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Age-dependent changes in blood pressure and arterial reactivity in obese Zucker rats

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Abstract

The purpose of the present investigation was to determine whether there is an association between changes in arterial reactivity to vasoactive agents and the development of hypertension in obese Zucker rats. At 20 weeks of age, obese rats were mildly hypotensive compared to their lean littermate controls. Maximum contractile responses of endothelium-intact mesenteric arteries from these rats to noradrenaline, endothelin-1 and KCl were depressed, although there was no change in relaxation to acetylcholine. By 32 weeks of age, obese rats had developed hypertension compared to their lean littermate controls. Maximum contractile responses of mesenteric arteries from 32-week-old obese rats to noradrenaline and endothelin-1 were no longer significantly different than control, although contractile responses to KCl remained depressed. In addition, there was a small increase in sensitivity to endothelin-1, while endothelium-dependent relaxation to acetylcholine was impaired. In contrast, there were no changes in contractile responses of endothelium-intact aortas from either 20- or 32-week-old obese rats to noradrenaline, endothelin-1 or KCl, while endothelium-dependent relaxation of this artery to acetylcholine was slightly enhanced at both ages. Therefore, changes in the reactivity of the mesenteric artery but not the aorta from obese Zucker rats parallel changes in blood pressure in these animals.

Keywords: Zucker rat; Blood pressure; Mesenteric artery; Aorta; Noradrenaline; Endothelin-1; Endothelium

1. Introduction

Obesity is recognized as a major risk factor for the development of hypertension, and the two often occur together with insulin resistance, hyperinsulinemia and dyslipidemia, a cluster of abnormalities known as the metabolic syndrome (Reaven, 1988; Gurnell et al., 2003). A close association between insulin resistance/hyperinsulinemia and hypertension has been noted in a number of studies, although whether they are causally related is still not clear. However, insulin resistance has been reported to result in a number of changes that could promote elevation of blood pressure, including activation of the sympathetic nervous system, local activation of the renin–angiotensin system, and direct vascular dysfunction with impaired endothelium-dependent relaxation and increased intracellular calcium leading to enhanced vasoconstriction (reviewed in Kirpichnikov and Sowers, 2001).

The obese Zucker rat develops many of the characteristics associated with the metabolic syndrome in humans, including insulin resistance associated with marked hyperinsulinemia, dyslipidemia and hypertension. It has been widely used to investigate changes in responsiveness of the vasculature to vasoconstrictor and vasodilator hormones in an attempt to more clearly characterize the vascular dysfunction associated with obesity and insulin resistance. However, the results of these studies have not given a clear picture of the nature of the changes in vascular responsiveness or their association with the development of hypertension in this model. For instance, although a number of studies have reported that endothelium-dependent relaxation to acetylcholine is preserved or even enhanced in vascular preparations from obese Zucker rats (Zemel et al., 1990; Auguet et al., 1989; Cox and Kikta, 1992; Sexl et al., 1995; Turner et al., 1995; Hopfner et al., 1999; Andrews et al., 2000), impaired acetylcholine-in-

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duced relaxation has also been reported (Zanchi et al., 1995; Walker et al., 1997; Arvola et al., 1999). These differences have likely arisen in part because of differences between studies in the arterial preparation and the age of the animals investigated. Indeed, in the only two studies in which vascular responsiveness was determined at different ages, endothelium-dependent relaxation was found to be enhanced in aortas from 12-week-old obese animals but not in those from older rats (Cox and Kikta, 1992; Sexl et al., 1995). Few studies have compared the responsiveness of different vascular preparations from obese rats of the same age. However, Hopfner et al. (1999) reported increased reactivity of the aorta from 15-week-old obese Zucker rats to methoxamine and endothelin-1, but no change in responsiveness of the perfused mesenteric arterial bed to these agonists or to acetylcholine. To further complicate matters, the age of onset of hypertension in the obese Zucker rat seems to vary greatly in different studies, with some investigators detecting elevated blood pressure at 6–8 weeks (Zemel et al., 1992), while others have found it to be delayed beyond 24 weeks of age (Cox and Kikta, 1992). This makes it difficult to relate changes in vascular reactivity with blood pressure in those studies in which blood pressure was not reported.

The purpose of the present investigation was to clarify whether there is an association between changes in arterial reactivity to vasoactive agents and the development of hypertension in obese Zucker rats. To this end, we compared the reactivity of the aortas and mesenteric arteries from male obese Zucker rats and their lean littermate controls, prior to and following the development of hypertension in the obese rats.

2. Materials and methods

Male obese (fa/fa) Zucker rats and their lean male littermates were obtained at the age of 8–10 weeks from the Department of Physiology, University of British Columbia, Vancouver, Canada. Animals were treated in accordance with the Animal Care Guidelines of the Canadian Council on Animal Care; protocols involving animals were approved by the University of British Columbia Animal Care Committee. Lean and obese rats were housed separately in a room with a 12-h dark/light cycle (light on 0700–1900). Food and water was provided ad libitum.

Table 1
Characteristics of 20- and 32-week-old male lean and obese Zucker rats

	n	20 weeks		*n*	32 weeks	
		Lean	Obese		Lean	Obese
Body weight (g)	19	393 ± 6	516 ± 10[a]	14	441 ± 8	619 ± 19[a]
Systolic blood pressure (mm Hg)	15	149 ± 2	139 ± 2[a]	12	146 ± 3	174 ± 3[a]
Glucose (mM)	19	7.7 ± 0.5	9.0 ± 0.5	7	10.2 ± 0.3	10.3 ± 0.7
Insulin (ng/ml)	19	2.9 ± 0.4	10.7 ± 0.9[a]	14	4.7 ± 0.5	6.6 ± 1.0
Cross-sectional area (mm^2)						
Aorta	19	0.80 ± 0.03	0.74 ± 0.04	14	0.91 ± 0.04	1.07 ± 0.04[a]
Mesenteric artery	18	0.17 ± 0.02	0.16 ± 0.02	14	0.33 ± 0.02	0.37 ± 0.02

Values are mean ± S.E., *n* = number of animals.
[a] $P < 0.05$ (one-way ANOVA) compared to lean of same age.

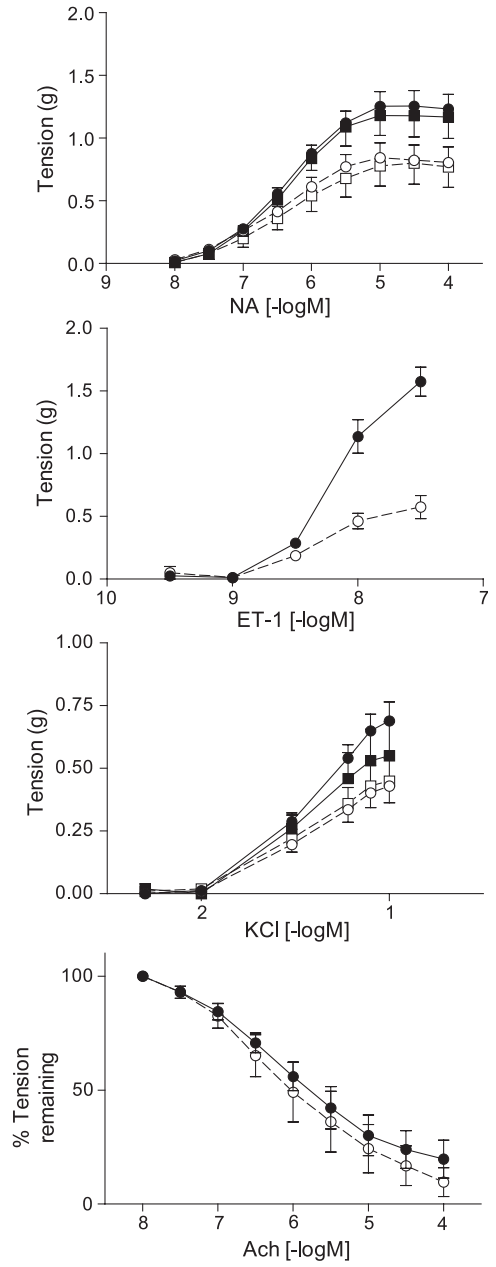

Fig. 1. Concentration–response curves of mesenteric arteries from 20-week-old lean and obese Zucker rats to noradrenaline, endothelin-1, KCl and acetylcholine. Filled circles: endothelium-intact lean, filled squares: endothelium-denuded lean, open circles: endothelium-intact obese and open squares: endothelium-denuded obese.

Table 2
Agonist pD_2 and R_{max} values in mesenteric arteries from 20-week-old lean and obese rats

	Lean		Obese	
	Endothelium intact	Endothelium denuded	Endothelium intact	Endothelium denuded
R_{max} (g)				
Noradrenaline	1.3 ± 0.1 (13)	1.2 ± 0.2 (5)	0.9 ± 0.1[a] (12)	0.8 ± 0.1 (5)
Endothelin-1	1.6 ± 0.1 (4)	ND	0.6 ± 0.09[a] (4)	ND
KCl	0.7 ± 0.08 (13)	0.6 ± 0.1 (5)	0.4 ± 0.07[a] (12)	0.5 ± 0.09 (5)
pD_2				
Noradrenaline	6.4 ± 0.08	6.5 ± 0.2	6.6 ± 0.08	6.4 ± 0.12
Endothelin-1	7.9 ± 0.09	ND	8.1 ± 0.07	ND
KCl	1.4 ± 0.04	1.5 ± 0.03	1.5 ± 0.03	1.5 ± 0.02

Values in parentheses represent number of animals. ND, not determined.
[a] $P < 0.05$ compared to corresponding lean value.

2.1. Measurement of systolic blood pressure

Systolic blood pressure of lean and obese Zucker rats was measured within 7 days of the experiment by the indirect tail cuff method. Briefly, the animals were placed in perspex restraining tubes and housed in an incubator maintained at 25–28 °C. Following a 15-min incubation period, the blood pressure was measured with an inflatable cuff and pulse sensor placed around the tail of the animals and coupled to a semiautomatic blood pressure system (IITC, Woodland Hills, CA, USA). The inflated cuff pressure was set at 250 mm Hg and pressure released at a rate of 500 mm Hg min^{-1}. Systolic blood pressure was calculated as the mean of at least three readings. To accustom them to the setting, rats were placed in the apparatus once each day for 3 days prior to the day that blood pressure was actually recorded.

2.2. Experimental protocol

Animals were sacrificed at 20 or 32 weeks of age by a single intraperitoneal injection of pentobarbital and blood was collected from the chest cavity for estimation of plasma glucose and insulin levels. A section of the thoracic aorta between the aortic arch and the diaphragm, and the superior mesenteric artery were removed and immediately placed in modified Krebs–Henseleit solution of the following composition (mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 1.8, NaH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0 and dextrose 11.0. After carefully cleaning the loosely adherent fat and connective tissue, the arteries were cut into 4-mm rings and placed on wire tissue hooks in 20-ml jacketed organ baths containing modified Krebs–Henseleit solution, continuously oxygenated with a mixture of 95%O_2/5% CO_2 at 37 °C. One hook was connected to a fixed tissue support and the other hook was connected to a Grass FT.03 (Grass Systems, Quincy, MA) force displacement transducer. The endothelium was removed in some aortic and mesenteric arterial rings by gently rubbing the inside of the rings on a stainless steel wire.

The arterial rings were equilibrated for at least 60 min under a resting tension of 1.0 (mesenteric arteries) or 2.0 (aorta) g. During the equilibration period, the Krebs solution in the bath was changed every 20 min. At the end of equilibration, the functional integrity of endothelium was tested by determining the ability of rings precontracted

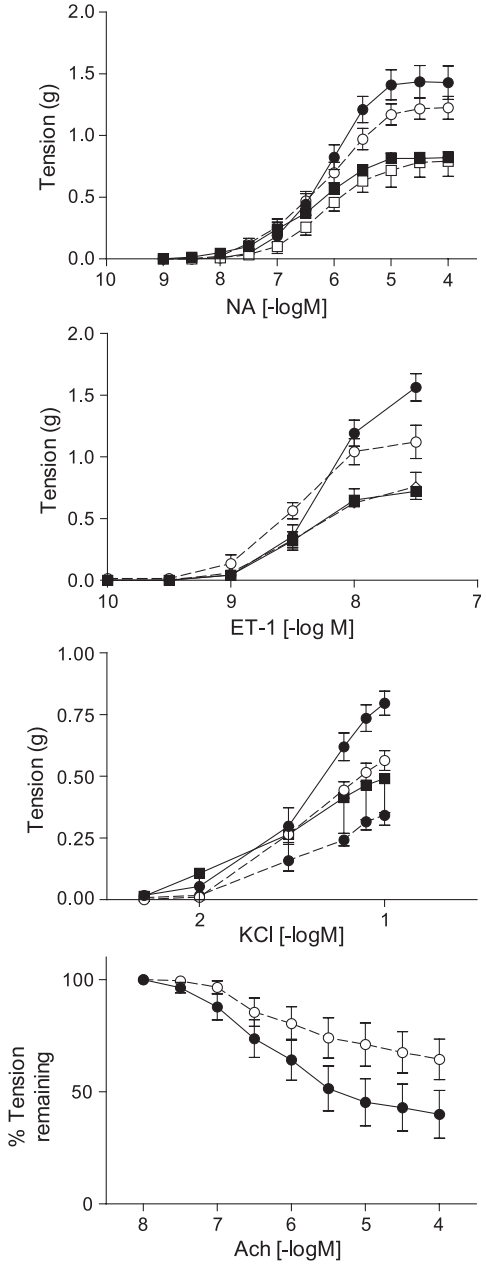

Fig. 2. Concentration–response curves of mesenteric arteries from 32-week-old lean and obese Zucker rats to noradrenaline, endothelin-1, KCl and acetylcholine. Filled circles: endothelium-intact lean, filled squares: endothelium-denuded lean, open circles: endothelium-intact obese and open squares: endothelium-denuded obese.

with an ED_{70} concentration of phenylephrine to relax in response to 10^{-5} M acetylcholine.

Contractile responses to noradrenaline, endothelin-1 and KCl were determined by cumulative addition of the agonists to the tissue bath to give the final concentration indicated in the figures. Tissues were pre-incubated with 10^{-5} M phentolamine for 15 min before concentration–response curves to KCl were obtained. Where the vasorelaxant responses to acetylcholine were studied, the tissues were precontracted with an ED_{70} concentration of phenylephrine. When the contractile response to phenylephrine reached a plateau, acetylcholine was added in a cumulative manner. In experiments where the effects of N^G-monomethyl-L-arginine (L-NMMA) on responses to noradrenaline, endothelin-1 and KCl were determined, tissues were pretreated with the nitric oxide (NO) synthase inhibitor (100 μM) for 60 min before constructing concentration–response curves to the agonists.

2.3. Statistical analysis

All data are presented as the mean ± S.E. Concentration–response curves were analyzed by nonlinear regression using GraphPad Prism version 3.00 (GraphPad Software, San Diego, CA) for calculation of pD_2 ($-\log EC_{50}$) values and maximum responses (R_{max}). Statistical significance was evaluated by one-way or two-way analysis of variance (ANOVA) followed by Newman–Keuls post hoc test for multiple comparisons, using NCSS. A $P<0.05$ was considered statistically significant.

2.4. Drugs

(−)-Noradrenaline hydrochloride, acetylcholine chloride, endothelin-1 and all chemicals were purchased from Sigma (St. Louis, MO). L-NMMA was purchased from Calbiochem (La Jolla, CA). A stock solution of noradrenaline was prepared daily in distilled water containing 1 mg/ml ascorbic acid, to prevent oxidation.

3. Results

Some characteristics of the rats used in this study are shown in Table 1. At both 20 and 32 weeks of age, obese rats weighed significantly more than their lean littermate controls. Interestingly, 20-week-old obese rats were slightly hypotensive in comparison to their lean controls, but this situation was reversed in 32-week-old obese rats, which had significantly elevated blood pressure. There were no differences in plasma glucose between lean and control rats at either age, although 20-week-old obese rats had very elevated insulin levels. However, by 32 weeks of age, insulin levels in the obese rats were similar to those of their lean littermates.

There were no significant differences in cross-sectional areas of mesenteric arteries from 20- and 32-week-old obese rats and their lean controls, although a marked increase in cross-sectional area between 20 and 32 weeks was evident in mesenteric arteries from both lean and obese rats (Table 1). The cross-sectional area of aortas from 20-week-old obese rats was not significantly different from control, but by 32 weeks of age the cross-sectional area of aortas from obese rats was significantly greater than that of aortas from age-matched controls (Table 1).

3.1. Mesenteric artery

Maximum contractile responses (R_{max}) of endothelium-intact mesenteric arteries from 20-week-old obese rats to noradrenaline, endothelin-1 and KCl were impaired compared to responses of arteries from age-matched lean animals, although no significant differences between obese and lean arteries in sensitivities (pD_2 or $-\log ED_{50}$ values) to any of these agonists were detected (Fig. 1, Table 2). In contrast, there were no significant differences between mesenteric arteries from 20-week-old lean and obese rats in endothelium-dependent relaxation to acetylcholine (Fig. 1). Removal of the endothelium had no significant effect on

Table 3
Agonist pD_2 and R_{max} values in mesenteric arteries from 32-week-old lean and obese rats

	Lean			Obese		
	+Endo ($n=14$)	−Endo ($n=7$)	+L-NMMA ($n=7$)	+Endo ($n=14$)	−Endo ($n=6$)	+L-NMMA ($n=7$)
R_{max} (g)						
Noradrenaline	1.5 ± 0.1	0.8 ± 0.04[a]	1.2 ± 0.2	1.2 ± 0.09	0.8 ± 0.1[a]	1.3 ± 0.1
Endothelin-1	1.3 ± 0.1	0.7 ± 0.07[a]	1.2 ± 0.2	1.1 ± 0.1	0.8 ± 0.1	1.3 ± 0.1
KCl	0.8 ± 0.05	0.5 ± 0.1[a]	0.6 ± 0.08	0.6 ± 0.04[b]	0.3 ± 0.04[a]	0.5 ± 0.05
pD_2						
Noradrenaline	6.2 ± 0.09	6.4 ± 0.1	6.7 ± 0.05[a]	6.2 ± 0.1	6.2 ± 0.04	6.8 ± 0.09[a]
Endothelin-1	8.2 ± 0.05	8.5 ± 0.09[a]	8.5 ± 0.1	8.4 ± 0.05[b]	8.4 ± 0.05	8.4 ± 0.08
KCl	1.4 ± 0.07	1.4 ± 0.07	1.4 ± 0.04	1.5 ± 0.06	1.4 ± 0.06	1.5 ± 0.03

+Endo, endothelium intact, −Endo, endothelium denuded, +L-NMMA, endothelium intact plus L-NMMA.
[a] $P<0.05$ compared to corresponding intact endothelium value.
[b] $P<0.05$ compared to corresponding lean value.

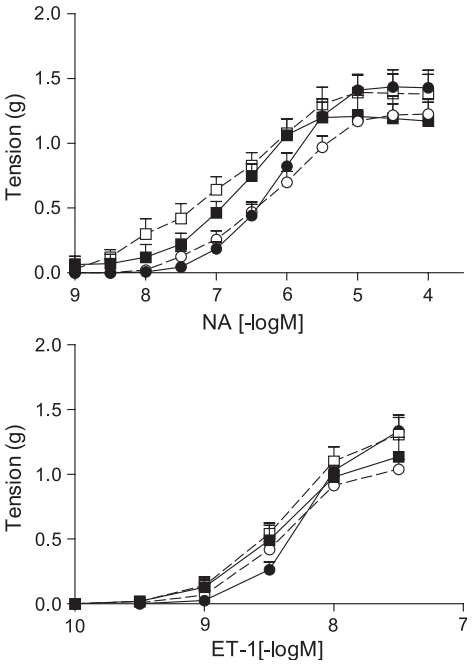

Fig. 3. Concentration–response curves of endothelium intact arteries from lean (filled symbols) and obese (open symbols) rats to noradrenaline and endothelin-1 in the absence (circles) and presence (squares) of L-NMMA.

either maximum responses or sensitivities of mesenteric arteries from lean or obese rats to noradrenaline or KCl (Fig. 1, Table 2).

At 32 weeks of age, the R_{max} of endothelium intact mesenteric arteries from obese rats to KCl remained significantly impaired while maximum responses to noradrenaline and to endothelin-1 did not significantly differ from those in mesenteric arteries from lean rats (Fig. 2, Table 3). While the sensitivities of mesenteric arteries from obese and lean rats to noradrenaline and KCl were not significantly different, there was a small but significant increase in sensitivity to endothelin-1 in mesenteric arteries from obese compared to lean rats (Table 3). Overall, mesenteric arteries from 32-week-old Zucker rats appeared to be less responsive than those from 20-week-old rats to endothelium-dependent relaxation with acetylcholine. When concentration–response curves were compared by two-way repeated measures ANOVA, the ability of endothelium-intact mesenteric arteries from obese 32-week-old rats to relax in response to acetylcholine was impaired compared to control (Fig. 2), although no significant differences in the acetylcholine R_{max} or pD_2 values were detected.

Removal of the endothelium from mesenteric arteries from 32-week-old rats resulted in impairment of maximum contractile responses of mesenteric arteries from lean and obese rats to all three agonists tested (Fig. 2, Table 3), although the difference was not statistically significant in the case of endothelin-1 in mesenteric arteries from obese rats. Endothelium removal had no effect on the sensitivities of mesenteric arteries from either lean or obese rats to KCl or noradrenaline, or of obese rats to endothelin-1. However, endothelium removal resulted in a significant increase in the endothelin-1 pD_2 value in mesenteric arteries from lean rats (Table 3).

In order to gain further insight into the regulation by the endothelium of contractile responses of mesenteric arteries to agonists, responses to noradrenaline, KCl and endothelin-

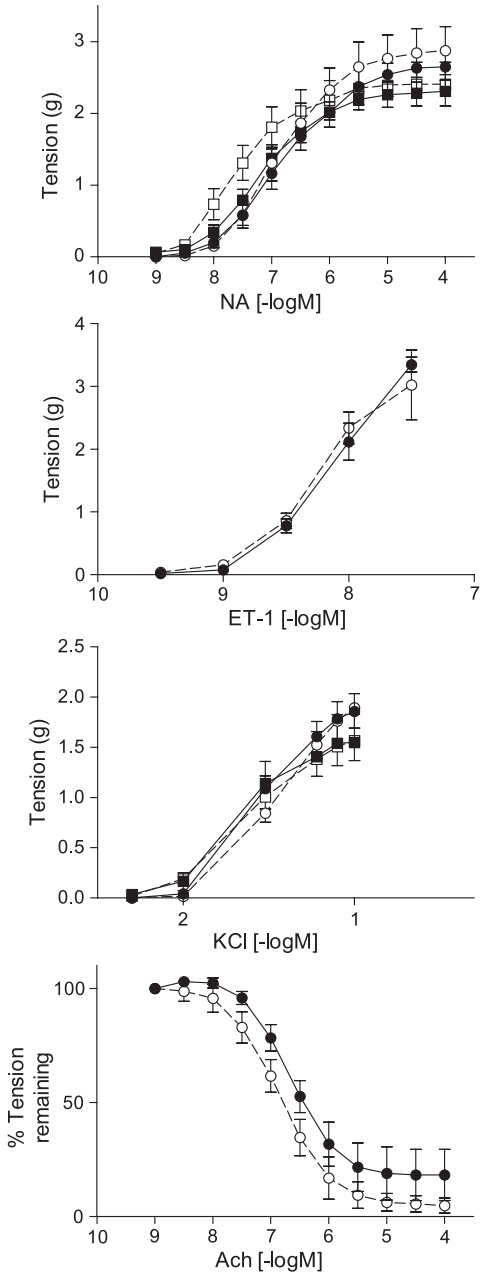

Fig. 4. Concentration–response curves of aortas from 20-week-old lean and obese Zucker rats to noradrenaline, endothelin-1, KCl and acetylcholine. Filled circles: endothelium-intact lean, filled squares: endothelium-denuded lean, open circles: endothelium-intact obese and open squares: endothelium-denuded obese.

Table 4
Agonist pD_2 and R_{max} values in aortas from 20-week-old lean and obese rats

	Lean		Obese	
	Endothelium intact	Endothelium denuded	Endothelium intact	Endothelium denuded
R_{max} (g)				
Noradrenaline	2.9 ± 0.2 (13)	2.3 ± 0.2 (6)	2.9 ± 0.2 (13)	2.4 ± 0.3 (6)
Endothelin-1	3.3 ± 0.1 (4)	ND	3.0 ± 0.6 (4)	ND
KCl	1.9 ± 0.2 (13)	1.6 ± 0.3 (6)	1.9 ± 0.2 (13)	1.6 ± 0.2 (6)
pD_2				
Noradrenaline	7.0 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.7 ± 0.1[a]
Endothelin-1	8.2 ± 0.05	ND	8.3 ± 0.04	ND
KCl	1.6 ± 0.04	1.7 ± 0.02	1.5 ± 0.04	1.6 ± 0.02

ND, not determined.
[a] $P < 0.05$ compared to corresponding intact endothelium value.

1 were determined in the presence and absence of the NO synthase inhibitor, L-NMMA. Incubation of endothelium-intact mesenteric arteries from both lean and obese rats with L-NMMA had no significant effect on responses to KCl (Table 3). However, while L-NMMA had no significant effect on maximum responses of mesenteric arteries from either lean or obese rats to noradrenaline, it produced a leftward shift in the noradrenaline concentration–response curve (Fig. 3), associated with a significant increase in noradrenaline pD_2 values in arteries from both lean and obese animals (Table 3). L-NMMA had much less effect on responses to endothelin-1 (Fig. 3). It produced a small increase in the endothelin-1 pD_2 value in mesenteric arteries from lean rats, similar in magnitude to that produced by endothelium removal, but was without effect on the endothelin-1 pD_2 value in mesenteric arteries from obese rats (Table 3).

3.2. Aorta

In contrast to mesenteric artery, contractile responses of endothelium-intact aorta from 20-week-old lean and obese rats to noradrenaline, endothelin-1 and KCl did not differ significantly from each other (Fig. 4, Table 4). Removal of the endothelium had no effect on contractile responses of aortas from lean and obese rats to KCl or on contractile responses of aortas from lean rats to noradrenaline (Table 4). However, removal of the endothelium from aortas of obese rats resulted in a leftward shift in the noradrenaline dose–response curve, with a significant increase in sensitivity to this agonist (Fig. 4, Table 4). Acetylcholine produced endothelium-dependent relaxation in aortas from both lean and obese rats (Fig. 4). Overall, the relaxation produced by acetylcholine in aortas from obese rats was significantly greater than that in lean (two-way repeated measures ANOVA), although no significant difference in acetylcholine pD_2 or R_{max} values was found.

Contractile responses of endothelium-intact aortas from 32-week-old hypertensive obese Zucker rats to noradrenaline, endothelin-1 and KCl also did not differ from those of aortas from lean animals (Fig. 5, Table 5). However, as was found at 20 weeks, endothelium-dependent relaxation to acetylcholine was significantly greater in aortas from obese rats by two-way repeated measures ANOVA (Fig. 5). Endothelium removal had no significant effect on

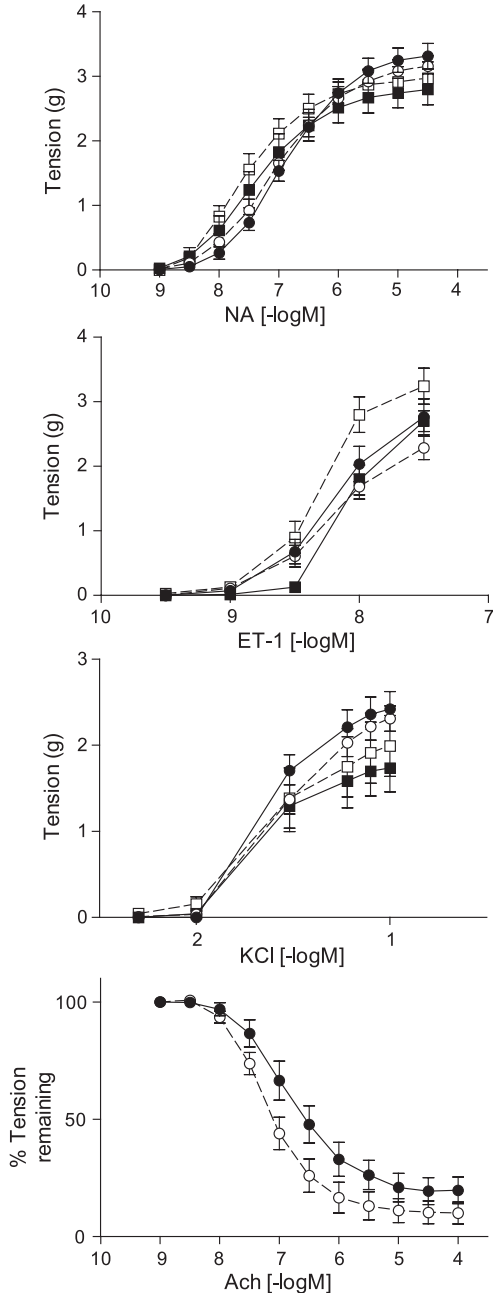

Fig. 5. Concentration–response curves of aortas from 32-week-old lean and obese Zucker rats to noradrenaline, endothelin-1, KCl and acetylcholine. Filled circles: endothelium-intact lean, filled squares: endothelium-denuded lean, open circles: endothelium-intact obese and open squares: endothelium-denuded obese.

Table 5
Agonist pD_2 and R_{max} values in aortas from 32-week-old lean and obese rats

	Lean			Obese		
	+Endo ($n=14$)	−Endo ($n=7$)	+L-NMMA ($n=7$)	+Endo ($n=14$)	−Endo ($n=7$)	+L-NMMA ($n=7$)
R_{max} (g)						
Noradrenaline	3.3 ± 0.2	2.8 ± 0.2	3.8 ± 0.2	3.2 ± 0.2	2.9 ± 0.2	3.6 ± 0.2
Endothelin-1	2.8 ± 0.3	2.7 ± 0.2	3.5 ± 0.2	2.3 ± 0.2	3.3 ± 0.3[a]	3.2 ± 0.2
KCl	2.4 ± 0.2	1.8 ± 0.3	2.2 ± 0.2	2.4 ± 0.1	2.2 ± 0.3	2.0 ± 0.2
pD_2						
Noradrenaline	7.0 ± 0.1	7.5 ± 0.2	7.9 ± 0.2[a]	7.1 ± 0.1	7.7 ± 0.2[a]	8.3 ± 0.1[a]
Endothelin-1	8.2 ± 0.05	8.1 ± 0.03	8.6 ± 0.2[a]	8.2 ± 0.05	8.3 ± 0.1	8.7 ± 0.1[a]
KCl	1.6 ± 0.01	1.6 ± 0.04	1.6 ± 0.02	1.5 ± 0.03	1.6 ± 0.06	1.6 ± 0.02

+Endo, endothelium intact, −Endo, endothelium denuded, +L-NMMA, endothelium intact plus L-NMMA.
[a] $P<0.05$ compared to corresponding intact endothelium value.

contractile responses of aortas from lean or obese rats to KCl, or on contractile responses of aortas from lean rats to noradrenaline or endothelin-1 (Fig. 5, Table 5). However, removal of the endothelium from the aortas of obese rats resulted in a significant increase in the noradrenaline pD_2 value and in the R_{max} to endothelin-1 (Fig. 5, Table 5). Incubation with L-NMMA resulted in a marked leftward shift in the dose–response curves to noradrenaline and endothelin-1 in endothelium-intact aortas from both lean and obese rats (Fig. 6, Table 5), although it had no effect on contractile responses of aortas from lean or obese rats to KCl (Table 5).

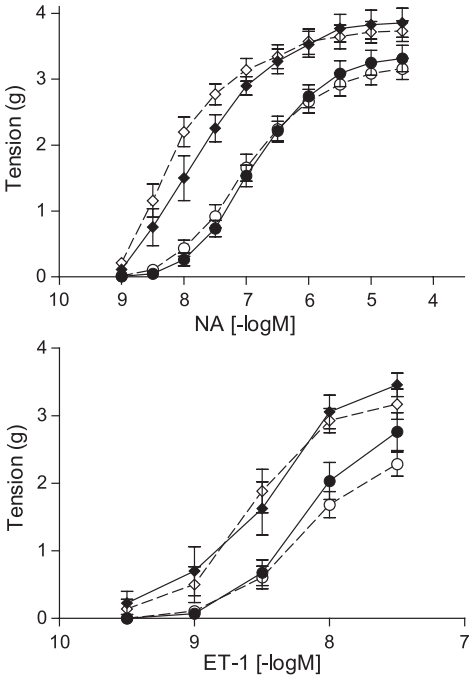

Fig. 6. Concentration–response curves of endothelium intact aortas from lean (filled symbols) and obese (open symbols) rats to noradrenaline and endothelin-1 in the absence (circles) and presence (diamonds) of L-NMMA.

4. Discussion

The results of the present study demonstrate that changes in the reactivity of the mesenteric artery, but not the aorta parallel changes in blood pressure in obese Zucker rats. At an age at which these rats were slightly hypotensive, the reactivity of the mesenteric artery to agonists was nonselectively depressed. Three months later, when the obese rats had well-established hypertension, contractile responses to noradrenaline and endothelin-1 had recovered, while endothelium-dependent relaxation to acetylcholine was impaired. In contrast, there were no differences in responsiveness of the aorta from obese Zucker rats of either age to vasoconstrictors, while endothelium-dependent relaxation to acetylcholine was slightly enhanced, regardless of age or blood pressure status.

The change in blood pressure of obese Zucker rats from hypotensive at 20 weeks old to hypertensive at 32 weeks old found in the present study is consistent with the previous observations of Cox and Kikta (1992), who reported that blood pressure was significantly lower in young obese rats than in their lean controls, but that it increased at a more rapid rate with age in the obese animals, so that eventually the obese animals became hypertensive. In the study of Cox and Kikta (1992), the linear regression curves of age vs. blood pressure in lean and obese rats crossed over at approximately 24 weeks of age. We have recently reported that 25-week-old male obese Zucker rats are mildly hypertensive in comparison with their age- and gender-matched lean littermates (He and MacLeod, 2002), indicating that in the animals used in our study, the cross over must occur at a similar or slightly younger age. Elevated blood pressure in obese Zucker rats 25 weeks of age and older seems to be a relatively consistent (Turner et al., 1995; Wu et al., 1996; Yuen et al., 1996; Arvola et al., 1999), though not universal (Zanchi et al., 1995; Turner and White, 1996) finding. In contrast, blood pressure status in obese Zucker rats younger than 20 weeks of age has varied widely in

different studies, with reports of no change (Walker et al., 1997; Verma et al., 2001), decreased (Cox and Kikta, 1992) and increased blood pressure (Zemel et al., 1990, 1992; Ouchi et al., 1996; Hopfner et al., 1999). The reason for these differences is not entirely clear, but in part it may reflect methodological differences between studies.

It is not clear from our results whether the increase in systolic blood pressure found in 32-week-old obese rats occurred alone or with a concurrent increase in diastolic pressure. However, in another investigation in animals from the same colony, direct blood pressure measurements were made in 27-week-old conscious, unrestrained obese Zucker rats (Yuen et al., 1996). The previously reported increase in systolic blood pressure found in the obese animals compared to their lean controls (Yuen et al., 1996) was associated with a significant increase in both diastolic pressure (from 108 ± 10 in lean to 142 ± 7 in obese rats, $P < 0.05$, $n = 7$) and mean arterial pressure (from 114 ± 10 in lean to 154 ± 6 in obese rats, $P < 0.05$) (Yuen and McNeill, personal communication). These results suggest that the increased blood pressure in older obese Zucker rats results primarily from an increase in peripheral vascular resistance.

The mechanism underlying the nonselective depression of maximum contractile responses of mesenteric arteries from 20-week-old obese rats to vasoconstrictors is unknown. It cannot be attributed to altered release of endothelium-dependent vasoactive substances, since removal of the endothelium had no effect on contractile responses of mesenteric arteries from either lean or obese rats to noradrenaline or KCl. However, this result is consistent with the findings of our previous study, in which maximum responses of the perfused mesenteric arterial bed from 25-week-old obese Zucker rats to KCl and to noradrenaline were also significantly impaired, although the noradrenaline response was only slightly reduced (He and MacLeod, 2002). The observation that responses of mesenteric arteries from 32-week-old obese rats to noradrenaline or endothelin-1 were no longer significantly depressed although those to KCl remained impaired, suggests that with increasing age there is a gradual increase in responsiveness of the mesenteric artery to receptor stimulation, but not to K^+-depolarization.

The lack of effect of endothelium removal on responses of mesenteric arteries from 20-week-old Zucker rats to noradrenaline contrasts with the situation in mesenteric arteries from Wistar rats, where endothelium removal results in enhanced contractile responses to α-adrenoceptor stimulation because of loss of the inhibitory effect of concurrent NO release (MacLeod et al., 1987). Although it is possible that the noradrenaline response is not modulated by release of endothelium-derived NO in mesenteric arteries from 20-week-old Zucker rats, the effects of endothelium removal in the present study are similar to those we observed on simultaneous inhibition of both NO and vasoconstrictor prostanoid production in the mesenteric arterial bed from 25-week-old lean and obese Zucker rats (He and MacLeod, 2002). Therefore, an alternate explanation is that NO is released but its inhibitory effect on the noradrenaline response is balanced by concurrent release of vasoconstrictor prostanoids. This suggestion is supported by the observation that the noradrenaline response of mesenteric arteries from 32-week-old rats was shifted to the left by L-NMMA. Furthermore, if there was a relatively greater contribution of endothelium-derived vasoconstrictor prostanoids than NO to the contractile response to agonists in mesenteric arteries from 32-week-old rats, this would explain why, despite the enhancement of the noradrenaline response by L-NMMA, removal of the endothelium resulted in impairment of maximum contractile responses of mesenteric arteries from 32-week-old rats to this agonist. The diminished response to endothelin-1 on endothelium removal is also consistent with the possibility that release of vasoconstrictor substances normally contributes to the maintenance of maximum contractile responses to this agonist, although the relative lack of effect of L-NMMA suggests that the release and/or action of NO is much smaller in the presence of endothelin-1 than noradrenaline.

The similarity of the effects of endothelium removal and of L-NMMA on noradrenaline responses in mesenteric arteries from lean compared to obese 32-week-old rats suggests that there is no change in the modulatory effects of NO and other endothelium-derived substances on the response to noradrenaline. In contrast, both endothelium removal and L-NMMA treatment produced a small increase in endothelin-1 pD_2 in mesenteric arteries from lean but not obese rats, suggesting that the increased sensitivity of mesenteric arteries from 32-week-old obese rats to this agonist may arise because the modest inhibition of the endothelin-1 response by endothelium-derived NO in mesenteric arteries from lean rats is absent in arteries from obese rats.

The decreased responsiveness to acetylcholine of mesenteric arteries from 32-week-old compared to 20-week-old lean and obese rats suggests that the acetylcholine-induced release or action of endothelium-derived vasodilator substances in mesenteric arteries diminishes with age. The further impairment of the vasodilator response to acetylcholine in mesenteric arteries from 32-week-old obese compared to lean rats, which was not seen in the younger animals, is consistent with previous studies in which defective acetylcholine-induced relaxation of mesenteric arteries from older obese rats was reported (Wu et al., 1996; Arvola et al., 1999). Since preliminary results (not shown) suggest that this difference remains following pretreatment of the arteries with L-NMMA, it is unlikely to be due to decreased release of nitric oxide. Although the mechanism underlying this impairment was not further investigated in the present study, it has been suggested to be due to reduced endothelium-dependent hyperpolar-

ization of smooth muscle in response to acetylcholine (Wu et al., 1996; Arvola et al., 1999).

Increased acetylcholine-induced relaxation of aortas from young (12–15 week old) obese Zucker rats has been reported in other studies, although in contrast to the present study, this effect did not persist with age (Cox and Kikta, 1992; Sexl et al., 1995). The increased response of aortas from 20-week-old obese rats to acetylcholine was not accompanied by a change in relaxation to the endothelium-independent vasodilator SNP (not shown), suggesting that sensitivity of the aorta to NO is unaltered. These data, together with the observation in preliminary studies that the enhanced relaxation of aortas from 32-week-old obese rats to acetylcholine was abolished by pretreatment with L-NMMA, suggest that acetylcholine-induced release of NO is actually enhanced in aorta from obese Zucker rats, both prior to and following the development of hypertension. The selective increase in noradrenaline pD_2 in aortas from obese but not lean rats on removal of the endothelium suggests that there may also be relatively greater basal release of NO in aortas from obese rats of both ages. Since L-NMMA produced a marked leftward shift in the dose–response curves to noradrenaline and endothelin-1 in aortas from both lean and obese 32-week-old rats, it is clear that release of NO modulates contractile responses of lean as well as obese rats at this age. Therefore, as in mesenteric artery, it seems likely that the effects of NO in aortas from lean and obese rats are balanced by concurrent release of an endothelium-dependent vasoconstrictor.

The lack of difference in contractile responses to noradrenaline, endothelin-1 or KCl of endothelium-intact aortas from either 20 or 32-week-old obese rats compared to their lean controls is in contrast to previous reports of enhanced sensitivity and/or R_{max} of aortas from obese rats to α-adrenoceptor agonists, endothelin-1 and KCl (Zemel et al., 1990; Cox and Kikta, 1992; Harker et al., 1993; Hopfner et al., 1998, 1999). However, those studies were conducted in rats younger (ranging in age from 10 to 16 weeks) than those used in the present investigation. In contrast, studies in obese rats older than 20 weeks have generally found either no change, or a decrease in sensitivity of the aorta to α-adrenoceptor agonists and KCl (Cox and Kikta, 1992; Harker et al., 1993; Sexl et al., 1995; Turner and White, 1996).

In conclusion, the results of the present investigation demonstrate that responses of arteries from obese Zucker rats to vasoactive agents vary with age, blood pressure and the vessel investigated, and that changes in the reactivity of the mesenteric artery but not the aorta from obese Zucker rats parallel changes in blood pressure in these animals.

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