Effects of high-sucrose feeding on insulin resistance and hemodynamic responses to insulin in spontaneously hypertensive rats

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INSULIN IS CHARACTERISTICALLY recognized for its ability to stimulate glucose uptake into insulin-sensitive tissues. On a quantitative basis, skeletal muscle has been identified as the main site of insulin-stimulated glucose disposal and as a major tissue responsible for postprandial hyperglycemia in insulin-resistant states (11). In addition to its effects on glucose metabolism, insulin was shown to vasodilate skeletal muscle vasculature in insulin-sensitive, but not in insulin-resistant, subjects (3). The vasodilating action of insulin has been confirmed by us in conscious rats (35) and by others in humans over a range of physiological insulin concentrations and by using different techniques (1, 51, 52). There is evidence that, over a range of physiological insulin concentrations and by different techniques (1, 51, 52). There is evidence that, among the most likely mechanisms responsible for the hemodynamic responses, there is a role for endothelium-derived nitric oxide (NO) in the mediation of insulin-induced skeletal muscle vasodilation (44, 46). Furthermore, insulin was shown to modulate the expression of the enzyme endothelial NO synthase (eNOS) and to stimulate NO production in both cultured endothelial cells and microvessels from lean rats, whereas in microvessels from insulin-resistant rats, the effects of the hormone on eNOS expression and NO production are blunted, resulting in the loss of its vasodilatory effects (28). The vasodilator effect of insulin has been suggested to play a physiological role in its glucose-lowering action by increasing glucose delivery to metabolically active tissue (3). A defect in this vascular action would contribute to insulin resistance by reducing glucose delivery (3).

An association between insulin resistance/hyperinsulinemia and hypertension has been demonstrated in obesity, Type 2 diabetes (10), and in lean, nondiabetic persons with essential hypertension (4). The observation of an association between insulin resistance and hypertension in humans has stimulated interest and research in the role of insulin in genetic and acquired models of hypertension in animals. Impaired glucose tolerance, reduced glucose-lowering effect of insulin, and hyperinsulinemia have been described in spontaneously hypertensive rats (SHR) (35, 47), in Milan hypertensive rats (9), and in rats made hypertensive by being fed a fructose or sucrose-enriched diet (38). Although these findings remain controversial, recent data have indicated that insulin resistance/hyperinsulinemia could serve as the trigger for the development of endothelial dysfunction and subsequent hypertension (26, 49).

In the present study, we were particularly interested in the SHR, as they represent an animal model of genetic predisposition to develop arterial hypertension during aging, and they have numerous similarities to the human counterpart of essential hypertension (22, 31, 54). The SHR have been used extensively to study blood pressure and represent an interesting model to further investigate the interrelation between hypertension and insulin resistance/hyperinsulinemia. In a previous study, we (35) showed that SHR are insulin resistant and have altered vascular responses to insulin when compared with their normotensive counterpart, the Wistar Kyoto (WKY) rats. Furthermore, high-fructose or high-sucrose diets were reported to further increase blood pressure in those animals (14) and to possibly potentiate the genetic predisposition to insulin resistance (43, 49). Therefore, in the light of these previous data, the present study was initiated to further characterize metabolic responses.
and vascular dysregulations in response to insulin in SHR and to examine the effect of an environmental factor, that is, a high-carbohydrate diet feeding, on these responses. Sucrose was chosen as the carbohydrate in the diet because it provides a significant portion of the calories in the Western diet.

MATERIALS AND METHODS

Animals and feeding protocol. All surgical and experimental procedures were approved by the Animal Care and Handling Committee of Laval University. The research and the care of animals conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). Male WKY rats (n = 36) and SHR (n = 55) aged 6 wk were purchased from Charles River (Saint-Constant, Quebec, Canada) and were housed individually in stainless steel cages. They were placed in a temperature-controlled room (22 ± 1°C) on a 12:12-h light-dark cycle (lights on at 0600). The SHR were randomly divided into two groups. One group (n = 40) was fed standard laboratory rat chow (rodent chow 5075, Charles River, and the other (n = 15) was fed a purified high-sucrose diet. The composition of the diets is given in Table 1. The WKY rats were fed standard laboratory rat chow and were used as normotensive control. The animals were allowed to acclimate to their environmental conditions and diets for 3 wk before the experiments were initiated. During this time, the animals had free access to water and the diet. Body weight and water and food intake were recorded every other day.

Surgical preparation. At the end of the acclimation period, the rats from each group were anesthetized with a mixture of ketamine and xylazine (100 and 10 mg/kg ip, respectively) and had pulsed Doppler flow probes implanted around the left renal and superior mesenteric arteries and the lower abdominal aorta to monitor changes in renal, mesenteric, and hindquarter blood flows, according to the method developed by Gardiner and Bennett (17) and as previously described in detail (35). After surgery, the rats were given a subcutaneous injection of buprenorphine (0.05 mg/kg), were returned to their cages, and allowed to recover for at least 7 days. The chow or sucrose diet continued during postsurgical recovery, and the latter was deemed satisfactory by the resumption of growth and normalization of 24-h food intake. After the recovery period, the rats were reanesthetized (as above), and the leads of the implanted probes were soldered to a microconnector (Microtech). Two separate catheters were implanted in the right jugular vein (for glucose and insulin infusions), and one catheter was implanted in the distal abdominal aorta via the left femoral artery (for measurement of blood pressure and heart rate). The catheters were tunneled subcutaneously to emerge at the same point as the probe wires. The rats were given subcutaneous injections of buprenorphine (0.05 mg/kg) and returned to their cages. The diet continued during this second postsurgical recovery. Experiments began at least 72 h after this last surgical step.

Euglycemic hyperinsulinemic clamp studies. The rats were deprived of food for 10–12 h before the glucose clamp study. Before each experiment, blood glucose and plasma insulin were determined, and the resting heart rate, blood pressure, and regional blood flows were recorded over 30 min in quiet, unrestrained, and unsedated rats. The three groups of rats were divided into three subgroups. The first subgroup was represented by chow-fed WKY rats (n = 13), chow-fed SHR (n = 12), and sucrose-fed SHR (n = 8) receiving insulin at a rate of 4 mU·kg⁻¹·min⁻¹. The second subgroup was represented by chow-fed WKY rats (n = 12), chow-fed SHR (n = 18), and sucrose-fed SHR (n = 7) receiving insulin at a rate of 16 mU·kg⁻¹·min⁻¹. The insulin solution was diluted to the appropriate concentration in saline (0.9% NaCl) containing 0.2% BSA to prevent the adsorption of insulin to the glassware and plastic surfaces. In control experiments, a third subgroup represented by chow-fed WKY rats (n = 11) and chow-fed SHR (n = 10) was infused with saline-0.2% BSA instead of insulin and dextrose to match approximately the saline load delivered during the clamp studies. The control animals were treated in the same way as the groups receiving insulin. After basal measurements of blood glucose and plasma insulin, the euglycemic hyperinsulinemic clamp was then carried out over 2 h, whereas heart rate, blood pressure, and blood flows were measured continuously as previously described (35).

Analytic methods. Blood samples for plasma glucose and insulin determinations in the basal state and during insulin infusion were obtained, placed in untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Minimax, International Equipment). The plasma was stored at −20°C until assay. The glucose concentration of the supernatant was measured by the glucose oxidase method (40) using a glucose analyzer (Technicon RA-XT), and the plasma insulin level was measured by radioimmunoassay (RIA) using porcine insulin standards and polyethylene glycol for separation.

Glucose transport activity in isolated rat skeletal muscles. A second series of experiments was performed in additional groups of chow-fed WKY rats (n = 16), chow-fed SHR (n = 10), and sucrose-fed SHR (n = 11) to characterize the effect of hypertension and high-sucrose diet (4 wk) on glucose transport activity in isolated skeletal muscles. Basal and insulin-stimulated glucose utilization was examined in isolated soleus and extensor digitorum longus (EDL) skeletal muscles from overnight-fasted rats. Glucose transport was measured by use of the glucose analog 2-deoxy-3-[¹⁴C]glucose, according to the method developed by Hansen et al. (19) and as previously described (42). The rats were anesthetized with ketamine-xylazine. Soleus and EDL muscles were dissected out and rapidly cut into 20- to 30-mg strips and incubated for 30 min at 30°C in a shaking water bath into 25-ml flasks containing 3 ml of oxygenated Krebs-Ringer bicarbonate (KRB) buffer supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA (RIA grade). Flasks were gassed continuously with 95% O₂-5% CO₂ throughout the experiment. The rats were then killed by decapitation, and vascular tissues (descending thoracic aorta and mesenteric vascular bed) and gastrocnemius skeletal muscles were quickly removed, clamp frozen, and stored at −80°C for further analysis (see Determination of eNOS protein expression and Western blot analysis).

After the initial incubation, muscles were incubated for 30 min in oxygenated KRB buffer in the absence or presence of insulin (Humulin R) at four different concentrations (0.002, 0.02, 0.2, and 2 nM/mL). Muscles were next washed for 10 min at 29°C in 3 ml of KRB buffer containing 40 mM mannitol and 0.1% BSA. Muscles were then incubated for 20 min at 29°C in 3 ml KRB buffer containing 8 mM 2-deoxy-3-[¹⁴C]glucose (2.25 μCi/ml), 32 mM 1[¹⁴C]mannitol (0.3 μCi/ml), 2 mM sodium pyruvate, and 0.1% BSA.

### Table 1. Diet composition derived from nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Composition, %wt</th>
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<tbody>
<tr>
<td>Carbohydrate</td>
<td>57.3  62.5</td>
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<tr>
<td>Starch</td>
<td>41.2  4.9  20.3</td>
</tr>
<tr>
<td>Sucrose</td>
<td>4.9  6.0  6.5</td>
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<tr>
<td>Protein</td>
<td>18.9  1.0</td>
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<tr>
<td>Fat</td>
<td>4.8  4.7</td>
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<tr>
<td>Vitamins</td>
<td>4.6  5.0</td>
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<tr>
<td>Minerals</td>
<td>4.6  5.0</td>
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<tr>
<td>Fiber</td>
<td>4.6  5.0</td>
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Chow is standard laboratory rat chow (2018 Teklad Global 18% protein rodent diet, Harlan Teklad). Purified high-sucrose diet is composed of the following: protein (casein (purified high nitrogen, ICN Biochemicals, Montreal, Quebec, Canada) and 0.3% L-methionine); vitamin mixture (no. 40060, Teklad, Madison, WI); mineral mixture (AIN-76 mineral mix, ICN Biochemicals); and fiber (cellulose, Alphael, ICN Biochemicals). Gross energy (in kcal/g) for chow-fed and sucrose-fed rats is 4.04 and 4.02, respectively.
Insulin was present throughout the wash and uptake incubations (if it was present in the previous incubation medium). After the incubation, muscles were rapidly blotted at 4°C, clamp frozen, and stored at −80°C until processed. Muscles were processed by boiling for 10 min in 1 ml of water. Extracts were transferred to an ice bath, vortexed, and then centrifuged at 1,000 g. Triplicate 200-μl aliquots of the muscle extract supernatant and of the incubation medium were counted for radioactivity using a Wallac 1409 counter. 2-deoxy-D-[14C]glucose uptake rates were corrected for extracellular trapping using [14C]mannitol (19).

**Discussion**

**Determination of eNOS protein expression and Western blot analysis.** Approximately 200 mg of gastrocnemius were homogenized in 1 ml of homogenization buffer containing 50 mM Tris·HCl (pH 7.5), 1 mM EDTA, 1 mM EGTA, 1 mM DTT, 50 mM NaF, 5 mM Na-pyrophosphate, 10% glycerol, 1% Triton X-100, 100 μg/ml phenylmethylsulfonyl fluoride (PMSF), and protease inhibitors (1 mM pepstatin A, 10 mM E64, and 1 mM leupeptin). The homogenate was centrifuged at 10,000 g for 10 min at 4°C to remove nonhomogenized material (crude homogenate). Protein concentrations of the supernatant were determined by the bichinchoninic acid method (Pierce), using BSA as the standard.

Muscle lysates (2 mg of protein) were used to purify eNOS enzyme with the use of 2',5'-ADP-Sepharose resin as previously described (32). Briefly, the muscle extracts were incubated (2 h, 4°C) with 5 mg (dry weight) of 2',5'-ADP-Sepharose (4B) beads (Amersham Pharmacia) equilibrated in PBS. ADP-Sepharose beads were collected by centrifugation and washed three times with PBS containing 2% Triton X-100. The beads were then boiled 10 min in Laemmli buffer, centrifuged at 6,000 g for 1 min, and subjected to Western blot analysis. Thus samples of muscle homogenates were separated on 6% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Subsequently, proteins were electrothermally transferred (100 V, 2 h) to a polyvinylidene difluoride (PVDF) filter membrane. The PVDF membranes were then incubated for 1 h at room temperature with buffer I [50 mM Tris·HCl (pH 7.5), 150 mM NaCl, 0.04% Igepal, and 0.02% Tween-20] containing 5% nonfat milk, followed by overnight incubation at 4°C with the specific primary antibodies. Monoclonal eNOS antibodies were purchased from Transduction Laboratories (Lexington, KY). Dilution of eNOS antibodies was 1:500 in buffer I containing 1% BSA. PVDF membranes were then washed for 45 min in buffer I (at room temperature), followed by 1-h incubation with anti-mouse immunoglobulin G conjugated to horseradish peroxidase (Amersham, Oakville, Ontario, Canada) in buffer I containing 5% nonfat milk. After PVDF membranes were washed for 45 min in buffer I (at room temperature), the immunoreactive bands were detected by the enhanced chemiluminescence method (Renaincence ECL kit, NEN Life Science, Boston, MA). Autoradiograms were analyzed by laser scanning densitometry using a tabletop Agfa scanner (Arcus II, Etobicoke, Ontario, Canada) and quantified with the NIH Image program (available by anonymous ftp at rsbweb.nih.gov).

**Measurement of immunoreactive endothelin-1 in vascular tissues.** Frozen tissues (mesenteric vascular bed) were homogenized twice with a Tissue-Tearor for 15 s in 2 ml of ice-cold extraction solution [1 M HCl, 1% acetic acid, 1% trifluoroacetic acid (TFA), and 1% NaCl], as previously performed (42). The homogenate was centrifuged at 3,000 g for 30 min at 4°C. The supernatant was then collected, and 100 μl of [125I]-labeled endothelin-1 (ET-1) (~1,000 counts/min) were added before extraction on a C18 Sep-Pak column. The Sep-Pak column was activated with 4 ml 60% acetonitrile and 0.1% TFA and then rinsed twice with 10 ml 0.1% TFA. After sample loading, the column was washed twice with 10 ml of 0.1% TFA, and the immunoreactive ET-1 (ir-ET-1) fraction was eluted with 3 ml of 60% acetonitrile-0.1% TFA and then counted in a gamma counter (recov- ery was 90–95%). The sample extracts were dried overnight in a Speed-Vac and reconstituted in 500 μl RIA buffer. Aliquots of 100 and 200 μl of extracted samples or 200 μl of standards (ET-1, Peninsula Laboratories) were added to 100 μl of anti-ET-1 antibody, and the final reaction volume was adjusted to 300 μl with RIA buffer. After a 24-h incubation period at 4°C, 100 μl of [125I]-labeled ET-1 (15,000 counts/min) in RIA buffer were added, and the tubes were incubated for an additional 24 h at 4°C. Bound and free radioactivity was separated by the second antibody method. After a 2-h incubation period at room temperature, 0.5 ml of RIA buffer were added, and the tubes were centrifuged at 2,500 g for 20 min at 4°C. The supernatant was then discarded, and the pellet was counted in a gamma counter. ir-ET-1 concentrations were corrected for losses in extraction.

**Data analysis.** Values are means ± SE; n is the number of observations. Data were analyzed for statistical significance by ANOVA for repeated measurements. Post hoc comparisons were made using Fisher’s test. A P value < 0.05 was taken to indicate a significant difference.

**RESULTS**

**Hemodynamic responses to insulin infusion during euglycemic hyperinsulinemic clamp.** The baseline values (before any intravenous infusion) for cardiovascular variables are listed in Table 2 for the three groups of rats. As expected, the basal mean blood pressure in SHR rats was higher than in WKY rats. This was accompanied by a smaller basal renal flow in SHR than in WKY rats, whereas there was no significant difference in basal heart rate or basal superior mesenteric and hindquarter flows between SHR and WKY rats. Moreover, we found lower basal renal and superior mesenteric vascular conductances in SHR than in WKY rats but similar basal hindquarter vascular conductance in both strains. Furthermore, as illustrated in Table 2, sucrose feeding in SHR was not associated with significant changes in mean arterial blood pressure, heart rate, regional blood flows, or vascular conductances when compared with values measured in the chow-fed SHR group.

Important cardiovascular responses to insulin were observed in conscious, unrestrained chow-fed WKY rats, chow-fed SHR, and sucrose-fed SHR in which the arterial blood glucose levels were maintained at baseline levels throughout the experiments by administering dextrose during insulin infusion

**Table 2. Baseline values of HR, MAP, regional Doppler shift, and vascular conductance in WKY rats, chow-fed SHR, and sucrose-fed SHR**

<table>
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<th></th>
<th>n</th>
<th>HR, beats/min</th>
<th>MAP, mmHg</th>
<th>Doppler Shift, kHz</th>
<th>Vascular Conductance, kHz/mmHg × 10³</th>
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<td></td>
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<td></td>
<td>Renal</td>
<td>Mesenteric</td>
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<td>WKY</td>
<td>36</td>
<td>328±6</td>
<td>98±2</td>
<td>6.8±0.4</td>
<td>7.9±0.4</td>
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<td>4.0±0.4</td>
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<td>70±4</td>
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<tr>
<td>Chow-fed SHR</td>
<td>40</td>
<td>335±7</td>
<td>135±2*</td>
<td>5.6±0.3*</td>
<td>8.0±1.1</td>
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<td>4.5±0.3</td>
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<td>42±3*</td>
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<tr>
<td>Sucrose-fed SHR</td>
<td>16</td>
<td>317±8</td>
<td>133±3*</td>
<td>6.0±0.7</td>
<td>7.2±0.5</td>
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<td>5.4±0.4</td>
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<td>45±2.5*</td>
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Values are means ± SE; n, no. of rats. Groups represent those used to assess hemodynamic effects of insulin intravenously infused during euglycemic hyperinsulinemic clamp studies. HR, heart rate; MAP, mean arterial blood pressure; WKY, Wistar-Kyoto; SHR, spontaneously hypertensive rat. *P < 0.05 vs. WKY by Student’s t-test for unpaired data.

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(Figs. 1–4). Figures 1 and 3 show that insulin infusion at a rate of 4 or 16 mU·kg⁻¹·min⁻¹ in WKY rats caused long-lasting increases in renal and hindquarter blood flows, but it had no significant effect on mean blood pressure, heart rate, or superior mesenteric flow when compared with measurements after a control infusion of vehicle (saline-0.2% BSA). These responses were associated with increases in renal and hindquarter vascular conductances, but no significant change was seen in superior mesenteric vascular conductance at both doses of insulin (Figs. 2 and 4).

In chow-fed SHR, the same infusions of insulin (at 4 or 16 mU·kg⁻¹·min⁻¹) had no effect on heart rate or hindquarter blood flow, whereas slight but significant decreases in superior mesenteric flow were observed when compared with the effects of control infusion of saline-0.2% BSA (Figs. 1 and 3). These blood flow responses differed significantly from those observed in WKY rats. Furthermore, in chow-fed SHR we found that insulin infusion at the dose of 4 mU·kg⁻¹·min⁻¹ had no effect on renal blood flow, whereas a slight but significant increase in renal flow was noted with the highest dose of insulin tested. However, the latter response was found to be significantly smaller than that observed in WKY rats. Moreover, insulin infusion at 16 mU·kg⁻¹·min⁻¹ in SHR caused a slight but significant increase in mean blood pressure (Fig. 3), which differed significantly from that observed in WKY rats, in which insulin infusion had no effect on mean blood pressure. These cardiovascular responses to insulin were associated with falls in superior mesenteric (at both doses of insulin) and hindquarter vascular conductances (at the dose of 16 mU·kg⁻¹·min⁻¹ only), but no change in renal vascular conductance occurred (at both doses of insulin) (Figs. 2 and 4). These responses differed significantly from those seen in WKY rats, in which insulin produced marked vasodilation in renal and hindquarter vascular beds but had no effect in the superior mesenteric vascular bed.

The sucrose-enriched diet was found to significantly alter some of the cardiovascular changes elicited by euglycemic infusion of insulin when compared with those observed in chow-fed SHR. Thus essentially we found that the pressor responses to both doses of insulin, as well as its hindquarter vasoconstrictor effects, were significantly higher in sucrose-fed SHR than in chow-fed SHR (Figs. 1–4).

**Responses during euglycemic hyperinsulinemic clamp.** Tables 3 and 4 show that, in the fasting state, basal arterial plasma glucose and insulin levels were similar in the three groups of rats studied. During the euglycemic hyperinsulinemic clamp, which was performed at an insulin infusion rate of 4 (Table 3) or 16 mU·kg⁻¹·min⁻¹ (Table 4), we found that fasting plasma insulin levels in the three groups of rats rose acutely and achieved similar plateaus, whereas normal plasma glucose levels were maintained in all groups of rats. However, at both doses of insulin infused, we noted that the average glucose infusion rates required to maintain euglycemia during the last

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**Fig. 1.** Cardiovascular changes elicited by control intravenous infusion of saline-0.2% BSA in conscious, chow-fed Wistar-Kyoto (WKY) rats (n = 11) or spontaneously hypertensive rats (SHR; n = 10), or by euglycemic infusion of insulin at a rate of 4 mU·kg⁻¹·min⁻¹ in chow-fed WKY rats (n = 13) or SHR (n = 12) or sucrose-fed SHR (n = 8). MAP, mean arterial blood pressure; HR, heart rate; bpm, beat per minute; Suc, sucrose. Values are means ± SE shown by vertical lines. Data were analyzed for statistical significance by ANOVA followed by Fisher’s test: *P < 0.05 for insulin-infused groups vs. their respective control saline-BSA groups; †P < 0.05 for sucrose-fed SHR group receiving same intravenous infusion of insulin vs. chow-fed SHR group receiving same intravenous infusion of insulin; §P < 0.05 for chow-fed or sucrose-fed SHR groups vs. chow-fed WKY rats receiving intravenous infusion of insulin.
hour of the clamp (GIR<sub>60-120</sub>) were significantly lower in both groups of SHR than in the group of WKY rats.

**Fig. 2.** Changes in regional vascular conductances elicited by control intravenous infusion of saline-0.2% BSA in conscious, chow-fed WKY rats (n = 11) or SHR (n = 10), or by euglycemic infusion of insulin at a rate of 4 mU·kg<sup>-1</sup>·min<sup>-1</sup> in chow-fed WKY rats (n = 13) or SHR (n = 12) or sucrose-fed SHR (n = 8). These data were derived from data shown in Fig. 1. Values are means ± SE shown by vertical lines. Data were analyzed for statistical significance by ANOVA followed by Fisher’s test: *P < 0.05 for insulin-infused groups vs. their respective control saline-BSA groups; †P < 0.05 for sucrose-fed SHR group receiving intravenous infusion of insulin vs. chow-fed SHR group receiving same intravenous infusion of insulin; §P < 0.05 for chow-fed or sucrose-fed SHR groups vs. chow-fed WKY rats receiving intravenous infusion of insulin.

**Fig. 3.** Cardiovascular changes elicited by control intravenous infusion of saline-0.2% BSA in conscious, chow-fed WKY rats (n = 11) or SHR (n = 10), or by euglycemic infusion of insulin at a rate of 16 mU·kg<sup>-1</sup>·min<sup>-1</sup> in chow-fed WKY rats (n = 12) or SHR (n = 18) or sucrose-fed SHR (n = 7). Values are means ± SE shown by vertical lines. Data were analyzed for statistical significance by ANOVA followed by Fisher’s test: *P < 0.05 for insulin-infused groups vs. their respective control saline-BSA groups; †P < 0.05 for sucrose-fed SHR group receiving intravenous infusion of insulin vs. chow-fed SHR group receiving same intravenous infusion of insulin; §P < 0.05 for chow-fed or sucrose-fed SHR groups vs. chow-fed WKY rats receiving intravenous infusion of insulin.

Effect of high-sucrose diet on 2-deoxy-D-[<sup>3</sup>H]glucose uptake in isolated skeletal muscles. Basal and insulin-stimulated glucose transport activity was measured in isolated skeletal muscles (soleus and EDL) from our three experimental groups. Thus, in the isolated soleus muscles, we found that sucrose feeding significantly increased basal glucose transport activity (2.1 ± 0.2 nmol·g<sup>-1</sup>·min<sup>-1</sup>; n = 11) compared with that observed in chow-fed SHR (1.7 ± 0.1 nmol·g<sup>-1</sup>·min<sup>-1</sup>; n =
Moreover, as illustrated in Fig. 5, we found that the high-sucrose diet in SHR had no effect on insulin-stimulated glucose uptake in isolated soleus muscle when compared with that observed in chow-fed SHR. However, the insulin-stimulated glucose transport activity measured in both groups of SHR was found to be significantly lower than that measured in soleus muscle from WKY rats, particularly at low doses of insulin (i.e., 0.002–0.02 mU/ml for chow-fed SHR and 0.002–0.2 mU/ml for sucrose-fed SHR).

Similarly to what we observed in soleus muscles, we found that sucrose feeding significantly increased basal glucose transport activity in EDL muscles (2.3 ± 0.2 mmol·kg⁻¹·min⁻¹; n = 11) when compared with that seen in chow-fed SHR (1.8 ± 0.1 mmol·kg⁻¹·min⁻¹; n = 10). The basal glucose transport activity measured in EDL muscles isolated from WKY rats (1.7 ± 0.1 mmol·kg⁻¹·min⁻¹; n = 16) was not different from that seen in chow-fed SHR but was significantly lower than that measured in sucrose-fed SHR. Furthermore, Fig. 5 indicates that the insulin-stimulated glucose transport activity (at all doses of insulin tested) measured in the EDL muscles isolated from WKY rats was not different from that observed in chow-fed SHR. However, sucrose feeding in SHR was associated with a reduction in insulin-stimulated glucose transport activity when compared with chow-fed SHR (significant at doses of 0.02–2 mU/ml of insulin) and WKY rats (significant at all doses of insulin tested).

Effect of high-sucrose diet on eNOS protein content in skeletal muscle. We examined the expression of eNOS protein in the gastrocnemius muscle isolated from the three groups of rats and determined whether the skeletal muscle eNOS protein content was affected by hypertension and the high-sucrose diet. Equivalent amounts of muscle proteins were resolved on SDS-PAGE, and immunoblotting was done using an eNOS-specific antibody. Western blot analysis showed that eNOS protein was detectable in the gastrocnemius muscle of the three groups of rats (the chow-fed SHR and WKY rats and the sucrose-fed SHR). The protein migrated as a single band of ~140,000 mol wt (Fig. 6). Immunoreactivity of eNOS was quantified by scanning densitometry, and the relative levels of eNOS protein expression are presented (Fig. 6). The results indicate that eNOS protein expression in SHR was reduced to the levels of 0.82-fold in the gastrocnemius muscle compared with that in WKY (Fig. 6). In sucrose-fed SHR, eNOS expression was reduced to the levels of 0.76-fold compared with that in WKY.

Fig. 4. Changes in regional vascular conductances elicited by control intravenous infusion of saline-0.2% BSA in conscious, chow-fed WKY rats (n = 11) or SHR (n = 10), or by euglycemic infusion of insulin at a rate of 16 mU·kg⁻¹·min⁻¹ in chow-fed WKY rats (n = 12) or SHR (n = 18) or sucrose-fed SHR (n = 7). These data were derived from data shown in Fig. 3. Values are means ± SE shown by vertical lines. Data were analyzed for statistical significance by ANOVA followed by Fisher’s test: *P < 0.05 for insulin-infused groups vs. their respective control saline-BSA groups; †P < 0.05 for control SHR group receiving same intravenous infusion of insulin; §P < 0.05 for chow-fed or sucrose-fed SHR groups vs. chow-fed WKY rats receiving intravenous infusion of insulin.

Table 3. Euglycemic infusion of insulin at a rate of 4 mU·kg⁻¹·min⁻¹ in conscious, unrestrained WKY rats, chow-fed SHR, and sucrose-fed SHR

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose</th>
<th></th>
<th>Plasma Insulin</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Basal, mM</td>
<td>60–120 min, mM</td>
<td>Basal, pM</td>
</tr>
<tr>
<td>WKY</td>
<td>11</td>
<td>5.6±0.1</td>
<td>5.4±0.2</td>
<td>57±6</td>
</tr>
<tr>
<td>Chow-fed SHR</td>
<td>12</td>
<td>5.4±0.2</td>
<td>5.3±0.2</td>
<td>59±10</td>
</tr>
<tr>
<td>Sucrose-fed SHR</td>
<td>7</td>
<td>5.3±0.1</td>
<td>5.4±0.2</td>
<td>82±14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. GIR60–120, glucose infusion rate required to maintain euglycemia during steady-state (60–120 min) plasma insulin concentration. *P < 0.05 vs. WKY rats by Student’s t-test for unpaired data.
DISCUSSION

In the present study, using chow-fed and high-sucrose-fed SHR chronically instrumented with intra-arterial catheters and pulsed Doppler flow probes to directly and continuously record intra-arterial blood pressure, heart rate, and regional blood flows in quiet, unrestrained rats, we noted that the cardiovascular responses to insulin were significantly altered when compared with the responses obtained in their age-matched chow-fed normotensive control. In WKY rats, the euglycemic infusion of insulin elicited vasodilation in renal and hindquarter vascular beds but no change in mean blood pressure, heart rate, or superior mesenteric flow or vascular conductance. In contrast, in chow-fed SHR, the same infusions of insulin caused a marked hindquarter vasoconstriction instead of vasodilation, a significant superior mesenteric vasoconstrictor effect, but had no effect on renal vascular conductance. Moreover, at the high dose of insulin, a slight but significant increase in blood pressure was noted, which was probably secondary to the vasoconstrictor effects of insulin in mesenteric and hindquarter vascular beds. Therefore, the physiological effect of insulin on vasodilate skeletal muscle and renal vasculatures is impaired in SHR. These cardiovascular changes are in keeping with our previous observations made in normotensive rats and SHR (35). The high-sucrose diet in SHR contributed to significantly enhance the pressor response to insulin previously noted in chow-fed SHR and its vasoconstrictor effect in the hindquarter vascular bed, whereas the other vascular responses were not further altered by the sucrose diet. The exact mechanism underlying the altered vascular responses seen in both dietary groups of SHR remains unclear. However, given that the SHR are characterized by sympathetic overactivity (22, 31) and that hyperinsulinemia and sucrose feeding are known to...

Table 4. Euglycemic infusion of insulin at a rate of 16 mU·kg⁻¹·min⁻¹ in WKY rats, chow-fed SHR, and sucrose-fed SHR

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose</th>
<th>Plasma Insulin</th>
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<tbody>
<tr>
<td></td>
<td>60–120 min, mM</td>
<td>60–120 min, pM</td>
</tr>
<tr>
<td>WKY</td>
<td>5.6±0.1</td>
<td>5.3±0.1</td>
</tr>
<tr>
<td>Chow-fed SHR</td>
<td>5.4±0.1</td>
<td>5.3±0.2</td>
</tr>
<tr>
<td>Sucrose-fed SHR</td>
<td>5.3±0.1</td>
<td>5.4±0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4,555±173</td>
</tr>
<tr>
<td></td>
<td>4,668±121</td>
<td>4,958±343</td>
</tr>
<tr>
<td></td>
<td>33±1*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. *P < 0.05 vs. WKY rats by Student’s t-test for unpaired data.
increased ET-1 concentration in the mesenteric arteries isolated from sucrose-fed rats. However, in the present study, we found that, in chow-fed and sucrose-fed SHR, the mesenteric artery content of ir-ET-1 was similar to that measured in WKY rats, suggesting that vascular ET-1 does not play an important role in the impaired endothelium-dependent responses to insulin in these two animal models. These results are consistent with a previous study (29) indicating that the ET system does not appear to be activated in SHR.

A significant reduction in whole body insulin sensitivity together with an alteration in the normal hindquarter vasodilator response to insulin was noted in our two groups of SHR. Given that, on a quantitative basis, skeletal muscle was pointed out as the predominant site of insulin-stimulated glucose disposal and as the major tissue responsible for postprandial hyperglycemia in insulin-resistant states (11), we believe that the impaired vasodilator response to insulin would contribute to insulin resistance by reducing delivery of glucose and insulin to skeletal muscle vascular beds. Although some debate took place over the physiological relevance of the vascular effects of insulin (55), a number of recent lines of evidence further support the concept of a significant functional relationship between the metabolic and vascular actions of insulin. Thus, with the use of higher-resolution methods and physiological concentrations of insulin, recent studies (8, 51) demonstrated the ability of insulin to increase muscle perfusion by recruiting microvascular beds and redistributing flow preferentially to areas with high rates of glucose uptake, suggesting that at least regionally, the metabolic and hemodynamic effects of insulin are coupled. The potential importance of these vascular actions is underscored by the observation that when the action of insulin on total blood flow (3) or capillary recruitment (37) is prevented in vivo, it concomitantly induces an acute state of insulin resistance.

The endothelium-derived NO has been proposed as the mediator coupling vasodilation to glucose metabolism (2). Striking parallels between metabolic insulin-signaling pathways and pathways related to vasodilator actions of insulin provide additional support for the hypothesis that the vascular endothelium is a physiological target of insulin that couples regulation of glucose metabolism with hemodynamics (58). Furthermore, a positive correlation was found between endothelial synthesis of NO and insulin sensitivity (33), and recently it was reported that the targeted disruption of NO production in the mouse induces insulin resistance and reduces insulin stimulation of muscle blood flow (12). These data provide genetic evidence that the enzyme NOS plays a role in modulating insulin sensitivity and carbohydrate metabolism and in mediating insulin vascular action. These findings further support the concept of a functional relationship between the metabolic and vascular actions of insulin, possibly at the endothelial level.

However, it is likely that factors unrelated to hemodynamics, such as an alteration in insulin regulation of glucose extraction at the level of skeletal muscle, could have contributed to insulin resistance noted in our two groups of SHR. Therefore, in the present study, the effect of hypertension and sucrose feeding on basal and insulin-stimulated glucose transport activity was examined in isolated muscles, thus in the absence of blood flow influence. In both soleus and EDL muscles of sucrose-fed SHR, we found that the basal rate of glucose
uptake was higher than that of chow-fed SHR and WKY rats. The precise mechanism for the higher basal glucose transport activity noted in our sucrose-fed rats cannot be assessed from the available data. However, this might represent an adaptive mechanism to the relative decrease in muscle perfusion present in insulin-resistant rats and then result from an increased expression of glucose transporter isoform-1 (GLUT-1), which is normally located in the sarcolemmal membrane and thought to be involved in basal glucose uptake, or from a dysregulation of GLUT-4 distribution, as occurs in muscle and fat of the hyperthyroid rat (45). Further studies are required to clarify this point. On exposure to insulin, we found that soleus muscles from SHR and sucrose-fed SHR exhibit a marked decrease in insulin-stimulated glucose transport activity compared with WKY rats. These findings are consistent with our previous data and with those of other authors indicating a reduced insulin action on glucose uptake in soleus muscles isolated from high-sucrose-fed normotensive rats (42) and from chow-fed SHR (23). However, in EDL muscles, a significant reduction in insulin-stimulated glucose transport activity was only seen in sucrose-fed rats, whereas no differences were noted between chow-fed SHR and WKY rats. The latter results are in line with those of a previous study (48) demonstrating that in vitro, muscle glucose transport was stimulated to a comparable degree by insulin in EDL strips from WKY and SHR. Thus together these findings support that both high-sucrose diet and hypertension cause resistance of skeletal muscle glucose transport to stimulation by insulin and highly suggest that environmental factors, that is, high-calorie diet feeding, can potentiate the genetic predisposition to insulin resistance in SHR at the level of skeletal muscle. The extent to which a reduction in the total cellular content of GLUT-4 proteins or an impairment in the translocation process of the GLUT-4 protein to the cell surface and/or a change in its intrinsic activity contributes toward the reduced insulin-stimulated glucose transport activity remains to be verified.

Another point that needs to be discussed is the fact that despite the significant effects of the sucrose diet on insulin hemodynamic responses in SHR, we did not observe any significant effect of the diet on resting values of mean arterial blood pressure, heart rate, regional blood flows, or vascular conductances in SHR. This appears to contradict some previous studies in which inducing insulin resistance with a high-sugar diet led to a rise in blood pressure in different rat strains. A pressor effect was reported whether glucose (38), sucrose (7, 16, 36, 38, 43, 56), or fructose (21, 39) was used, although sucrose and fructose appear to have greater effects (38). The reason for the different results is not clear, but it may depend on the length of the dietary periods, the content of carbohydrates in the diet and the amount ingested, and the age of the rat, as well as the nutritional status (fed state vs. fasted state), because fasting was reported to lower blood pressure (13). However, it would be important to consider as well that, in these previous studies reporting a hypertensive effect, the observations were almost always based on a simple and indirect measurement of tail systolic blood pressure in restrained animals. Although generally accepted, the tail-cuff method has limitations that may have confounded accurate assessment of blood pressure in these animals (15). Indeed, the elevation of systolic pressure, when measured only at the tail artery, may simply represent an alteration of pressure wave transmission without a significant change in intra-aortic pressure (41). Furthermore, the handling stress and stress associated with tail plethysmography (the installation of the tail cuff and the warming period during the test) may increase the likelihood that any acute measurement of blood pressure would result in a hypertensive reading, especially under conditions of increased blood pressure lability (6), added to the fact that SHR are heat sensitive (34). The results from our study, however, do not exclude the possibility that a high-sugar diet does increase blood pressure lability but suggest that under our experimental conditions (unrestrained rats housed in a quiet room), there may have been insufficient stimulus to cause a pressure response similar to that previously reported in studies using the tail-cuff technique. Our results are consistent with those of Brands et al. (5) and Kobayashi et al. (27), who failed to show any hypertensive effect when blood pressure was recorded intra-arterially in fructose-fed rats not acutely restrained. Our findings are also consistent with a previous report indicating that sucrose diet alone in SHR is not associated with an elevation of blood pressure (18).

In summary, the present study has disclosed some important features of sucrose feeding in the SHR. Notably, sucrose feeding in SHR significantly alters the cardiovascular responses to insulin when compared with their chow-fed hypertensive counterpart. Thus the high-sucrose diet in SHR was shown to markedly enhance the pressor response to insulin and its hindquarter vasoconstrictor effect. A reduction in eNOS protein content in muscle, but no change in vascular ET-1 protein, was also noted in sucrose-fed SHR when compared with WKY rats; however, these changes were not different from those noted in chow-fed SHR. In soleus and EDL muscles isolated from sucrose-fed SHR, we found higher rates of basal glucose transport activity than those noted in chow-fed SHR and WKY rats. Furthermore, a marked and similar decrease in insulin-stimulated glucose transport activity was noted in soleus muscles from both groups of SHR when compared with WKY rats, whereas in EDL muscles, a significant reduction in insulin-stimulated glucose transport was only seen in sucrose-fed rats when compared with the other groups of rats. Taken together, the present findings highly suggest that environmental factors, that is, high intake of simple sugars, can potentiate the genetic predisposition to endothelial dysfunction and insulin resistance in SHR at the skeletal muscle level.

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GRANTS

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