only on completion of the experiment (120 min) to prevent alteration of the blood flow from the hindlimb due to sampling and to minimize the effects of blood loss. The body temperature was maintained using a water-jacketed platform and a heating lamp positioned above the rat.

Following a 1-h equilibration, either a saline infusion (protocol 1) or hyperinsulinemic (20 mU · min⁻¹ · kg⁻¹)-isoglycemic clamp (protocol 2 (19)) was performed for 2 h (Fig. 1). Since 1-MX (SigmaAldrich) clearance was very rapid, it was necessary to partially inhibit the activity of xanthine oxidase. To do this, an injection of a specific xanthine oxidase inhibitor, allopurinol (10 μmole/kg) was administered as a bolus dose 5 min before commencing the 1-MX infusion (0.4 mg · kg⁻¹ · min⁻¹) (Fig. 1). This allowed constant arterial concentrations of 1-MX to be maintained throughout the experiment. At the end of the experiment, blood was sampled from the femoral vein and carotid artery. From the arteriovenous difference multiplied by 100, it was possible to determine 1-MX disappearance were calculated. The latter was used as an indicator of the removal of 2-DG from the blood.

Analytical methods. A glucose analyzer (Model 2300 Stat Plus; Yellow Springs Instruments) was used to determine whole-blood glucose (by the glucose oxidase method) during the insulin clamp. A blood sample of 25 μl was required for each determination. Insulin levels at the beginning and end of the experiment were determined from arterial plasma samples by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Sweden). Perchloric acid-treated plasma samples were centrifuged for 10 min, and the supernatant was required for each determination. Insulin levels at the beginning and end of the experiment were determined from arterial plasma samples by enzyme-linked immunosorbent assay (ELISA) (Mercodia, Sweden). Perchloric acid-treated plasma samples were centrifuged for 10 min, and the supernatant was used to determine 1-MX, allopurinol, and oxypurinol concentrations by reverse-phase high-performance liquid chromatography (HPLC) as previously described (19,20). Xanthine oxidase activity was assayed from muscle homogenates as described previously (21).

2-DG uptake assay. The frozen soleus, red gastrocnemius, white gastrocnemius, extensor digitorum longus, tibialis, and plantaris muscles were ground under liquid nitrogen and homogenized using an Ultra Turrax. Free and phosphorylated [3H]-2-DG were separated by ion exchange chromatography using an anion exchange resin (AG1-X8) (22,23). Biodegradable counting scintillant-BCA (Amersham) was added to each radioactive sample and radioactivity determined using a scintillation counter (LS3801; Beckman.). From this measurement and a knowledge of plasma glucose and the time

**FIG. 1. Study design.** Arterial and femoral venous blood samples were collected at the time indicated by the sample tube for HPLC analysis. The hand indicates the time at which allopurinol and 2-DG were injected. Arterial samples were taken at 80, 85, 90, 105, and 120 min to monitor radioactive 2-DG, and the ink drop indicates when arterial samples were taken for glucose determination. Venous infusions are indicated by the bars.
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TABLE 1
Characteristics of lean and obese Zucker rats

<table>
<thead>
<tr>
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<th>Lean</th>
<th>Obese</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>322 ± 4</td>
<td>445 ± 5*</td>
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<tr>
<td>Epididymal fat pad (g)</td>
<td>1.3 ± 0.1</td>
<td>4.4 ± 0.2*</td>
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<tr>
<td>Calf muscle mass (g)</td>
<td>1.58 ± 0.03</td>
<td>1.16 ± 0.03*</td>
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<tr>
<td>Plasma insulin (pmol/l)</td>
<td>182 ± 21</td>
<td>4158 ± 491*</td>
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<tr>
<td>Blood glucose (mmol/l)</td>
<td>4.8 ± 0.2</td>
<td>11.7 ± 0.6*</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>107 ± 2</td>
<td>107 ± 1</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>335 ± 9</td>
<td>329 ± 6</td>
</tr>
<tr>
<td>Femoral blood flow (ml/min)</td>
<td>0.69 ± 0.05</td>
<td>0.69 ± 0.05</td>
</tr>
<tr>
<td>Vascular resistance (RU)</td>
<td>164 ± 10</td>
<td>172 ± 14</td>
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</table>

Data are means ± SE for n = 14–17 rats in each group. *Significantly different (P < 0.05) from lean Zucker rats. Epididymal fat pad and calf muscles were removed from rats at the completion of the experiment. All other values were measured at the commencement of saline or insulin infusion. Calf muscle mass includes soleus, plantaris, and whole gastrocnemius.

course of plasma 2DG disappearance, \( R_p \), which reflects glucose uptake into the muscle, was calculated as previously described by others (22,23).

Data analysis. All data are expressed as means ± SE. Mean FBF, mean heart rate, and mean arterial blood pressure were calculated from 5-s subsamples of the data, representing 500 flow and pressure measurements every 15 min. Vascular resistance in the hindleg was calculated as mean arterial blood pressure in millimeters of mercury divided by FBF in milliliters per minute and expressed as resistance units (RUs). Glucose uptake in the hindlimb was calculated from arterial to venous glucose difference and multiplied by FBF and expressed as \( \mu \)mol/min. The \( 1 \)-MX disappearance was calculated from arterial to venous plasma \( 1 \)-MX difference and multiplied by FBF (corrected for the volume accessible to \( 1 \)-MX, 0.871, determined from plasma concentrations obtained after additions of standard \( 1 \)-MX to whole rat blood) and expressed as \( \mu \)mol/min.

Statistical Analysis. To ascertain differences between treatment groups at the end of the experiment (120 min), two-way ANOVA using the Student-Newman-Keuls method was performed. Significant differences (P < 0.05) between insulin and saline treatment in each phenotype of rat, as well as differences between lean and obese rats undergoing the same treatment, were reported. An unpaired Student’s t test was used to determine whether there was a significant difference (P < 0.05) between the glucose infusion rates (GIRs) at the conclusion of the experiments. All tests were performed using the SigmaStat statistical program (Jandel Software).

RESULTS

Characteristics. At the time of study, obese Zucker rats (445 ± 5 g) weighed significantly more that their lean littermates (322 ± 4 g; Table 1). Furthermore, the epididymal fat pad mass was significantly greater in the obese rats (Table 1). The total mass of the lower leg muscles was reduced by 27%, with individual muscle mass (mg wet wt) for the lean and obese rats as follows: soleus (106 ± 3, 98 ± 4), plantaris (229 ± 4, 176 ± 3, P < 0.001), red gastrocnemius (362 ± 16, 313 ± 13, P < 0.05), white gastrocnemius (380 ± 21, 572 ± 24, P < 0.001), EDL (145 ± 3, 103 ± 2, P < 0.001), and tibialis (470 ± 13, 345 ± 7, P < 0.001). Obese Zucker rats also exhibited hyperinsulinemia and hyperglycemia, before either insulin or saline infusions, when compared with their lean littermates (Table 1). In lean rats, plasma insulin levels were significantly higher following infusion of insulin compared with saline infusion (insulin 8,630 ± 665 pmol/l, saline 441 ± 70 pmol/l). Similarly, in obese Zuckers, plasma insulin levels were 20,190 ± 1,281 and 5,963 ± 815 pmol/l following insulin or saline infusions, respectively. The higher basal insulin levels may reflect the fed state and the fact that the obese rats eat more than the lean rats. Clearly, an insulin infusion of 20 mU · min⁻¹ · kg⁻¹ resulted in significantly higher insulin levels in obese rats compared with the lean rats.

Hemodynamic effects. There was no significant difference in the basal mean arterial pressure or heart rate between lean and obese rats (Table 1), nor did any difference develop as a result of a 2-h insulin or saline infusion (data not shown). Similarly, basal femoral arterial blood flow and vascular resistance also did not differ between lean and obese animals (Table 1). However, whereas there was very little increase in femoral arterial blood flow with saline infusion, insulin significantly increased flow in the lean Zuckers but not in the obese rats (Fig. 2A). Accordingly, vascular resistance was decreased by insulin only in the lean Zucker rats (Fig. 2B).

Glucose metabolism. Basal blood glucose was significantly higher in the obese (11.7 ± 0.6 mmol/l) than in the lean (4.8 ± 0.2 mmol/l; Table 1) animals, and GIRs were set to maintain these levels throughout the insulin clamp (Fig. 3A). As shown in Fig. 3B, and because of the marked insulin resistance of the obese animals, glucose infusions were much less. Plateau values for glucose infusion of lean and obese animals were 22.2 ± 1.1 and 7.7 ± 1.4 mg · kg⁻¹ · min⁻¹, respectively.

Glucose extraction across the hindleg was increased significantly by insulin in the lean rats, but the small
increase induced by insulin in the obese rats was not significant (Fig. 4A). HGU, which is the product of glucose extraction and FBF, showed a similar trend (Fig. 4B). Insulin resistance was also apparent in the obese rats when assessed by 2-DG uptake into individual muscles (Fig. 5). No significant effect was seen in soleus, plantaris, red or white gastrocnemius EDL, or tibialis muscles. Combined data for insulin-mediated 2-DG uptake in muscles of lean and obese animals were 25.9 ± 2.4 and 12.6 ± 2.6 μg·g⁻¹·min⁻¹, respectively. For each muscle and the combined data, 2-DG uptake following saline infusion (2 h) tended to be greater for the obese than the lean rats, but this was not significant.

**1-MX metabolism.** The arterial plasma concentrations of oxypurinol were similar in all groups (Fig. 6A). Arterial 1-MX was significantly greater in the insulin-treated lean animals compared with the insulin-treated obese animals, but the difference was only minor (24% lower in obese animals). Taken together these data suggested that the enzyme-xanthine oxidase was inhibited to the same extent in obese and lean animals. The extent of arterio-venous 1-MX difference due to insulin for lean versus obese animals was significant, with extraction nearly half that of the lean (Fig. 6C). When total femoral arterial blood flow was taken into account, the 1-MX disappearance during both saline and insulin infusion, was significantly lower in the obese rats. Insulin stimulated 1-MX metabolism only in the lean animals (Fig. 6D).

Xanthine oxidase activity measured in tissue homogenates from the thigh muscle tended to be lower in obese rats (32 ± 4 pmol·min⁻¹·mg protein⁻¹) than in the lean rats (41 ± 4 pmol·min⁻¹·mg protein⁻¹), but this was not significant.

**DISCUSSION**

The present study shows for the first time major impairments of insulin’s hemodynamic effects in Zucker obese rats in vivo. The impairments manifested as markedly decreased responses to insulin in terms of hindleg femoral arterial blood flow and capillary recruitment in obese animals when compared with age-matched lean rats. These findings add an important in vivo perspective to data already reported by others about impaired vascular responses to insulin in Zucker obese rat tissues in vitro. For example, Zemel et al. (16) noted that isolated thoracic aortae from obese rats were more sensitive to phenylephrine-induced constriction; moreover, the ability of insulin to attenuate this response was reduced. Similarly, Walker...
et al. (24) found that norepinephrine-induced vasoconstriction was attenuated by insulin in mesenteric arteries from lean rats, but not from obese rats. In addition, endothelial function was abnormal and the acetylcholine-induced dilation was reduced in obese rats (24).

The impaired capillary recruitment in the obese Zucker rats may contribute to the insulin resistance seen in these rats. Consistent with reports by others (6,7), we noted that the GIR was reduced by 65% with no significant effect of insulin to increase either HGU or 2-DG uptake in the obese rats, even at supraphysiological insulin levels. In addition, at more physiological levels (e.g., 3 mU/min/kg) we also found no response to insulin with respect to either glucose uptake or hemodynamic measurements (data not shown).

The present study also adds to the findings of our previous report which demonstrated that resting muscle oxygen uptake was impaired in the obese Zucker hindlimb muscle (25) and led to the likelihood that impaired access for insulin and glucose extends to oxygen. Thus, vascular changes involving regulation of flow distribution and/or dominance of nonnutritive flow may be contributory to both the obese phenotype and the insulin resistance. However, the mechanism responsible for the impaired response to insulin may relate more to impaired insulin signaling in the vasculature than to dominance of nonnutritive flow. This view is supported by data from preliminary studies that show that contraction-mediated capillary recruitment (1-MX metabolism) is increased by 85% and subsequently reaches levels of contraction similar to those in lean rats (26). Since insulin’s activation of the PI 3-kinase pathway, but not the MAP-kinase pathway, is diminished in vascular tissues from obese Zucker rats (27), and since nitric oxide (NO) has been implicated in insulin-mediated increases in both total flow (28,29) and capillary recruitment (30), defects in either NO production or the vascular smooth muscle cell (VSMC) responsiveness to NO may underlie the diminished vascular insulin response in vivo.

In the present study, capillary recruitment has been measured by determining arterio-venous extraction of 1-MX across the leg and femoral arterial blood flow. A number of findings emerged in addition to the clearly impaired response to insulin by the obese animals. First, it was evident that the stimulation of capillary recruitment in lean Zucker rats was muted (18) and may reflect partial insulin resistance of the genotype mix of the lean Zucker rats that may be either heterozygous (Fa/fa) or homozygous (Fa/Fa). A heterozygote effect is apparent for body weight, total body fat, and insulin secretion (31). Second, in saline-infused rats the hindlimb 1-MX disappearance was lower in obese rats compared with lean rats. This may result from reduced muscle mass, lower capillary density (32), or decreased perfusion of existing capillaries in the...
basal state. The xanthine oxidase activity in muscle homogenates from obese Zucker rats tended to be reduced. This may be a reflection of a lower capillary density in the obese rats (32), although there is no consensus on this issue (33).

The fact that the basal HGU and 2-DG uptake were not elevated in the lean Zucker rats in accordance with the higher 1-MX metabolism is not unexpected, since it is probably necessary to have insulin present to observe marked alterations in glucose uptake. Furthermore, it is likely that in the obese rats, the basal glucose uptake is partly increased by mass action since the glucose levels were significantly higher than those in the lean group. This would offset any differences in glucose uptake due to the extent of perfusion and would result in similar levels in both groups.

Failure to find a significant increase in hindleg or 2-DG uptake in the obese rats in response to insulin indicates that the insulin resistance is not solely due to the decreased muscle mass. Moreover, insulin resistance is apparent when muscles from obese rats are incubated, suggesting that there is an intrinsic defect in the tissue itself (8). Impairment of insulin's hemodynamic actions in vivo could contribute to the insulin resistance by reducing access for hormone and nutrient but would not be the sole cause of the insulin resistance in these animals.

The novel finding of impaired insulin-mediated hemodynamic effects would imply decreased access of insulin and glucose and therefore a gradient from plasma to interstitium. In contrast, Holmang et al. (34) by use of microdialysis measurements in skeletal muscle, found that the interstitial and plasma insulin concentrations were similar in both lean and obese Zucker rats, and that the insulin resistance of obese Zucker rats is a result of a cellular defect, rather than a hemodynamic one. At this stage it cannot be explained why the findings differ. Much depends on the accuracy of microdialysis in demonstrating small differences in insulin concentration and it is not known what effect changes in capillary recruitment as mediated by insulin have on average interstitial insulin levels.

Tumor necrosis factor-α (TNFα) expression is elevated in adipose tissue of obese individuals and has been implicated as a cause of the associated insulin resistance (35). In support of this, treatment of obese Zucker rats with a soluble TNFα receptor-immunoglobulin G chimeric protein improved GIR during a hyperinsulinemic-euglycemic clamp (36). Similarly, Cheung et al. (37) showed that

![FIG. 6. Arterial plasma concentrations of oxypurinol (A) and 1-MX (B) and arteriovenous 1-MX extraction (C) and 1-MX disappearance measured across the hindleg (D) of saline (□) and insulin clamp–treated (●) lean and obese Zucker rats. Data were collected 120 min after the start of saline or insulin infusion. Values are means ± SE for n = 7–9 in each group. *P < 0.05, significantly different from corresponding saline treatment control; #P < 0.05, significantly different from corresponding treatment in lean Zucker rats.](image-url)
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inhibition of TNFα activity through the adenovirus-5-mediated transfer of a TNF inhibitor gene improved peripheral and hepatic insulin sensitivity in obese Zucker rats. Raised plasma levels of TNFα have been reported in obese Zucker rats (38) and the blunted hemodynamic effects observed in these rats may be a consequence of this (18). However, elevated plasma free fatty acids may be more important (39) and responsible for the insulin resistance of the Zucker rat. Recent data from our laboratory support this view and show that acutely elevated free fatty acids in a nonobese strain markedly impair insulin-mediated capillary recruitment and muscle glucose uptake in vivo (40).

To summarize, insulin-resistant obese Zucker rats displayed blunted insulin-mediated increases in total FBF and capillary recruitment. These abnormalities may contribute to the insulin resistance of muscle in the obese animals.

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