British Journal of Pharmacology (2002) 137, 185–196

www.nature.com/bjp

Induction of insulin resistance by high-sucrose feeding does not raise mean arterial blood pressure but impairs haemodynamic responses to insulin in rats

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1 This study was undertaken to further investigate the effects of a sucrose-enriched diet on vascular function and insulin sensitivity in rats.

2 Male Sprague-Dawley rats were randomized to receive a sucrose- or regular rat chow-diet for 4 weeks. A first group of sucrose- and chow-fed rats was instrumented with pulsed Doppler flow probes and intravascular catheters to determine blood pressure, heart rate, regional blood flows and insulin sensitivity in conscious rats. Insulin sensitivity was assessed by the euglycemic hyperinsulinemic clamp technique. Glucose transport activity was examined in isolated muscles by using the glucose analogue [^{3}H]-2-deoxy-D-glucose. A second group of sucrose- and chow-fed rats was used to obtain information regarding nitric oxide synthase (NOS) isozymes protein expression in muscles, and determine endothelin content in vascular tissues isolated from both dietary groups.

3 Sucrose feeding was found to induce insulin resistance, but had no effect on resting blood pressure, heart rate, or regional haemodynamics. This insulin resistance was accompanied by alteration in the vascular responses to insulin. Insulin-mediated skeletal muscle vasodilation was impaired, whereas the mesenteric vasoconstrictor response was potentiated in sucrose-fed rats. A reduction in eNOS protein content in muscle and an increase in vascular endothelin peptide were noted in these animals. Moreover, a reduction in insulin-simulated glucose transport activity was also noted in muscles isolated from sucrose-fed rats.

4 Together these data suggest that a cluster of metabolic and haemodynamic abnormalities occur in response to the intake of simple sugars in rats.

British Journal of Pharmacology (2002) **137,** 185–196. doi:10.1038/sj.bjp.0704864

Keywords: Insulin resistance; blood pressure; regional blood flow; insulin vascular effects; sucrose diet; glucose transport; endothelin; nitric oxide synthase

Abbreviations: b.p.m., beat per minute; BSA, bovin serum albumin; EDL, extensor digitorum longus; eNOS, endothelial nitric oxide synthase; ET-1, endothelin; GIR, glucose infusion rate; HR, heart rate; iNOS, inducible nitric oxide synthase; ir-ET-1, immunoreactive endothelin; KRB, Krebs-Ringer bicarbonate; MAP, mean arterial pressure; NEFA, nonesterified fatty acids; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PMSF, phenylmethylsulphonyl fluoride; PVDF, polyvinylidene difluoride; RIA, radioimmunoassay; SDS–PAGE, sodium dodecyl sulphate polyacrylamide gel electrophoresis

Introduction

In addition to its effects on glucose metabolism, insulin has been shown to vasodilate skeletal muscle vasculature in insulin-sensitive, but not in insulin-resistant subjects (Baron *et al*., 1993). The vasodilating action of insulin has been confirmed by several groups over a range of physiological insulin concentrations and by using different techniques (Anderson *et al*., 1991; Vollenweider *et al*., 1993; Pitre *et al*., 1996). The endothelium-derived nitric oxide (NO) has been pointed out as the possible mediator of insulin vasodilatory effects in skeletal muscle (Steinberg *et al*., 1994). This vascular effect has been suggested to play an integral part in the glucose lowering action of insulin by increasing glucose delivery to metabolically active tissue, and defects in this action would contribute to insulin resistance by reducing glucose delivery (Baron *et al*., 1993). However, this area is certainly acknowledged to be highly controversial, with counter-arguments indicating that the blood flow effect of insulin has trivial, if any, role on glucose uptake (for review see Yki-Järvinen & Utriainen, 1998). Therefore, in continuity with our previous studies indicating a regulatory effect of insulin on blood flow in rats (Pitre *et al*., 1996;

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Gaudreault *et al*., 2001), and in regard with the 'flow controversy' (Yki-Järvinen & Utriainen, 1998), we were interested to further examine the regional haemodynamic effects of insulin in relation with its glucoregulatory action in both normal and pathophysiological conditions, by using an animal model of insulin resistance, the high sucrose fed rats.

Since the original work of Himsworth (1935) exploring the effects of high- and low-carbohydrate diets on glucose tolerance and insulin sensitivity, numerous studies in rats have clearly shown the capacity of diets high in simple sugars to reduce insulin sensitivity (Gutman *et al*., 1987; Hwang *et al*., 1987; Hulman & Falkner, 1994) and to induce a compensatory hyperinsulinemic response (Hwang *et al*., 1987; Reaven *et al*., 1989). In addition to insulin resistance, several studies, but not all (Brands *et al*., 1994), have reported that feeding rats a diet high in glucose (Reaven & Ho, 1991), sucrose (Reaven & Ho, 1991) or fructose (Hwang *et al*., 1987; Reaven *et al*., 1989) causes hypertension. The demonstration that dietary manipulation also leads to an increase in blood pressure lends further support to previous studies indicating the possibility that insulin resistance and hyperinsulinemia may have a causal role in the genesis of hypertension (Modan *et al*., 1985). Related to this are recent reports indicating a decreased endothelium-dependent relaxation in rats fed a diet high in carbohydrate (Verma *et al*., 1996). Accordingly, this animal model of insulin resistance, the sucrose-fed rats, with its normoglycaemic control, the chow-fed rats, were suitable models for the primary goal of the present study, which was to further explore the blood flow effect of insulin in relation with its glucoregulatory action in normal and pathophysiological conditions. Although several studies have investigated the effects of a high sugar diet on glucose metabolism, no studies have so far conducted experiments looking in parallel at vascular and metabolic actions of insulin in conscious, chronically instrumented rats. The haemodynamic and metabolic profiles associated with this intervention could provide information on possible mechanisms underlying altered insulin sensitivity. In this study, the rats were chronically instrumented with intravascular catheters and pulsed Doppler flow probes to permit a continuous recording of blood pressure, heart rate and regional blood flows. Insulin sensivity was assessed by using the euglycemic hyperinsulinemic clamp technique. Furthermore, we carried out an *in vitro* study to determine glucose transport activity in isolated skeletal muscles, and to obtain information regarding the effects of high-sucrose diets on NO synthase (NOS) isoforms expression in skeletal muscles and ET-1 protein content in vascular tissues.

Methods

Animals and feeding protocol

All surgical and experimental procedures followed institutional animal care guidelines. Fifty-three male Sprague-Dawley rats (Charles River, St-Constant, Canada) aged 5 weeks and initially weighing 200–250 g, were housed individually in stainless steel cages. They were placed in a temperature-controlled room ($22 \pm 1^{\circ}C$) on a 12 h/12 h light/dark cycle (lights on at 0600) and had free access to tap water. The animals were randomly divided into two groups. One group ($n=29$) was fed standard laboratory rat chow (rodent chow 5075, Charles River) and the other ($n=24$) was fed a purified high sucrose diet. The composition of the diets is given in Table 1. The animals were allowed to acclimate to their environmental conditions and diets for 3 weeks before the experiments were initiated. During this time the animals had free access to 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 of the two dietary groups were anaesthetized with a mixture of ketamine–xylazine (100 and 10 mg kg^{-1}, respectively, intraperitoneally (i.p.)) and implanted with pulsed Doppler flow probes to monitor changes in renal, mesenteric and hindquarter blood flows according to the method developed by Gardiner & Bennett (1988), as previously described in detail (Pitre *et al*., 1996). After surgery, the rats were given subcutaneous injections of ampicillin (150 mg kg^{-1}) and buprenorphine (0.1 mg kg^{-1}) and returned to their cages. 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. At least 7 days later, the rats were re-anaesthetized with a mixture of ketamine–xylazine (100 and 10 mg kg^{-1}, respectively, i.p.). The leads of the implanted probes were soldered to a microconnector (Microtech Inc.), and two separate catheters were implanted in the right jugular vein (for glucose and insulin infusions) and one catheter 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 ampicillin (150 mg kg^{-1}) and buprenorphine (0.1 mg kg^{-1}) and returned to their cages. The diet continued during this second postsurgical recovery. Experiments began at least 72 h after this last surgical step in conscious, unrestrained animals with free access to water but not food.

Table 1 Diet composition derived from nutrients

Composition % weight	*Chow**	*Sucrose†*
Carbohydrate	55.0	62.5
Starch	36.3	
Sucrose	3.13	62.5
Protein	20.1	20.3
Fat	5.1	6.5
Vitamins	1.0	1.0
Minerals	4.8	4.7
Fibre	4.6	5.0
Gross Energy kcal/g	4.04	4.02

*Standard laboratory rat chow (rodent 5075, Charles River). †Purified high sucrose diet: Protein is casein (purified high nitrogen, ICN Biochemicals, Montreal, Canada) and 0.3% dl-methionine. Vitamine mixture (No. 40060, Teklad, Madison, WI, U.S.A.). Mineral mixture (AIN-76 mineral mix, ICN Biochemicals). Fibre (cellulose, Alphacel, ICN Biochemicals).

Euglycemic hyperinsulinemic clamp studies

The rats were deprived of food for 12–14 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 flow were recorded over 30 min in quiet, unrestrained, and unsedated rats. Both dietary groups of rats were divided into three subgroups, with the first subgroup represented by chow-fed rats ($n=9$) and sucrose-fed rats ($n=8$) receiving insulin at a rate of 4 mU kg^{-1} min^{-1}, and the second subgroup represented by chow-fed rats ($n=10$) and sucrose-fed rats ($n=8$) receiving insulin at a rate of 16 mU kg^{-1} min^{-1}. In control experiments, a third subgroup of chow-fed rats ($n=10$) and sucrose-fed rats ($n=8$) was infused with saline–0.2% bovine serum albumin (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 as previously described (Pitre *et al*., 1996). Heart rate, blood pressure, and regional blood flow were continuously measured during the clamp study. At the end of the clamp, food (chow or sucrose diets) was returned to the rats. Two days later, some rats randomly chosen from both dietary groups were deprived again of food for 12 h and new experiments were carried out to measure glucose transport activity in isolated skeletal muscles.

Glucose transport activity in isolated rat skeletal muscles

Basal and insulin-stimulated glucose utilization were examined in isolated soleus and extensor digitorum longus (EDL) skeletal muscles from overnight fasted chow-fed ($n=5$) and sucrose-fed rats ($n=5$). Glucose transport in isolated muscles was measured by use of the glucose analogue [^{3}H]-2-deoxy-D-glucose, according to the method developed by Hansen *et al*. (1994) and as previously described (Santuré *et al*., 2000). [^{3}H]-2-deoxy-D-glucose uptake rates were corrected for extracellular trapping using [^{14}C]-mannitol (Hansen *et al*., 1994).

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 Company). The plasma was stored at $-20^{\circ}C$ until assay. The glucose concentration of the supernatant was measured by the glucose oxidase method (Richterich & Dauwalder, 1971) using a glucose analyser (Technicon RA-XT), and the plasma insulin level was measured by radioimmunoassay (RIA) using porcine insulin standards and polyethylene glycol for separation.

Diets and feeding protocol

A second series of experiments was performed in two additional dietary groups to characterize the effect of the high-sucrose diet on postprandial serum variables at a well-defined moment after food intake. Thus, male Sprague-Dawley rats ($n=20$), initially weighing 150–175 g, were placed in room at $22\pm1^{\circ}C$ lighted between 2000 and 0800 h and had free access to tap water. One group of rats ($n=10$) was fed standard laboratory rat chow, and the other ($n=10$) was fed the high sucrose diet for a total of 4 weeks. Body weight and food intake were recorded every other day. The animals had free access to the diets until the third day before the end of the experiment, at which time food intake was restricted to the dark period. Food was provided 30 min after the beginning of the dark period until lights were turned on. This dietary protocol did not alter total 24-h food intake and ensured that all rats began ingesting food at the same time after a fasting period of 12.5 h. Animals from each of the two dietary groups were equally divided into two subgroups. One subgroup ($n=5$) from each of the two dietary groups was killed by decapitation in the fasted state whereas the other ($n=5$) was killed after 2 h of a 30 min food intake period. Blood was collected and kept on ice until centrifuged ($1500\times g$, $4^{\circ}C$, 15 min). The separated serum was stored at $-70^{\circ}C$ until later biochemical determinations. Vascular tissues (descending thoracic aorta and mesenteric vascular bed) and gastrocnemius skeletal muscle were quickly removed, clamp-frozen and stored at $-80^{\circ}C$ for further analysis.

Triacylglycerol, nonesterified fatty acid, glucose and insulin measurements

Serum glucose concentration was determined using a Beckman glucose analyser (Beckman Instruments, Palo Alto, CA, U.S.A.). Insulin was measured by radioimmunoassay using a reagent kit from INCSTAR (Stillwater, MN, U.S.A.) with rat insulin standard. Serum triglycerides were assayed by an enzymatic method (Kohlmeier, 1986) using a reagent kit from Boehringer Mannheim (Montreal, Canada), which allowed correction for free glycerol. Free fatty acids were measured enzymatically using commercially available kits (Wako Chemicals, Dallas, U.S.A.).

Preparation of muscle homogenates

Approximately 1 g of gastrocnemius was homogenized in 5 ml of homogenization buffer containing 25 mmol l^{-1} Tris-HCl (pH 7.4), 1 mmol l^{-1} EDTA and protease inhibitors (100 μg ml^{-1} phenylmethylsulphonyl fluoride (PMSF), 1 mmol l^{-1} pepstatin A, 10 mmol l^{-1} E64 and 1 mmol l^{-1} leupeptin). The homogenate was centrifuged at $1200\times g$ for 10 min at $4^{\circ}C$ to remove non-homogenized material (crude homogenate). Protein concentrations of the supernatant were determined by bicinchoninic acid method (Pierce), using BSA as the standard.

Western blot analysis

Protein samples (150 μg) were subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) on 7.5 or 6% polyacrylamide gels, as described by Laemmli (1970), and electrophoretically transferred (100 V, 2 h) on polyvinylidene difluoride (PVDF) filter membranes. The PVDF membranes were incubated for 1 h at room temperature with buffer I (50 mmol l^{-1} Tris-HCl [pH 7.4],

150 mmol l^{-1} NaCl, 0.04 % Igepal, and 0.02% Tween-20) containing 5% non-fat milk, followed by overnight incubation at 4°C with a specific primary antibodies Dombrowski *et al*., 1996). Monoclonal (eNOS and iNOS) and polyclonal (nNOS) antibodies were purchased from Transduction Laboratories (Lexington, KY, U.S.A.). Dilutions of antibodies were: eNOS, 1 : 500, nNOS, 1 : 250 and iNOS 1 : 500, in buffer I containing 1% BSA. Then, PVDF membranes were washed for 45 min in buffer I (at room temperature), followed by 1 h incubation with either anti-mouse (Amersham, Ontario, Canada), anti-rabbit (Amersham) or anti-goat (Santa Cruz, CA, U.S.A.) immunoglobulin G (1 : 10,000 dilution) conjugated to horseradish peroxidase (Amersham) in buffer I containing 5% non-fat milk (for anti-mouse or anti-rabbit) or 1% BSA (for anti-goat). After PVDF membranes were washed for 45 min in buffer I (at room temperature) the immunoreactive bands were detected by the enhanced chemiluminescence method (Renaissance ECL kit, NEN Life Science, Boston, MA, U.S.A.). Autoradiographs were analysed by laser scanning densitometry using a tabletop Agfa scanner (Arcus II, Etobicoke, Ontario, Canada) and quantified with the NIH Image program (zippy.nmh.nih.gov).

Measurement of ir-ET-1 in vascular tissues

Frozen tissues (descending thoracic aorta or mesenteric vascular bed) were homogenized twice with a Tissue-Tearor for 15 s in 2 ml ice-cold extraction solution (1 M HCl, 1% acetic acid, 1% TFA, and 1% NaCl) (D'Amour *et al*., 1999). The homegenate was centrifuged at $3000 \times g$ for 30 min at 4°C. The supernatant was then collected, and 100 μl of [^{125}I]ET-1 (about 1000 c.p.m.) was added prior to extraction on a C18 Sep-Pak column. The Sep-Pak column was activated with 4 ml 60% acetonitrile and 0.1% TFA, then rinsed twice with 10 ml 0.1% TFA. After sample loading, the column was washed twice with 10 ml 0.1% TFA, and the ir-ET-1 fraction was eluted with 3 ml 60% acetonitrile and 0.1% TFA, then counted in a gamma counter (recovery is 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 adjusted to 300 μl with RIA buffer. After a 24 h incubation period at 4°C, 100 μl of [^{125}I]ET-1 (15,000 c.p.m.) in RIA buffer was added, and the tubes were incubated for an additional 24 h at 4°C. Bound and free radioactivity were separated by the second antibody method. After a 2 h incubation period at room temperature, 0.5 ml RIA buffer was added, and the tubes were centrifuged at $2500 \times g$ 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 ± s.e.mean; *n* is the number of observations. Data were analysed for statistical significance by an analysis of variance (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

Weight and metabolic changes

Table 2 illustrates the effects of the sucrose-enriched diet versus the normal chow diet on body weight, average daily food intake, and resting values for mean arterial blood pressure, heart rate and regional blood flows and vascular conductances. These results demonstrate that, after the 4 weeks of feeding, rats displayed comparable final body weight regardless of whether they had been fed the high sucrose or the normal chow diet. However, the average daily *ad libitum* intake was significantly lower for the sucrose-fed rats compared to their control chow-fed rats. Sucrose feeding was not associated with significant changes in mean arterial blood pressure, heart rate or regional blood flows or vascular conductances as compared with values measured in the normal chow-fed group. Table 3 shows the effects of the long-term diets and of meal intake on serum levels of glucose, insulin, triglycerides and nonesterified fatty acids. These results clearly indicate that diet and the nutritional status interacted on serum glucose and serum insulin. The interaction was due to the fact that acute food intake increased serum glucose and serum insulin more in the sucrose-fed than in chow-fed rats. The combination of higher glycemia and higher insulinemia in the postprandial state found in sucrose-fed rats compared to their chow-fed counterparts is indicative of glucose intolerance and insulin resistance. Triglyceridemia was elevated 2–3 fold by acute food intake, but no significant overall effect of diet composition was observed. Fasting serum levels of nonesterified fatty acids (NEFA) were similar in the two dietary cohorts, whereas food intake decreased serum NEFA levels to the same extent in both groups, indicating that adipose lipolysis was similarly sensitive to the inhibitory action of insulin.

Table 2 Body weight, average daily food intake and baseline values of heart rate, mean blood pressure and regional Doppler shift and vascular conductance in rats

Characteristics	*Chow-fed group* (n = 29)	*Sucrose-fed group* (n = 24)
Initial body weight (g)	235 ± 10	227 ± 9
Final body weight (g)	343 ± 7	337 ± 5
Food intake (g)	48 ± 1	35 ± 1*
Heart rate (beats min^{-1})	336 ± 6	327 ± 6
Mean arterial blood pressure (mm Hg)	87 ± 1	81 ± 2
Doppler Shift (kHz)		
Renal	8.6 ± 0.7	7.8 ± 0.4
Mesenteric	13.8 ± 0.8	13.1 ± 1.3
Hindquarter	6.6 ± 0.4	7.1 ± 0.4
Vascular Conductance (kHz $mmHg^{-1}$)10^3		
Renal	99 ± 8	97 ± 5
Mesenteric	160 ± 10	162 ± 17
Hindquarter	77 ± 6	88 ± 5

Values are means ± s.e.mean; *n* is the number of rats. (The chow-fed and sucrose-fed groups of rats represent those used to assess haemodynamic effects of insulin intravenously infused during the euglycemic hyperinsulinemic clamp studies). *$P<0.05$ sucrose-fed group versus chow-fed group.

Table 3 Fasting and postprandial serum levels of glucose, insulin, triglycerides and none sterified fatty acids of chow-fed and sucrose-fed rats

	Fasting		*Postprandial*[a]	
Parameter	*Chow-fed*	*Sucrose-fed*	*Chow-fed*	*Sucrose-fed*
Glucose (mmol l^{-1})	7.8±0.2	7.9±0.2	8.0±0.1	9.3±0.3*†
Insulin (nmol l^{-1})	0.21±0.04	0.26±0.02	0.57±0.07*	0.84±0.16*†
Triacylglycerols (mmol l^{-1})	1.10±0.18	0.88±0.14	3.18±0.44*	2.15±0.46*
Nonesterified fatty acids (mmol l^{-1})	0.60±0.04	0.59±0.05	0.22±0.04*	0.21±0.03*

Values are means±s.e.mean of five rats. [a]Postprandial values were measured in serum obtained 2 h after meal intake. *$P<0.05$ Chow- or sucrose-fed rats in postprandial state versus their respective fasting control. †$P<0.05$ sucrose-fed rats in postprandial state versus chow-fed rats in postprandial state.

Haemodynamic responses to insulin infusion during the euglycemic hyperinsulinemic clamp

Figure 1A shows that insulin infusion at a rate of 4 mU kg^{-1} min^{-1} in a group of chow-fed rats caused a significant increase in renal blood flow, but it had no effect on heart rate, mean arterial blood pressure, or superior mesenteric or hindquarter flows compared with measurements following a control infusion of vehicle (saline–0.2% BSA). Furthermore, there was no consistent effect on renal, superior mesenteric, or hindquarter vascular conductances, compared with the effects of control infusion of saline–0.2% BSA (Figure 2A). In sucrose-fed rats, the same infusion of insulin had no effect on heart rate, mean arterial blood pressure or superior mesenteric flow, while a significant increase in renal flow and a slight but significant fall in hindquarter flow were observed when compared with the effects of control infusion of saline–0.2% BSA in sucrose-fed rats (Figure 1A). The latter response differed significantly from our observations in chow-fed rats, in which insulin infusion had no effect on hindquarter blood flow. These responses were associated with a marked and long-lasting decrease in hindquarter vascular conductance, but no significant changes were seen in renal or superior mesenteric vascular conductances (Figure 2A). The hindquarter vasoconstrictor effect differed significantly from that seen in chow-fed rats, in which insulin had no effect in the hindquarter vascular bed.

The higher dose of insulin tested (16 mU kg^{-1} min^{-1}) in chow-fed rats produced cardiovascular changes characterized by long-lasting increases in renal and hindquarter flows, but no significant changes were seen in heart rate or mean arterial blood pressure (Figure 1B). Moreover, there was a slight but significant decrease in superior mesenteric flow. Furthermore, significant increases were noted in renal and hindquarter vascular conductances, whereas a significant decrease in superior mesenteric vascular conductance was observed (Figure 2B). In sucrose-fed rats, insulin infusion at the dose of 16 mU kg^{-1} min^{-1} had no effect on heart rate or mean arterial blood pressure, but caused a significant increase in renal flow when compared with the effects of control infusion of saline–0.2% BSA (Figure 1B). These responses were not significantly different from those seen in chow-fed rats. However, insulin infusion in sucrose-fed rats caused significant falls in superior mesenteric and hindquarter flows, which differed significantly from those observed in chow-fed rats (Figure 1B). These cardiovascular responses were associated with increases in renal vascular conductance, that was not different from that seen in chow-fed rats, and decreases in superior mesenteric and hindquarter vascular conductances, when compared with the effects of control infusion of saline–0.2% BSA (Figure 2B). These latter responses differed significantly from those seen in chow-fed rats, in which the same infusion of insulin produced a smaller vasoconstriction in the superior mesenteric vascular bed and a vasodilation in the hindquarter, instead of a vasoconstriction.

Responses during euglycemic hyperinsulinemic clamp

Figure 3 shows that, in the fasting state, basal arterial plasma glucose and insulin levels were similar in the four groups of rats studied. During the euglycemic hyperinsulinemic clamp, we found that for each dose of insulin tested (4 and 16 mU kg^{-1} min^{-1}), fasting plasma insulin levels in both dietary cohorts rose acutely and achieved similar plateaus, whereas normal plasma glucose levels were maintained in every subgroup of rats (Figure 3) However, the average glucose infusion rate required to maintain euglycemia were significantly smaller in the sucrose-fed rats than in their respective chow-fed control group at the two doses of insulin tested (4 and 16 mU kg^{-1} min^{-1}).

Effect of high-sucrose diet on [^{3}H]-2-deoxy-D-glucose uptake in isolated skeletal muscles

The effect of the long-term diets on basal and insulin-stimulated glucose uptake in isolated soleus and EDL muscles is shown in Figure 4. Thus, in both skeletal muscles we found that the sucrose-enriched diet had no influence on basal glucose transport activity compared with that observed in chow-fed rats. However, in the presence of low doses of insulin (i.e. 0.002 and 0.02 mU ml^{-1}), we found a significantly smaller insulin-stimulated glucose transport activity in soleus and EDL muscles isolated from sucrose-fed rats than in those isolated from chow-fed rats. These differences were no longer observed in the presence of higher doses of insulin (i.e. 0.2 and 2 mU ml^{-1}).

Effect of high-sucrose diet on NOS isoforms protein content in skeletal muscle

We examined the expression of NOS isozymes in gastrocnemius muscle isolated from both the sucrose-fed and chow-fed rats, and determined whether skeletal muscle eNOS, nNOS or iNOS protein contents were affected by the high-

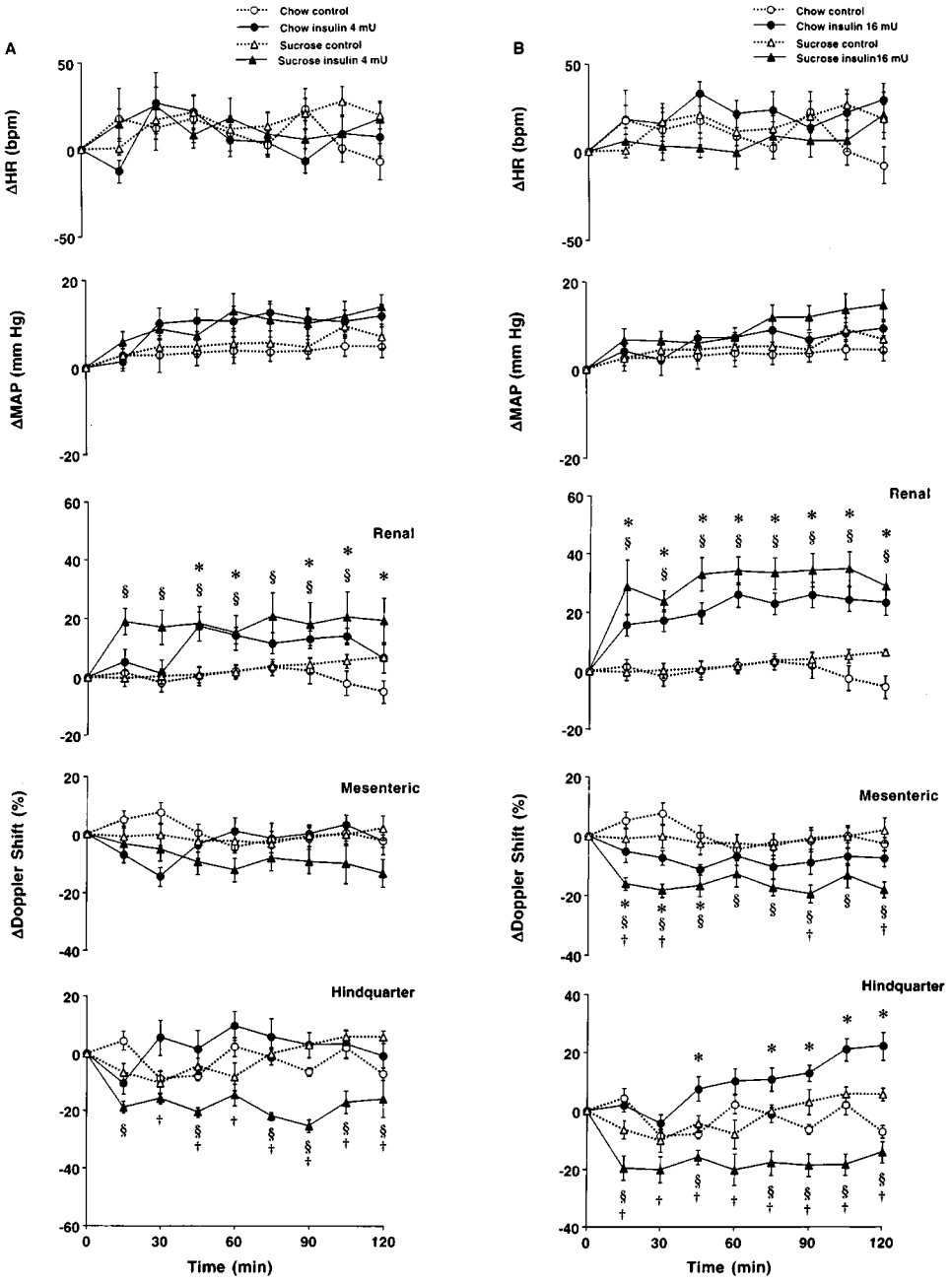

Figure 1 Cardiovascular changes elicited by control intravenous infusion of saline–0.2% bovine serum albumin (BSA, in A and B) in conscious chow- ($n=10$) or sucrose-fed ($n=8$) rats, or by euglycemic infusion of insulin at a rate of 4 (in A; chow-fed, $n=9$; sucrose-fed, $n=8$) or 16 (in B; chow-fed, $n=10$; sucrose-fed, $n=8$) mU kg^{-1} min^{-1} in conscious Sprague Dawley rats. Values are means with s.e.mean shown by vertical lines. *$P<0.05$ when the cardiovascular responses to insulin at the dose of 4 or 16 mU kg^{-1} min^{-1} are compared with those elicited by the control infusion of saline-BSA in the chow-fed group. §$P<0.05$ when the cardiovascular responses to insulin at the dose of 4 or 16 mU kg^{-1} min^{-1} are compared with those elicited by the control infusion of saline-BSA in the sucrose-fed group. †$P<0.05$ for the sucrose-fed group receiving i.v. infusion of insulin versus the chow-fed group receiving the same i.v. infusion of insulin. MAP, mean arterial blood pressure; HR, heart rate; b.p.m., beat per minute.

sucrose diet. Equivalent amounts of muscle proteins were resolved on SDS–PAGE and immunoblotting was done using eNOS, nNOS or iNOS specific antibodies. Western blot analysis showed that eNOS and nNOS proteins were all detectable in gastrocnemius muscle of both groups of rats (the chow-fed and the sucrose-fed groups), whereas iNOS protein was not detected in any group. The proteins migrated as a single band of about 140,000 M_r for eNOS, and 155,000 M_r for nNOS (Figure 5). Immunoreactivity of eNOS and nNOS was quantified by scanning densitometry and the mean data are presented (Figure 5). The results indicate that eNOS protein content was significantly lower in sucrose-fed rats than in chow-fed rats (Figure 5A). In contrast, nNOS protein concentration in gastrocnemius muscle was not found

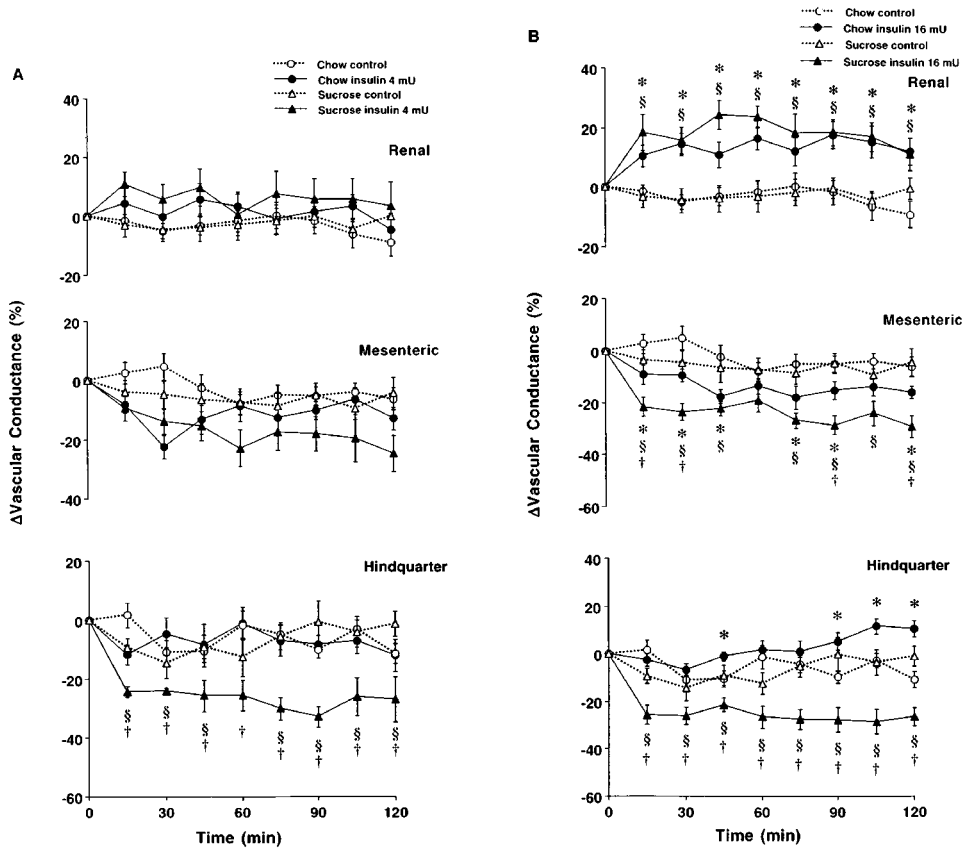

Figure 2 Changes in regional vascular conductances elicited by control i.v. infusion of saline-0.2% BSA (in A and B) in conscious chow- ($n=10$) or sucrose-fed ($n=8$) rats, or by euglycemic infusion of insulin at a rate of 4 (in A; chow-fed, $n=9$; sucrose-fed, $n=8$) or 16 (in B; chow-fed, $n=10$; sucrose-fed, $n=8$) mU kg^{-1} min^{-1} in conscious Sprague Dawley rats. These data were derived from the data shown in Figure 1. Values are means with s.e.mean shown by vertical lines. *$P<0.05$ when the cardiovascular responses to insulin at the dose of 4 or 16 mU kg^{-1} min^{-1} are compared with those elicited by the control infusion of saline–BSA in the chow-fed group. §$P<0.05$ when the cardiovascular responses to insulin at the dose of 4 or 16 mU kg^{-1} min^{-1} are compared with those elicited by the control infusion of saline–BSA in the sucrose-fed group. †$P<0.05$ for the sucrose-fed group receiving i.v. infusion of insulin versus the chow-fed group receiving the same i.v. infusion of insulin.

to be significantly affected by the sucrose enriched diet (Figure 5B).

Effect of high-sucrose diet on ET-1 protein content in vascular tissues

Figure 6 shows the effects of the long-term diets on immunoreactive ET-1 (ir-ET-1) concentration in vascular tissues. The results clearly indicate that sucrose feeding impairs ir-ET-1 concentration in the mesenteric arterial bed isolated from sucrose-fed rats. Thus, a significantly higher ir-ET-1 concentration was noted in the mesenteric arteries isolated from sucrose-fed rats than that from chow-fed rats, whereas similar concentrations were noted in the thoracic aorta of both dietary cohorts.

Discussion

The demonstration in this study that sucrose feeding produces insulin resistance, as determined using the euglycemic hyperinsulinemic clamp technique, is consistent with previous studies carried out in high-fructose or high-sucrose fed rats, and using different techniques to assess insulin resistance (Gutman *et al*., 1987; Hwang *et al*., 1987; Hulman & Falkner, 1994). In the present study, no significant diet effects were noted on plasma glucose or insulin levels in the fasted state, whereas marked differences were seen for postprandial glucose and insulin responses between diets. Indeed, rats fed a high-sucrose diet developed higher postprandial hyperglycemia and hyperinsulinemia than their chow-fed counterparts. Similar diet effects on postprandial glucose and insulin responses have been reported in rats and humans (Kaufman *et al*., 1991; Hulman & Falkner, 1994; Daly *et al*., 1998), and are indicative of glucose intolerance and insulin resistance. However, at variance with some other studies reporting hypertriglyceridemia and elevated blood level of free fatty acid in rats fed a sucrose- or fructose-rich diet (Gutman *et al*., 1987; Hwang *et al*., 1987), our results failed to show any differences in plasma triglyceride or NEFA levels between the two dietary cohorts in either the fasting or the postprandial condition. This discrepancy may best be explained by the fact that diet-induced modifications in the metabolic and hormonal profile are probably dependent on the duration of diet treatment and amount of carbohydrate in the diet (Gutman *et al*., 1987; Lombardo *et*

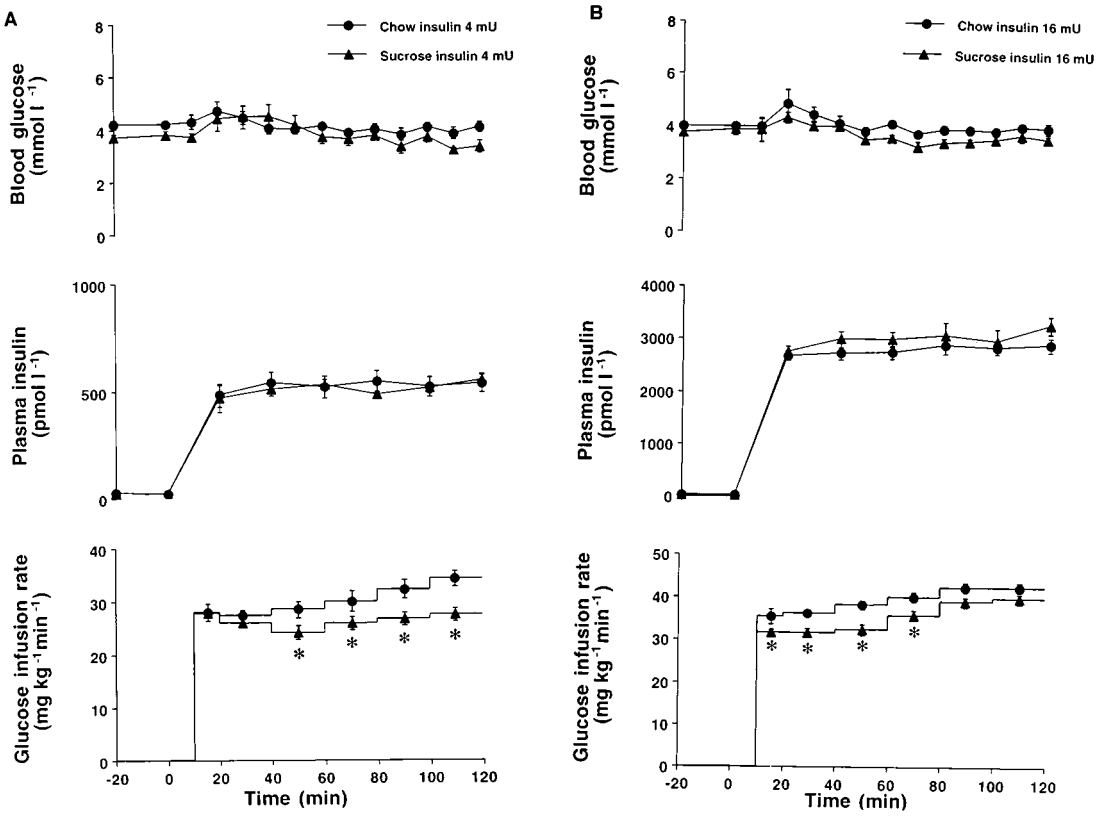

Figure 3 Summary of steady-state blood glucose and plasma insulin concentrations and glucose infusion rate during a euglycemic hyperinsulinemic clamp performed in conscious, unrestrained Sprague Dawley rats. Insulin was infused at a rate of 4 (in A; chow-fed, $n=9$; sucrose-fed, $n=8$) or 16 (in B; chow-fed, $n=10$; sucrose-fed, $n=8$) mU kg^{-1} min^{-1}. Data are presented as means$\pm$s.e.mean. Glucose infusion rates necessary to maintain euglycemia during steady-state hyprinsulinemia is significantly lower in sucrose-fed rats than in chow-fed rats. $^*P<0.05$ for the sucrose-fed rats versus the chow-fed rats.

al., 1996; Pagliassotti *et al*., 2000), and the age of the animals (Pagliassotti *et al*., 2000) at the time of blood sampling.

In this study, sucrose feeding and the concomitant induction of insulin resistance did not lead to a significant rise in blood pressure, or changes in heart rate or regional haemodynamics. 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 (Hwang *et al*., 1987; Reaven & Ho, 1991). A potential problem with those studies reporting a hypertensive effect of high sugar intake, however, is that blood pressure was measured acutely, almost always with the tail-cuff technique. Although generally accepted, this method has limitations that may have confounded accurate assessment of blood pressure in these animals (Ferrari *et al*., 1990). Consistent with our observations, other investigators have also failed to show any hypertension when blood pressure was directly recorded from an intra-arterial catheter in fructose-fed rats not acutely restrained (Brands *et al*., 1994). Therefore, these findings suggest that insulin resistance can be induced by a high-sugar diet in rats without causing hypertension or significant alterations in heart rate or regional haemodynamics.

Although no diet effects were noted on resting blood pressure, heart rate, or regional blood flow, the vascular responses to insulin were significantly altered by the diet. In chow-fed rats, the euglycemic infusion of insulin elicited vasodilation in renal and hindquarter vascular beds and a slight vasoconstriction in the superior mesenteric vascular bed, but no change in mean blood pressure, or heart rate. These cardiovascular changes are consistent with those we previously reported in normal Wistar, Wistar Kyoto and Sprague Dawley rats (Pitre *et al*., 1996; Gaudreault *et al*., 2001). In contrast, in high sucrose-fed rats the same infusions of insulin caused a marked hindquarter vasoconstriction instead of vasodilation, and the superior mesenteric vasoconstrictor effect was potentiated. However, the renal vasodilator response was not altered by the sucrose diet. The reason why different vascular responses were observed in both dietary groups is unclear. However, given that hyperinsulinemia and simple carbohydrate feeding in the rat are known to increase sympathetic activity (Fournier *et al*., 1986; Anderson *et al*., 1991), it is tempting to propose that the difference was caused by a general enhanced vasoconstrictor effect of insulin in sucrose-fed rats due to an abnormal sympathetic overactivity in response to hyperinsulinemia. This would have prevented the insulin-mediated hindquarter vasodilator effect and potentiated the superior mesenteric vasoconstriction.

A second explanation could be an impaired endothelium-mediated relaxation in sucrose-fed rats, as previously shown in fructose-fed rats (Verma *et al*., 1996). Thus, a reduced expression of NOS enzymes with subsequent alteration in production of NO could be one of the factors that contribute to the impaired hemodynamic responses to NO-dependent vasodilator such as insulin and acetylcholine, as previously

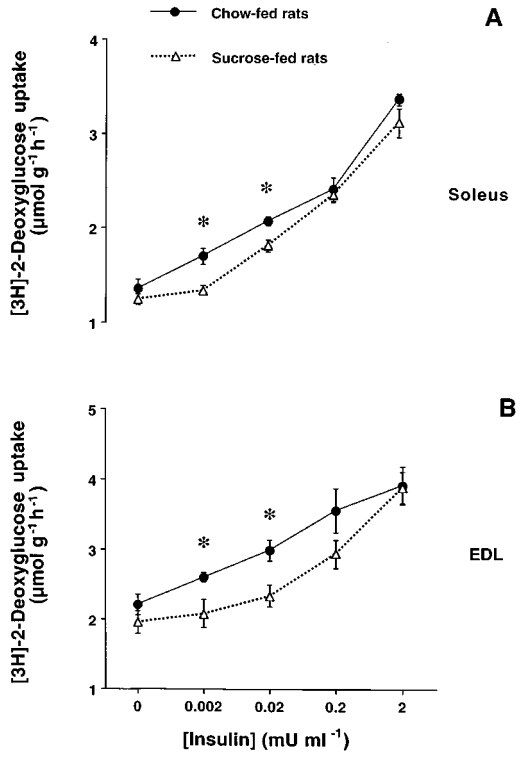

Figure 4 Insulin dose-response curve for stimulation of glucose uptake in soleus and EDL muscles. Muscles were dissected out from chow-fed rats ($n=5$) and sucrose-fed rats ($n=5$). Values are means ± s.e.mean shown by vertical lines. Comparisons were made between insulin evoked responses in chow-fed rats and those in sucrose-fed rats. $^*P<0.05$ for the sucrose-fed rats versus the chow-fed rats.

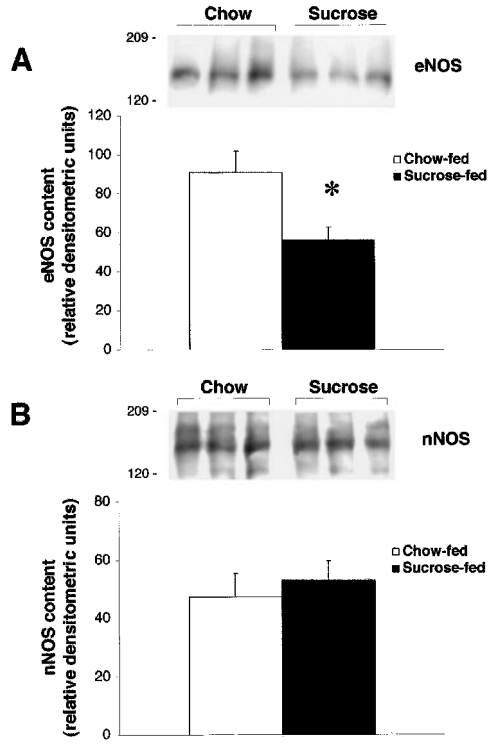

Figure 5 Effects of high-sucrose diet on nitric oxide synthase (NOS) isoforms content in gastrocnemius muscle isolated from overnight fasted sucrose- and chow-fed rats. A representative immunoblot showing eNOS (in A) and nNOS (in B) immunoreactivity in gastrocnemius muscles from three different animals for each dietary group is shown. Muscle protein extracts (150 μg) were subjected to SDS–PAGE on 6% polyacrylamide gels as described in Methods. Immunoblotting was performed with isoform-specific antibodies against eNOS or nNOS, and the reactive bands were detected by enhanced chemiluminescence. Scanning data of eNOS and nNOS contents (in relative densitometric units) are from five individual gastrocnemius muscles in each dietary group and are represented as means ± s.e.mean. $^*P<0.05$ represent a significant difference between chow and sucrose-fed rats.

reported in muscle vascular bed of obese subjects and diabetic patients (McVeigh *et al*., 1992; Steinberg *et al*., 1994; 1996), and in arteries isolated from fructose-fed rats, and diabetic rats (Meraji *et al*., 1987; Verma *et al*., 1996). Indeed, a reduced eNOS expression has been reported in patients with type 2 diabetes and in animal models with insulin resistance (Pieper, 1998; Perreault *et al*., 2000). Furthermore, insulin was shown to modulate eNOS expression and stimulate NO production both in cultured endothelial cells and in microvessels from lean rats, whereas in microvessels from insulin-resistant rats the effects of insulin on eNOS expression and NO production were found to be blunted, resulting in the loss of its vasodilatory effects (Zeng & Quon, 1996; Kuboki *et al*., 2000). In agreement with these previous data, our results show a decreased abundance of eNOS protein in skeletal muscle of sucrose-fed rats. Thus, the impaired hindquarter haemodynamic responses to insulin in sucrose-fed rats could be explained, at least in part, by the down-regulation of eNOS protein in skeletal muscle. This defect could be selective for skeletal muscle vascular beds as the vasodilator responses to insulin was not altered in renal beds of sucrose-fed rats. Although skeletal muscle expresses all isoforms of the NOS family, the endothelial-type, eNOS, is predominantly found in the vascular endothelial cells in skeletal muscle. Whereas only one report suggests that eNOS is also expressed in muscle cells (Kobzik *et al*., 1995), most studies failed to detect eNOS in these cells by immunocytochemistry (Frandsen *et al*., 1996; Segal *et al*., 1999), suggesting that the impaired eNOS expression in sucrose-fed rats is mainly localized to the endothelial cells of the muscle vasculature.

On the other hand, the impaired vascular response to insulin in the sucrose-fed group could be related to an exaggerated production of endothelium-derived contracting factors, such as ET-1 (in the face of normal or altered NO production), which would tend to prevent the insulin-mediated hindquarter vasodilator effects and potentiate superior mesenteric vasoconstriction. Consistent with this is the demonstration here that the mesenteric arteries from the sucrose-fed rats contained a greater amount of ET-1 protein than the chow-fed rats. Similar results have been documented recently in the mesenteric arteries of fructose-fed rats (Verma *et al*., 1996). Insulin is known to stimulate gene expression of vascular ET-1 *in vitro*, and modulates ET-1 production and release (Frank *et al*., 1992; Hu *et al*., 1993). Moreover, elevated vascular expression of ET-1 and ET_A receptor mRNA and increased plasma levels of ET-1 have been reported in fructose-fed rats (Juan *et al*., 1998). Thus, it is possible that an increased production of ET-1 in response to

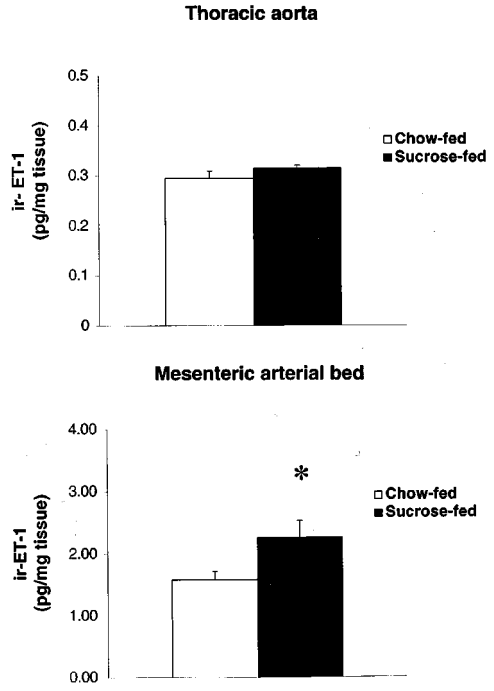

Figure 6 Immunoreactive endothelin 1 (ir-ET-1) concentration in thoracic aorta and mesenteric arterial bed of chow- and sucrose-fed rats. Arteries were dissected out from overnight fasted chow-fed rats ($n=4$) and sucrose-fed rats ($n=4$). Values are means$\pm$s.e.mean shown by vertical lines. *$P<0.05$ for sucrose-fed rats versus chow-fed rats.

insulin stimulation could explain some of the differences seen in the vascular responses noted in sucrose- and chow-fed rats.

Together, the results presented here coupled with those of previous studies suggest that the conjunction of sympathetic overactivity, decreased expression of eNOS protein (and possibly impaired NO production), and elevated expression of ET-1 in vascular tissues may have contributed to blunt the insulin-mediated hindquarter vasodilation and to potentiate the superior mesenteric vasoconstriction in the sucrose-fed rats. The absence of significant change in blood pressure in sucrose-fed rats despite marked hindquarter and enhanced superior mesenteric vasoconstrictor responses to insulin might be explained by a differential change in cardiac output. Thus a reduction in cardiac output accompanied by an increased peripheral vascular conductance in sucrose-fed rats would result in no change in blood pressure. The reason why in the present study the renal vasodilator response to insulin was not altered by the sucrose diet is unclear. However, it is possible that individual vascular tissues are differentially affected by the diet, probably as a function of different NO synthase activity or autoregulation of the balance between vasoactive agents, or a different susceptibility to nutrients. It is also possible that the impairment of insulin's action in the vascular system occurs at different rates in individual vascular tissues, depending on the amounts of sucrose in the diet and the duration of feeding. Further studies will be necessary to clarify this point.

A significant reduction in whole-body insulin sensitivity together with an alteration in the normal hindquarter haemodynamic response to insulin was noted in sucrose-fed rats. According to the haemodynamic concept put forth by Alain Baron and his associates (Baron *et al*., 1993), the impaired haemodynamic response to insulin could have contributed, at least in part, to the insulin resistance observed in sucrose-fed rats, by reducing glucose delivery to skeletal muscle cells. On a quantitative basis, skeletal muscle has been identified as the predominant site of insulin-stimulated glucose disposal, and as the major tissue responsible for postprandial hyperglycemia in insulin resistant states (Baron *et al*., 1993). However, the role of defects in insulin stimulated blood flow in the pathogenesis of insulin resistance is highly controversial, as other investigators failed to demonstrate an association between the blood flow effect of insulin and glucose uptake (for review see Yki-Järvinen & Utriainen, 1998). Therefore, in order to distinguish the direct effects of insulin (on glucose uptake) from its effects on blood flow in the different experimental groups, we carried out experiments in isolated skeletal muscles to examine the effect of sucrose feeding on glucose transport activity, thus in the absence of blood flow influence. The soleus and EDL muscles were obtained from chow- and sucrose-fed rats. We found that, compared with a rat chow diet, the high-sucrose diet caused resistance of skeletal muscle glucose transport to stimulation by insulin. These results do not necessarily rule out the possibility of a haemodynamic contribution to insulin resistance in sucrose-fed rats, but indicate that other factors, such as insulin receptor disturbance (downregulation/desensitization/uncoupling), or alterations in glucose transporter expression, or subcellular distribution, and various functional characteristics, could also contribute to insulin resistance in sucrose-fed rats, and have been involved in other models of insulin-resistance, such as obesity and diabetes (Kahn & Saad, 1992; James & Piper, 1994).

In summary, the present study has disclosed three important features of sucrose feeding in the rat: (1) it induces insulin resistance, but has no effect on mean blood pressure, heart rate, or regional haemodynamics measured continuously in conscious, unrestrained rats; (2) this insulin resistance is accompanied by alteration in the vascular responses to insulin. The insulin-mediated skeletal muscle vasodilation, shown to be NO-dependent, was impaired in sucrose-fed rats. Interestingly, a reduction in eNOS protein content in muscle, and an increase in vascular ET-1 protein were also noted in these animals; (3) sucrose-induced insulin resistance translates into a reduction in insulin-simulated glucose transport activity in isolated muscles, particularly at low concentrations of insulin. These data, taken together with those of previous studies in high-fructose or high-sucrose fed rats, suggest that a cluster of metabolic and haemodynamic abnormalities occur in response to the intake of simple sugars in rats.

The authors wish to thank Marie Tremblay and Josée Lalonde for their expert assistance. This work was supported by grants to Dr H. Bachelard from the Medical Research Council of Canada and the Heart and Stroke Foundation of Québec. H. Bachelard is a chercheur-boursier of the Fonds de la recherche en santé du Québec.

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(Received January 1, 2002
Revised April 29, 2002
Accepted June 28, 2002)