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Defective Endothelium-Dependent Relaxation in Fructose-Hypertensive Rats

Subodh Verma, Sanjay Bhanot, Linfu Yao, and John H. McNeill

The present study examined the endothelium-dependent and -independent responses of isolated mesenteric arteries to acetylcholine and the endothelium-independent vasodilator sodium nitroprusside in mesenteric arteries from fructose-induced hypertensive rats. Fructose feeding resulted in hyperinsulinemia and elevated blood pressure when compared to controls (plasma insulin, 5.9 ± 0.4 *v* control 3.6 ± 0.4 ng/mL, $P <$ .05; systolic blood pressure, 154 ± 5 *v* control 127 ± 7 mm Hg, $P <$ .05). The maximum contractile response of mesenteric arteries to norepinephrine did not differ between the control and fructose groups, either with or without the endothelium. In arteries with intact endothelia, precontracted with the approximate ED_{50} of norepinephrine, the percent maximum relaxation produced by acetylcholine in hypertensive rats was lower than the control arteries (62 ± 7 *v* control 95 ± 5, $P <$ .05) without any change in sensitivity. In arteries precontracted with norepinephrine, the endothelium-independent vasodilator sodium nitroprusside produced a dose-dependent relaxation in arteries obtained from control and fructose groups, both with and without the endothelium. The maximum relaxation produced by sodium nitroprusside did not differ between control and fructose arteries, either with or without the endothelium; however, removal of the endothelium caused an increase in sensitivity of this agonist. These data suggest that in the insulin resistant and hyperinsulinemic fructose-hypertensive rats, there is a defective endothelium-dependent yet preserved endothelium-independent relaxation. Am J Hypertens 1996;9:370–376

KEY WORDS: **Insulin resistance, hyperinsulinemia, fructose-hypertension, endothelial function.**

The association between hyperinsulinemia, insulin resistance, and hypertension has been documented extensively; however, the mechanisms or mediators linking insulin resistance and hyperinsulinemia to elevated blood pressure (BP) are poorly understood and need further clarification.[1–7] Recently, we have become interested in the role of endothelium-derived contracting and relaxing factors in insulin-resistant and hyperinsulinemic rodent models of hypertension. Our interest stems from reports suggesting that plasma insulin may be an important endogenous vasodilator.[8–11] Moreover, insulin-mediated vasodilation has been shown to be nitric oxide (NO) dependent.[12] Hence, it is plausible that in states of insulin resistance, insulin-mediated vasodilation may be blunted, which may contribute toward the development or maintenance of elevated BP.[8,9,13] Thus, it would be relevant to study the endothelium-dependent and -independent responses in an insulin-resistant and hyperinsulinemic model of hypertension. The fructose-hypertensive (FH) rat model has been used to study the interrela-

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tionship between hyperinsulinemia, insulin resistance, and hypertension, wherein feeding normal Sprague-Dawley rats a high fructose-diet results in hyperinsulinemia, marked insulin resistance, and elevated BP.[3,7,14] In the present study responses of isolated mesenteric arteries from FH rats to the endothelium-dependent vasodilator, acetylcholine (ACh) and the endothelium-independent vasodilator, sodium nitroprusside (NaNP) were examined. Here, we show that mesenteric arteries from FH rats exhibit a decreased relaxation to ACh but not to NaNP, suggesting an impaired endothelium-dependent relaxation in FH rats.

RESEARCH DESIGN AND METHODS

Animals and Experimental Design Male Sprague-Dawley rats were procured at 5 weeks of age (University of British Columbia animal care unit) and were assigned to two experimental groups: control (C, n = 14) and fructose (F, n = 14). At week 6 (weeks signify the age of the rats) systolic BP, plasma glucose, and plasma insulin (5-h fasted) were measured. Starting at week 7, the rats in the F group were started on a 66% fructose diet for 3 weeks. The sodium content of the diet was reasonably similar to that of the standard rat chow (standard chow, 4 g / kg; fructose diet, 4.2 g / kg). At week 11, mesenteric arteries from both control and fructose rats were removed and endothelium-dependent and -independent relaxation to ACh and NaNP was studied in vessels precontracted with norepinephrine (NE, described below).

BP Measurement Systolic BP was measured in conscious rats using the indirect tail-cuff method without external preheating.[15] The animals were preconditioned to the experimental procedure before the actual measurements were conducted. In this method, the reappearance of pulsations on gradual deflation of the BP cuff are detected by a photoelectric sensor and are amplified and recorded digitally as the systolic BP. An average of five such readings was taken to obtain the individual systolic BP. We have validated readings obtained by this method by comparison with those obtained by direct intraarterial cannulation. Recorded pressures were similar (within 5 mm Hg) to those obtained by direct cannulation; similar results have been reported by other laboratories.[14–16]

Tissue Preparation and Protocol At week 11, rats from both C and F groups were killed with an overdose of pentobarbital and the superior mesenteric artery was carefully dissected out, cleaned of adherent connective tissue, and cut into rings. Two rings, approximately 3 mm in length, were dissected from each rat. One ring of each pair was left intact, and the other ring was gently rotated on a stainless steel rod to remove the endothelium. The tissues were suspended in an isolated tissue bath containing modified Krebs-Ringer bicarbonate solution with the composition (in mmol / L): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $NaHCO_3$(25), dextrose (11.1), and edetate calcium disodium (0.026), maintained at 37°C, and oxygenated with 95% O_2 / 5% CO_2. Each ring was placed under a resting tension of 1.0 *g*. The tissues were then allowed to equilibrate for 90 to 120 min before the experiments were conducted. Isometric responses were recorded on a Grass polygraph (Grass Instruments, Quincy, MA).

In an initial study, cumulative concentration-response curves to NE were obtained in rats from C and F groups, both with and without the endothelium. The approximate median effective dose (ED_{50}) was calculated for both C and F groups (with and without endothelium) and was used to precontract the arteries in the relaxation studies. The approximate ED_{50} was determined to be 5×10^{-7} mol / L with the endothelium intact and 4×10^{-8} mol / L with the endothelium denuded. The value did not differ between the C and F groups.

Cumulative concentration-response curves to ACh were obtained in arteries from C and F rats (precontracted with approximately the same ED_{50}) with and without the endothelium. After a 60-min wash period, the arteries were contracted with NE and the cumulative concentration-response curves to NaNP were obtained. At the completion of each experiment, the length of the tissues was measured. The tissues were then placed on a wet filter paper, lightly blotted and weighed. The cross-sectional area of each tissue was calculated using the formula: Cross-sectional area (mm^2) = Weight (mg) / [Length (mm) × Density (mg / mm^3)]. The density of the arteries was assumed to be 1.05 mg / mm^3. Responses of each preparation were then calculated as the tension (g) in response to each agonist / cross-sectional area of the tissue (mm^2). Agonist pD_2 ($-\log ED_{50}$) were also calculated by nonlinear regression analysis of the dose-response curves.

Drugs All drugs used in the present study (ACh and NaNP) were obtained from Sigma (St. Louis, MO). The fructose diet was obtained from Harlan Teklad Laboratories (Madison, WI).

Biochemical Measurements Plasma glucose was measured by the glucose oxidase method, using a diagnostic kit from Boehringer Mannheim Laval (Québec, Canada). Plasma insulin was assayed using a double-antibody radioimmunoassay, using a kit from Linco Research Inc. (St. Louis, MO).

Statistics Results are expressed as mean ± SEM. The variable "n" represents the number of animals in each group. Results were compared using a one-way analysis of variance (ANOVA) followed by a Newman-Keuls test. A probability of $P < .05$ was taken to indicate a significant difference between means.

RESULTS

The F rats were hyperinsulinemic and hypertensive when compared to the C rats (plasma insulin, 5.9 ± 0.4 ng/mL *v* C 3.6 ± 0.4 ng/mL, $P < .05$; systolic BP, 154 ± 5 mm Hg *v* C 127 ± 7 mm Hg, $P < .05$; Table 1). Body weight, plasma glucose, and mesenteric cross-sectional area did not differ between C and F groups (Table 1).

The maximum contractile response of mesenteric arteries to NE did not differ between C and F groups either with or without the endothelium (with endothelium, C 1.8 ± 0.14 g/mm^2 *v* F 1.7 ± 0.15 g/mm^2, $P > .05$; without endothelium, C 2.09 ± 0.07 g/mm^2 *v* F 2.18 ± 0.06 g/mm^2, $P > .05$; Figure 1). However, both the maximum response to NE and the sensitivity significantly increased after removal of the endothelium (pD_2 values, C(−) endothelium 7.77 ± 0.1 *v* C(+) endothelium 6.93 ± 0.15, $P < .05$; F(−) endothelium 7.76 ± 0.14 *v* F(+) endothelium 7.07 ± 0.16, $P < .05$; Table 2).

In arteries precontracted with NE, ACh produced a dose-dependent relaxation of both C and F arteries with intact endothelia (Figure 2). This effect was abolished in both vessels by removal of the endothelium (Figure 2). The percent maximum relaxation produced by ACh in F arteries was significantly lower than the control arteries (62 ± 7 *v* C 95 ± 5, $P < .05$; Table 2), without any change in sensitivity (6.53 ± 0.16 *v* C 7.12 ± 0.43, $P > .05$; Table 2).

In arteries precontracted with NE, the endothelium-independent vasodilator NaNP produced a dose-dependent relaxation in both C and F groups both with and without the endothelium (Figure 3). The maximum relaxation produced by NaNP did not differ between C and F arteries either with or without endothelia (Table 2). However, removal of the endothelium caused a significant increase in sensitivity in both C and F rats (pD_2 values: C(−) endothelium 8.12 ± 0.06 *v* C(+) endothelium 7.44 ± 0.08, $P < .05$; F(−) endothelium 8.17 ± 0.17 *v* F(+) endothelium 7.50 ± 0.08, $P < .05$; Table 2).

TABLE 1. GENERAL CHARACTERISTICS OF THE RATS IN THE TWO GROUPS AT WEEK 11

Characteristics	Control	Fructose
Weight (g)	438 ± 8	451 ± 12
Systolic blood pressure (mm Hg)	127 ± 7	154 ± 5*
Plasma glucose (mmol/L)	5.8 ± 0.2	5.5 ± 0.5
Plasma insulin (ng/mL)	3.6 ± 0.4	5.9 ± 0.4*
Cross-sectional area of mesenteric arteries (mm^2)	0.45 ± 0.03	0.51 ± 0.06

* $P < .05$ *different from control.*

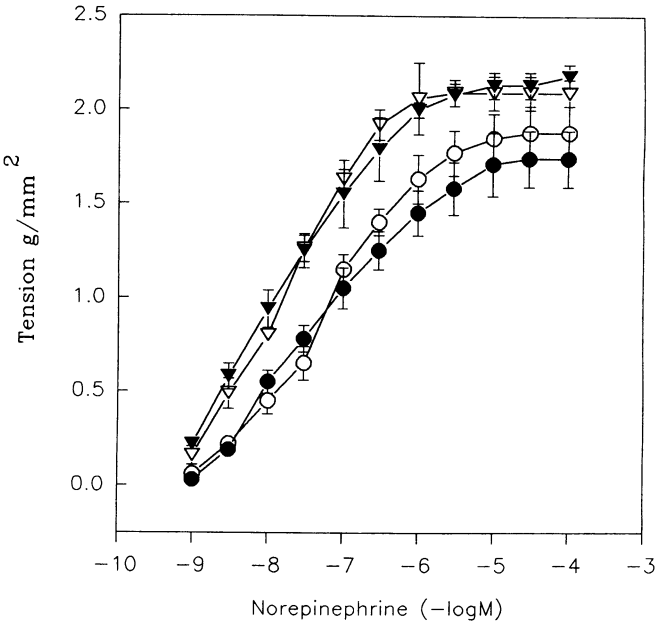

FIGURE 1. *Dose-response curves of mesenteric arteries from control (C) and fructose (F) rats to norepinephrine, in the presence (C, open circles; F, closed circles) and absence (C, open triangles; F, closed triangles) of the endothelium. Each curve represents the mean of data obtained from five arteries. Each point is represented as the mean ± SE.*

DISCUSSION

The primary finding of this study is that superior mesenteric arteries from FH rats exhibit depressed endothelium-dependent relaxation to ACh. The decreased maximum relaxation appears to be chiefly endothelium dependent, as the responses to the endothelium-independent vasodilator NaNP were preserved. A diminished ability of vascular smooth muscle (VSM) to relax has been implicated as an important factor that may contribute to the elevated peripheral resistance in hypertension.[17–19] Data from the present experiment are consistent with a similar phenomenon in the FH rat.

The endothelium plays a central role in the maintenance of VSM tone and reactivity by secreting several endothelium-derived contracting and relaxing factors such as nitric oxide (NO), prostacyclin, endothelin, angiotensin II, and cyclooxygenase-derived contracting factors.[20,21] Two discoveries in the last decade, namely, NO (endothelium-derived relaxing factor) and endothelin have captured the focus of current research; the former capable of fully relaxing blood vessels and the latter being the most potent endogenous vasoconstrictor known to date.[20,21] Recent reports suggest that a critical balance between endothelium-derived contracting and relaxing factors is crucial in maintaining normal VSM tone and reactivity. Essential hypertension is characterized by both mor-

TABLE 2. SENSITIVITIES AND MAXIMUM RELAXATION OF MESENTERIC ARTERIES FROM CONTROL (C) AND FRUCTOSE (F) RATS WITH AND WITHOUT ENDOTHELIA

	Sensitivity (pD_2)				Maximum Decrease in Tension (% of Contraction to Norepinephrine)			
	With Endothelium		Without Endothelium		With Endothelium		Without Endothelium	
	C	F	C	F	C	F	C	F
Norepinephrine	6.93 ± 0.15	7.07 ± 0.16	7.77 ± 0.10*	7.76 ± 0.14*	—	—	—	—
Acetylcholine	7.12 ± 0.43	6.53 ± 0.16	—	—	95 ± 5	62 ± 7†	—	—
Sodium nitroprusside	7.44 ± 0.08	7.50 ± 0.08	8.12 ± 0.06*	8.17 ± 0.17*	100 ± 0	100 ± 0	100 ± 0	100 ± 0

* $P < .05$ *different from C and F with endothelium.*
† $P < .05$ *different from C.*

phologic and functional alterations of the endothelium leading to an imbalance of endothelium-derived contracting and relaxing factors.[17,20,22,23] Such an imbalance may be central to the development or reinforcement of the hemodynamic alterations, which are characteristic of essential hypertension.[21]

ACh-evoked relaxation of isolated blood vessels is known to be endothelium dependent and mediated by NO formation from L-arginine.[24] Hence, a decreased endothelium-dependent relaxation in FH rats may be attributable to a reduced production of NO from L-arginine, a reduced responsiveness of blood vessels to NO or an exaggerated production of endothelium-derived contracting factors (ET-1 and prostaglandin H_2).[20,21,25] As both ACh and NaNP vasodilate through the same second messenger system (cyclic GMP), the observation that the relaxation to ACh is impaired, whereas the responses to the endothelium-independent vasodilator NaNP are preserved in mesenteric arteries from FH rats, suggest that the sensitivity of the VSM to NO may be similar between the C and FH rats. As NO was not quantitated in this study, the extent to which a reduced production of NO contributes toward the decreased endothelium-dependent relaxation cannot be commented on. A decreased NO production form of L-arginine has been observed in the mesenteric arteries of the spontaneously hypertensive rat (SHR) and the 2-kidney, 1-clip renovascular hypertensive rat.[26–28] Alternatively, it is possible that the decreased endothelium-dependent relaxation may be related to an exaggerated production of endothelium-derived contracting factors such as ET-1 and prostaglandin H_2 (in the face of normal or altered NO production). An important observation that stems from studies in the aorta of the SHR is that the impaired endothelium-dependent responses to ACh and other agonists appear to be related to a simultaneous production of endothelium-derived contracting factors in addition to a decrease in NO production.[29–31] This observation is based on studies demonstrating that in the aorta of the SHR, ACh causes endothelium-dependent contractions mediated by prostaglandin H_2; blockade of this pathway (by cyclooxygenase inhibitors) restores endothelium-dependent relaxation to ACh. We are currently pursuing studies to delineate the exact mechanisms underlying the defective endothelium-dependent relaxation in FH rats.

Fructose feeding resulted in hyperinsulinemia and elevated BP, an observation that has been documented previously.[3,7,14] We have demonstrated previously that fructose feeding for similar periods of time as those used in this study, results in hyperinsulinemia and marked insulin resistance, as measured by the euglycemic hyperinsulinemic clamp.[3] In an attempt to establish a cause-effect relationship between hyperinsulinemia, insulin resistance, and hypertension, we examined the effects of multiple drug interventions (vanadyl sulfate, metformin, and organic vanadium compounds) that improve these defects(ie, lower plasma insulin levels and improve insulin sensitivity) on blood pressure in rats.[3–7] Interestingly, all interventions used prevented the development of high BP in both the SHR and the FH rat models. Moreover, restoration of the plasma insulin levels in drug-treated rats to levels that existed before treatment, reversed the effect on BP. These studies clearly indicated that either hyperinsulinemia and insulin resistance are causally related to the development of high BP in rats, or that the underlying mechanisms are closely associated with expression of both of these disorders. However, the primary question that needs to be addressed is what the particular mechanisms or mediators are that link hyperinsulinemia or insulin resistance to hypertension in rats.

Although hyperinsulinemia has been proposed to increase BP through several mechanisms including activation of the sympathetic nervous system, sodium retention, VSM hypertrophy, and alterations of VSM calcium handling,[32–35] the precise nature of these mechanisms remain elusive. Although the endothelium (by secreting contracting and relaxing factors) plays a pivotal role in the regulation of VSM tone in

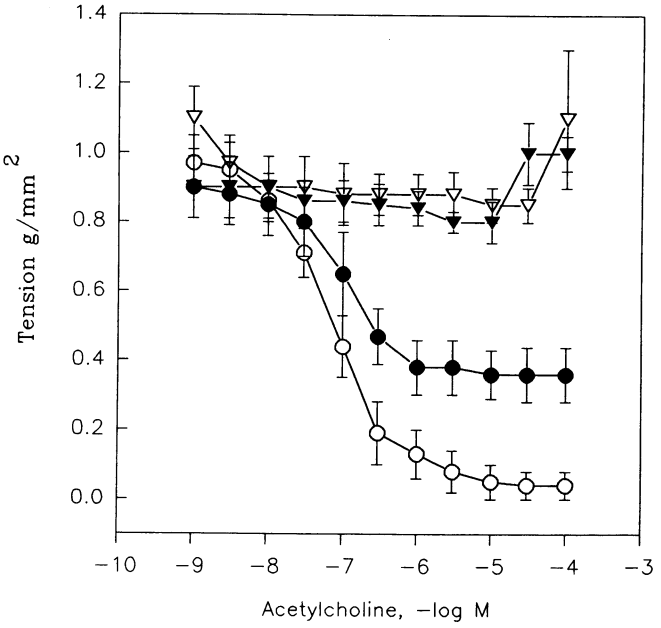

FIGURE 2. *Dose-response curves of mesenteric arteries from control (C) and fructose (F) rats to acetylcholine, in the presence (C, open circles; F, closed circles) and absence (C, open triangles; F, closed triangles) of the endothelium. Each point is represented as the mean ± SE. Arteries with intact endothelium were precontracted with 5 × 10^{-7} mol/L norepinephrine and produced an increase in tension of 0.94 ± 0.06 g/mm^2 in C (n = 8) and 0.90 ± 0.07 g/mm^2 in F (n = 7). Arteries without endothelium were precontracted with 4 × 10^{-8} mol/L norepinephrine and produced an increase in tension of 1.0 ± 0.12 g/mm^2 in C (n = 6) and 0.92 ± 0.15 g/mm^2 in F (n = 7).*

both physiologic and pathophysiologic states, information on endothelial function in states of hypertension associated with hyperinsulinemia and insulin resistance is lacking. Of particular importance are two recent concepts suggesting a link between insulin resistance, hyperinsulinemia, endothelial dysfunction, and elevated BP. First, recent studies suggest that insulin stimulates endothelin release both in vivo and in vitro.[36–38] More important, plasma ET-1 levels are increased in insulin-treated diabetic and nondiabetic rats.[39] Elevated ET-1 levels have also been reported in type II diabetic subjects.[40] These studies led us to hypothesize that hyperinsulinemia (induced by fructose feeding) may provide a continual stimulus for ET release, which may increase BP by altering plasma or local concentrations of ET. In an attempt to gain insight into the role of ET in FH hypertension, we have recently studied the effects of chronic endothelin receptor blockade (using bosentan) on FH rats.[41] Chronic bosentan treatment prevented the increase in BP in these rats, which suggests that endothelin may play a role in the development of FH in rats. Second, at the other end of the spectra are studies documenting that insulin may be an important endogenous vasodilator and that insulin-mediated vasodilation may represent a physiologic action of insulin.[8–11]

Although the exact mechanisms through which insulin causes vasodilation remain to be defined, of interest are studies demonstrating that under conditions of euglycemic hyperinsulinemia, insulin-mediated vasodilation is NO dependent[12] and suppression of such vasodilation leads to increases in arterial pressure.[42] If insulin-mediated vasodilation represents a physiologic action of insulin, it is plausible that in states of insulin resistance a defect in the insulin-induced stimulation of NO could be one of the factors that contributes to altered vascular function and hypertension. Although the results from the present study do not allow us to answer this question unequivocally, these data may suggest that induction of insulin resistance (by chronic fructose feeding) may blunt insulin's vasodilatory effects and contribute toward the development or maintenance of elevated BP in these rats. Indeed, insulin-mediated increases in skeletal muscle blood flow are blunted in the insulin-resistant states of obesity, noninsulin dependent diabetes mellitus, and hypertension.[8,9,43] Taken together, data from

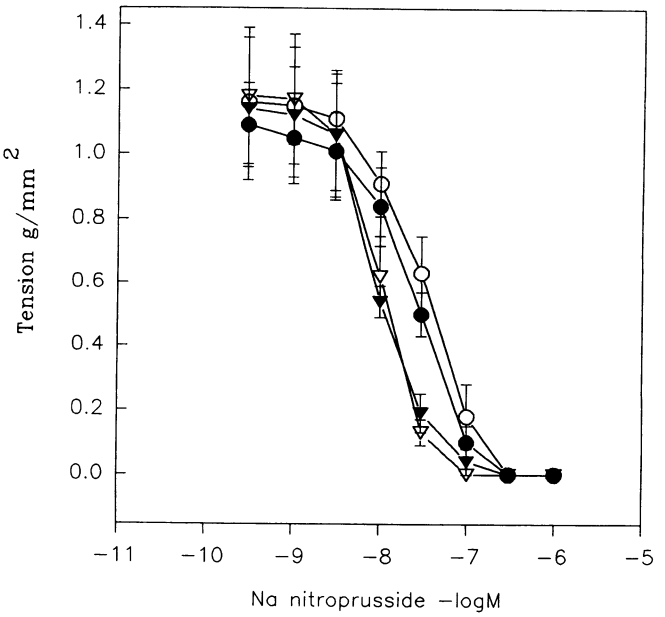

FIGURE 3. *Dose-response curves of mesenteric arteries from control (C) and fructose (F) rats to sodium nitroprusside, in the presence (C, open circles; F, closed circles) and absence (C, open triangles; F, closed triangles) of the endothelium. Each point is represented as the mean ± SE. Arteries with intact endothelium were precontracted with 5 × 10^{-7} mol/L norepinephrine and produced an increase in tension of 1.17 ± 0.1 g/mm^2 in C (n = 8) and 1.06 ±. 0.9 g/mm^2 in F (n = 7). Arteries without endothelium were precontracted with 4 × 10^{-8} mol/L norepinephrine and produced an increase in tension of 1.2 ± 0.16 g/mm^2 in C (n = 6) and 1.1 ± 0.13 g/mm^2 in F (n = 7).*

our previous study demonstrating a role of endothelin in FH rats, coupled with results from the present study demonstrating a defective endothelium-dependent relaxation in FH rats, suggests an attractive hypothesis whereby an imbalance in the NO and endothelin pathways may contribute toward the development or maintenance of hypertension in these rats.

It is important to note that the involvement of cyclooxygenase-constricting factors (mainly prostaglandin H_2) in mediating the decreased response to ACh cannot be excluded (as the study was conducted in the absence of a cyclooxygenase inhibitor). Of particular importance in this regard are recent studies documenting that the mesenteric vascular effects of insulin are not associated with ET-1 release but appear to be linked to an increased release of endothelial-derived contracting factors from the cyclooxygenase pathway.[44] This extensive study by Wu et al[44] demonstrated that perfusion of isolated mesenteric arteries with insulin and insulinlike growth factor-I resulted in the potentiation of the arginine-vasopressin dose-response curve. These responses were not affected by ET blockade (by BQ-123), but were markedly inhibited by indomethacin, suggesting the involvement of the cyclooxygenase pathway in mediating this effect. As the study was conducted in perfused mesenteric arteries from insulin-sensitive and normotensive rats, the extent to which both insulin resistance and hypertension chronically modulate these pathways remains to be determined.

Whether insulin resistance or hyperinsulinemia, or both, account for the functional changes in the endothelium, or whether these changes are a consequence of hypertension, is a question that remains to be answered. Furthermore, as insulin resistance, hyperinsulinemia, and hypertension can impair endothelial function independently, the extent to which each of these factors contribute either individually or additively toward the altered endothelium-dependent relaxation remains unclear. However, what is perhaps more important is the observation that in FH rats there is a specific defect in the ability of resistance vessels to relax; this defect appears to be mainly endothelium dependent as the responses to the endothelium-independent vasodilator NaNP are preserved. Further studies are needed to delineate the exact mechanisms underlying the defective endothelium-dependent relaxation and to examine whether these defects are attributable to hyperinsulinemia and insulin resistance or whether they are simply a consequence of elevated BP in these rats.

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