Dietary obesity in the rat induces endothelial dysfunction without causing insulin resistance: a possible role for triacylglycerols

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ABSTRACT

Impaired arterial vasorelaxation, due primarily to endothelial dysfunction, is associated with obesity. To clarify the relationship with insulin resistance and other metabolic disturbances, we studied endothelial-dependent and -independent vascular responses in rats with dietary-induced obesity. Dietary-obese rats had significantly higher body weights (10–32%; $P < 0.001$) and fat-pad masses (220–280%; $P < 0.001$) than lean controls, together with raised plasma levels of triacylglycerols (15–80%; $P < 0.001$), non-esterified fatty acids (13–38%; $P < 0.05$) and leptin (85–180%; $P < 0.001$). However, measures of insulin sensitivity (including the hyperinsulinaemic–euglycaemic clamp in a parallel experiment) were comparable with those in controls. Contractions induced in mesenteric arteries by noradrenaline (0.5–8 μmol/l) were comparable in lean and obese groups, but vasorelaxation in noradrenaline-preconstricted arteries was markedly reduced in dietary-obese rats of both sexes. Concentration–response curves to endothelium-dependent vasorelaxants (acetylcholine, A23187 and insulin) showed significant reductions in maximal relaxation (20–95% less than in leans; $P < 0.001$) and significant rightward shifts in EC_{40} (concentration giving 40% of maximal response) ($P < 0.01$). Relaxation in response to the direct NO donor, sodium nitroprusside, showed a lesser impairment (12%; $P < 0.01$) in dietary-obese rats. Maximal relaxation to acetylcholine was correlated inversely in both sexes with fat-pad mass ($r^2 = 0.37$, $P < 0.05$) and plasma triacylglycerols ($r^2 = 0.51$, $P < 0.01$), and with leptin in males only ($r^2 = 0.35$, $P < 0.05$). Independent determinants of acetylcholine-induced relaxation were fat mass and plasma triacylglycerols; plasma insulin and insulin sensitivity had no effect. Dietary-induced obesity severely impaired arterial relaxation in both sexes, particularly at the endothelial level. This is not attributable to insulin resistance, but may be related to moderate hypertriglyceridaemia.

INTRODUCTION

Obesity is an important risk factor for atheroma, and predisposes to hypertension and dyslipidaemia, in which hypertriglyceridaemia is now thought to be important [1,2]. Obesity is also accompanied by specific abnormalities of endothelial function that also occur in other pro-atherogenic conditions [3,4]. The endothelium is

Key words: diet-induced obesity, insulin resistance, vascular dysfunction.

Abbreviations: ACh, acetylcholine; CCh, carbamylcholine; EC_{40}, concentration giving 40% of maximal response; M-value, mean steady-state glucose infusion rate during the last 30 min of the 180 min clamp divided by terminal plasma insulin concentration; NA, noradrenaline; NEFA, non-esterified fatty acids; SNP, sodium nitroprusside; TG, triacylglycerol(s); ZDF, Zucker diabetic fatty.

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crucial to key arterial functions, including the regulation of vascular tone [5]. Endothelial cells produce various vasoactive mediators, notably the vasodilator nitric oxide (NO), which acts on the underlying vascular smooth muscle [6]. NO production is regulated by the endothelial form of NO synthase, which is stimulated by mediators and hormones that include acetylcholine (ACh), bradykinin and insulin [6,7].

Impaired NO-mediated vasorelaxation has been demonstrated in obesity, Type II diabetes and hypertension [8–10], with the most striking abnormality being blunted vasodilatation in response to ACh. Impaired endothelium-mediated vasorelaxation could contribute to hypertension [11], and its presence in subjects at high risk of atherogenesis suggests that it may predict atheroma formation, and ultimately coronary and peripheral arterial disease [11,12].

The causes of endothelial dysfunction and its pathophysiological relationship with other pro-atherogenic conditions are unknown. Obesity (especially abdominal), hypertension, Type II diabetes and dyslipidaemia are all commonly associated with reduced sensitivity to the metabolic actions of insulin [3,13]. This has led to the hypothesis that their co-segregation represents a specific 'metabolic syndrome' (syndrome X), whose fundamental cause is insulin resistance [3,13–15]. It has also been suggested that insulin resistance is the cause of vascular dysfunction in these conditions [13–17]. This hypothesis remains unproven.

Much of the work relating defects in endothelial function and vascular reactivity to insulin resistance has been done in genetically obese rats [*fa/fa* Zucker, JCR-LA *cp/cp* and ZDF (Zucker diabetic fatty)], which have massive insulin resistance that is due ultimately to mutations affecting the leptin receptors [18,19]. However, these mutants are imperfect models of human obesity, which is not due to a primary loss of leptin signalling and in which insulin resistance is less marked. We have therefore studied a rat model more relevant to human obesity, namely that induced by voluntary overeating of a palatable diet. This diet-induced obesity is characterized by a selective increase in body fat mass (> 100% above lean values), with proportionate rises in plasma leptin, and by variable but relatively mild insensitivity to insulin and leptin [20].

Here, we have characterized in detail the vascular responses to endothelium-dependent vasodilatation [ACh, carbamylcholine (CCh), A23187 and insulin] and to the direct NO donor, sodium nitroprusside (SNP). Endothelial function has not been reported previously in dietary-induced obesity. To try to clarify the causes of endothelial dysfunction in obesity, we also sought to identify the metabolic determinants of impaired relaxation in response to ACh. In particular, we assessed changes in insulin sensitivity from plasma insulin and glucose levels, and in a parallel study using the hyperinsulinaemic–euglycaemic clamp.

MATERIALS AND METHODS

Animals and experimental protocol

In experiment 1, adult male Wistar rats ($n = 28$) were randomly assigned to a control group ($n = 10$) or a dietary-obese group ($n = 18$). In experiment 2, adult female Wistar rats ($n = 18$) were randomly assigned to a control ($n = 5$) or a dietary-obese ($n = 13$) group. All had free access to water and were housed in groups of two or three under controlled environmental conditions (19–22 °C; 30–40% humidity) and a 12-h light/dark cycle (lights on at 07.00 hours). Controls were fed on standard pelletted laboratory chow (CRM Biosure, Cambridge, U.K.), while dietary-obese groups had free access to a highly palatable, high-energy diet consisting of 33% (by weight) ground chow, 33% Nestlé condensed milk, 7% sucrose and 27% water. Male rats were studied for 16 weeks and females for 12 weeks.

The rats were killed by CO_2 inhalation, and the gonadal and perirenal fat masses and the gastrocnemius muscle were dissected and weighed. Blood was collected for measurement of glucose, insulin, leptin, non-esterified ('free') fatty acids (NEFA) and triacylglycerols (TG; triglycerides). The plasma glucose concentration was determined by the glucose oxidase method, and NEFA and TG concentrations were measured with commercial diagnostic kits from Boehringer Mannheim and Sigma Diagnostics respectively. Insulin and leptin concentrations were measured by RIA kits (Pharmacia/Upjohn Diagnostics and Linco Research respectively).

In experiment 3, 20 adult male Wistar rats were randomly assigned to a control ($n = 10$) or a dietary-obese ($n = 10$) group and treated as described above for 12 weeks. They underwent a hyperinsulinaemic–euglycaemic clamp as a terminal procedure [21]. Rats were anaesthetized with an intraperitoneal injection of a Diazemuls (Dumex Ltd)/Hypnorm (Janssen Pharmaceutical Ltd)/water mixture (1:1:2, by vol.). Human soluble insulin (Humulin-S; Eli Lilly, Basingstoke, Hants., U.K.), in isotonic saline containing 1% (w/v) BSA (Sigma), was infused at a constant rate of 14 m-units/min into the right jugular vein to produce hyperinsulinaemia. Glucose solution (20% in water) was co-infused with the insulin, and the rate was adjusted to maintain blood glucose at 5 mmol/l; tail-vein blood glucose concentrations were measured every 5 min using an Exactech® electrochemical meter (Medisense, Oxford, U.K.). The M-value was derived from the mean steady-state glucose infusion rate during the last 30 min of the 180 min clamp divided by the terminal plasma insulin concentration [21]. At the end of the clamp, rats were

killed by exsanguination, and plasma glucose and insulin concentrations were measured as above.

Assessment of vascular function

In experiments 1 and 2, eight third-order mesenteric arteries were carefully dissected from each animal and mounted on two 40 μm-diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, U.K.). Pairs of arteries were incubated in a 5 ml organ bath containing physiological salt solution [composition (in mmol/l): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5] gassed with 95 % O_2/5 % CO_2 at 37 °C.

After a 30 min equilibration period, the length–tension characteristics for each vessel were determined [22]. Arteries were allowed a further 30 min to equilibrate before being depolarized twice with high-potassium physiological salt solution, in which NaCl in normal physiological salt solution was replaced by an equimolar concentration of KCl (125 mmol/l). Cumulative concentration–response curves to either KCl (10–125 mmol/l) or noradrenaline (NA; 0.5–8 μmol/l) were then carried out. Any vessel failing to reach its predetermined target tension in response to KCl (125 mmol/l) was discarded.

Assessment of endothelium-dependent and -independent vascular relaxation

To characterize endothelial defects, we measured the effects of selected endothelium-dependent vasodilators (ACh, CCh, the calcium ionophore A23187 and insulin) in arteries preconstricted with 8 μmol/l NA. We similarly investigated direct effects on vascular smooth muscle using the endothelium-independent vasodilator SNP.

Reagents

NA, ACh, CCh, A23187 and SNP were all obtained from Sigma. All solutions except A23187 were freshly made up before use in distilled water. A23187 was prepared in ethanol; working concentrations of ethanol in the bath (< 0.01 %, v/v) do not affect vascular contractility [22,23].

Data interpretation and statistical analyses

Vasoconstriction in response to NA and KCl is expressed as absolute force generated. Relaxation in response to ACh, CCh, A23187, SNP or insulin was calculated as the percentage reduction from the maximal tension generated in response to a supramaximal concentration of NA (8 μmol/l).

Data are means ± S.E.M. Statistical significance was tested using repeated-measures ANOVA or the Mann–Whitney test, as appropriate. Univariate correlations between maximal ACh-induced vasorelaxation and various metabolic parameters were determined by linear regression followed by two-tailed analyses, using Arcus Pro-stat (Version 3.23; Iain Buchan, University of Liverpool, U.K.). Independent determinants of ACh-induced vasorelaxation were identified from partial correlation coefficients, derived from stepwise analysis using the SPSS statistical package (Version 8; SPSS Inc.). Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS

Body weight and metabolic data

Rats of either sex given the highly palatable diet gained progressively more weight than chow-fed controls, being significantly heavier ($P < 0.01$) after 4 weeks. Diet-induced weight gain was significantly greater in males (experiment 1) than in females (experiment 2), with the final body weights of diet-fed rats being 32 % (males) and 9 % (females) greater than those of their respective chow-fed controls (Figure 1, Table 1). The weight gain of the dietary-obese male rats in experiment 3 (18 % over control) fell mid-way between those in experiments 1 and 2 (Table 2). The epididymal and perirenal fat masses weighed significantly more (> 200 %; $P < 0.001$) in all dietary-obese groups than in controls, while gastrocnemius muscle weight was comparable (Table 1).

In dietary-obese rats of either sex, terminal plasma TG levels were significantly higher (by 15–80 %; $P < 0.001$) than in lean controls, as were NEFA levels (by 13–38 %; $P < 0.05$). Leptin levels were also significantly raised above those in controls, by 180 % in males and 85 % in females (Table 1).

There were no significant differences in either plasma insulin or glucose levels between dietary-obese rats and controls in either experiment 1 or 2 (Table 1), suggesting that the moderate obesity in these rats was not accompanied by insulin resistance. This was confirmed by

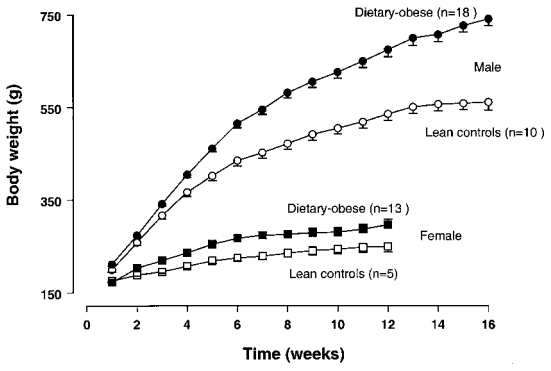

Figure 1 Effects of a highly palatable diet on weight gain in male and female Wistar rats

Lean controls were fed standard laboratory pelleted diet, while dietary-obese rats were given a highly palatable diet throughout the experimental period. Data represent means ± S.E.M.

Table 1 Physiological and metabolic characteristics of chow-fed and dietary-obese male and female rats

Data are means ± S.E.M. NS, not significant.

	Males			Females		
Parameter	Chow-fed ($n = 10$)	Dietary-obese ($n = 18$)	P value	Chow-fed ($n = 5$)	Dietary-obese ($n = 13$)	P value
Body weight (g)						
Initial	200 ± 5	211 ± 3	NS	175.7 ± 2.8	172.9 ± 1.9	0.98 (NS)
Final	562.8 ± 17.7	742.0 ± 14.4	< 0.0001	250.1 ± 5.5	271.6 ± 10.6	< 0.05
Gain	363.0 ± 16.3	530.1 ± 14.9	< 0.001	74.4 ± 3.6	99.1 ± 10.8	< 0.05
Epididymal fat pad mass (g)	8.38 ± 1.0	18.6 ± 0.8	< 0.0001	–	–	–
Gonadal fat mass (g)	–	–	–	0.76 ± 0.05	2.12 ± 0.17	< 0.0001
Perirenal fat mass (g)	11.5 ± 1.8	25.7 ± 1.8	< 0.0001	2.20 ± 0.08	5.12 ± 0.48	< 0.001
Gastrocnemius muscle mass (g)	3.4 ± 0.1	3.5 ± 0.1	NS	1.60 ± 0.06	1.72 ± 0.07	0.1126 (NS)
Plasma glucose (mmol/l)	11.2 ± 0.8	12.2 ± 0.5	NS	8.0 ± 0.4	8.3 ± 0.4	0.114
Plasma insulin (m-units/l)	2.00 ± 0.42	2.21 ± 0.38	NS	12.69 ± 2.39	12.86 ± 1.4	0.239
Plasma leptin (ng/ml)	25.30 ± 5.15	75.63 ± 7.0	< 0.0001	5.97 ± 0.60	10.98 ± 0.30	< 0.001
Plasma TG (mmol/l)	0.44 ± 0.03	0.79 ± 0.07	< 0.001	0.23 ± 0.06	0.59 ± 0.13	< 0.01
Plasma NEFA (mmol/l)	0.34 ± 0.04	0.47 ± 0.04	< 0.05	0.33 ± 0.08	0.38 ± 0.03	0.05

Table 2 Physiological and metabolic characteristics of chow-fed and dietary-obese male rats used for glucose clamping

Data are means ± S.E.M. NS, not significant.

Parameter	Chow-fed ($n = 10$)	Dietary-obese ($n = 10$)	P value
Body weight (g)			
Initial	244.6 ± 5.1	252.0 ± 2.3	NS
Final	465.6 ± 8.6	523.5 ± 11.1	< 0.001
Gain	221.5 ± 10.3	271 ± 14.9	< 0.01
Plasma glucose (mmol/l)	5.48 ± 0.38	6.07 ± 0.44	NS
Post-clamp plasma insulin (μ-units/ml)	175 ± 21	205 ± 42	NS
M-value	0.50 ± 0.07	0.52 ± 0.06	NS

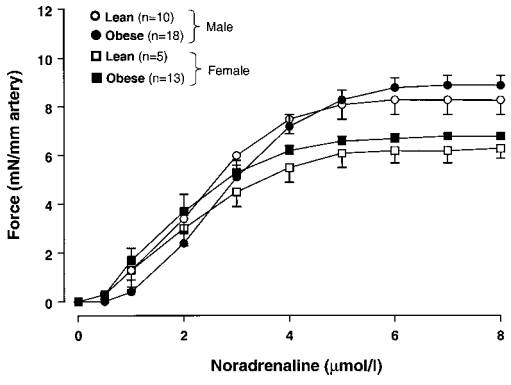

Figure 2 Effects of NA on arteries from dietary-obese and lean control rats

Data represent means ± S.E.M. The concentration–response curves for the two groups did not differ significantly for either sex.

the clamp study in experiment 3. Steady-state glucose and insulin concentrations during the clamp were closely similar in lean and obese rats (Table 2), and the M values were almost identical (lean, 0.50 ± 0.07; obese, 0.52 ± 0.06; $P > 0.1$).

Vascular contractile responses

There were no significant differences in arterial diameter between the obese and lean groups [males: obese, 235 ± 15 μm; lean, 230 ± 11 μm ($P > 0.5$); females: obese, 230 ± 15 μm; lean, 231 ± 10 μm ($P > 0.5$)].

The contractile responses of arteries to increasing concentrations of NA (0.5–8 μmol/l) were measured in all the rats, and displayed the characteristic sigmoid relationship. Maximal tension generated was consistently greater in males, but there were no significant differences between any lean and obese groups (Figure 2). KCl concentration–response curves (tested in arteries from female rats) similarly showed no significant differences between lean and obese groups (results not shown).

Endothelium-dependent relaxation

In both sexes, arteries from lean rats that were pre-contracted with NA (8 μmol/l) demonstrated pro-

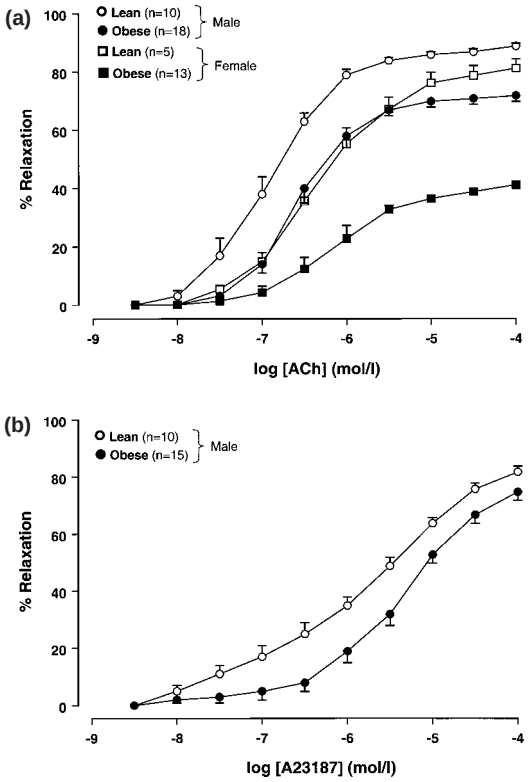

Figure 3 Relaxation curves for (a) ACh and (b) A23187 on NA-precontracted arteries from lean control and dietary-obese rats

When contraction reached a plateau after 2 min, concentration–response curves to ACh were carried out. Data represent means ± S.E.M. The concentration–response curves for the two groups differ significantly ($P < 0.01$) for each sex.

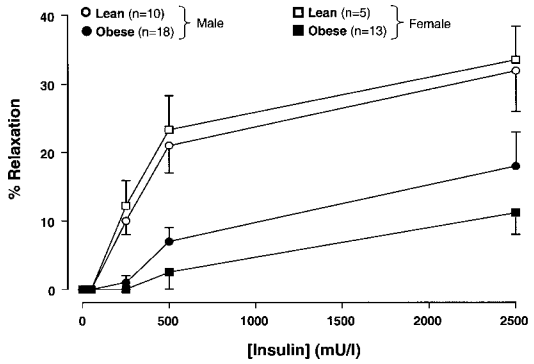

Figure 4 Relaxation curves for insulin on NA-precontracted arteries from lean control and dietary-obese rats

When contraction reached a plateau after 2 min, concentration–response curves to insulin were carried out. Data represent means ± S.E.M. The concentration–response curves for the two groups differ significantly ($P < 0.001$) for each sex.

gressive relaxation to cumulative additions of Ach (10 nmol/l–100 μmol/l). Maximal relaxation at 100 μmol/l ACh was 89 ± 3 % and 83 ± 3 % for arteries from male and female rats respectively (Figure 3a). Arteries from dietary-obese rats that were similarly exposed to ACh displayed marked impairment of the relaxation responses (Figure 3a), with maximum relaxation of only 72 ± 4 % in males and 42 ± 1 % in female rats (both $P < 0.001$ compared with lean controls). The dose–response curves for arteries from obese rats also displayed significant rightward shifts as compared with those from lean controls. EC_{40} values (concentration giving 40 % of maximal response) were as follows: obese males, 0.27 ± 0.1 μmol/l; lean males, 0.09 ± 0.01 μmol/l ($P < 0.001$); obese females, 97.53 ± 0.48 μmol/l; lean females, 0.36 ± 0.02 μmol/l ($P < 0.001$).

The effects of CCh, tested in arteries from males, were similar to those of ACh. Maximum relaxation in response to 100 μmol/l CCh was 94 ± 1 % in arteries from lean rats, but only 75 ± 4 % ($P < 0.001$) in arteries from obese rats, and there was a marked rightward shift of the dose–response curve (EC_{40} values: obese, 1.2 ± 0.10 μmol/l; lean, 0.22 ± 0.02 μmol/l; $P < 0.001$).

A23187, tested in arteries from males, induced the concentration-dependent relaxation of NA-preconstricted arteries. This effect was significantly attenuated in arteries from obese rats at A23187 concentrations of between 3.16 nmol/l and 31.6 μmol/l, but the responses were comparable at the highest concentration (100 μmol/l) (Figure 3b). The EC_{40} was significantly higher in the dietary-obese group than in the controls (4.64 ± 0.08 and 1.37 ± 0.02 μmol/l respectively; $P < 0.01$).

Insulin induced concentration-dependent arterial relaxation in arteries from lean rats of both sexes. Arteries from dietary-obese rats consistently showed a marked loss of insulin-induced relaxation, with reductions of > 90 % ($P < 0.001$) at a concentration of 250 m-units/l compared with those from their lean counterparts (Figure 4).

Endothelium-independent relaxation

The direct NO donor SNP, tested in females, also induced vasorelaxation that was attenuated in dietary obesity. The maximum relaxation at 100 μmol/l SNP of arteries from chow-fed rats was 81 ± 3 %, but only 71 ± 2 % in those from obese rats ($P < 0.01$). In addition, the concentration–response curve showed a significant rightward shift (EC_{40} values: obese, 2.05 ± 0.02 μmol/l; lean, 0.21 ± 0.03 μmol/l; $P < 0.001$) (Figure 5).

Metabolic determinants of vascular dysfunction

We examined the relationships between endothelial-mediated vasorelaxation (measured as the percentage reduction of maximal NA-induced vasorelaxation induced by 100 μmol/l ACh) and major metabolic changes of obesity, namely body weight, fat-pad mass (sum of

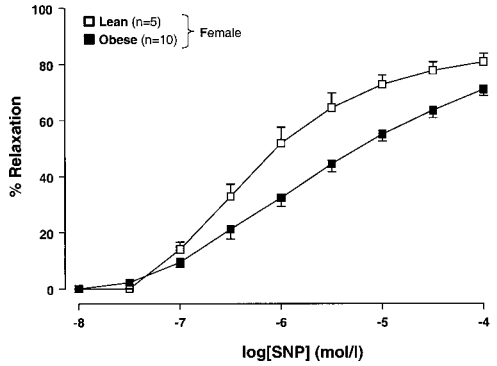

Figure 5 Relaxation curves for SNP on NA-precontracted arteries from lean control and dietary-obese female rats

When contraction reached a plateau after 2 min, concentration–response curves to SNP were performed. Data represent means ± S.E.M. The concentration–response curves for the two groups differ significantly ($P < 0.001$).

perirenal and gonadal fat pads) and plasma levels of TG, NEFA, leptin and insulin (Table 3).

There was an inverse correlation between body weight and maximal ACh-induced vasorelaxation in male but not female rats, whereas both sexes showed highly significant inverse correlations between ACh-induced vasorelaxation and fat-pad mass. In both sexes, plasma TG levels were also significantly inversely correlated with maximal ACh-induced vasorelaxation (Table 3). Stepwise multivariate analysis on data from both sexes demonstrated that the independent determinants for endothelial dysfunction in dietary obesity were total fat-pad mass and plasma TG levels. Univariate analysis showed that body weight and plasma leptin levels were inversely related to maximal ACh-induced vasorelaxation in male rats, but not in females. Moreover, stepwise analysis also indicated a significant independent contribution of leptin to vascular dysfunction in male rats only (Table 3).

These analyses also confirmed that endothelial dysfunction was not related to metabolic insulin resistance. There was no significant correlation between attenuation of maximal ACh-induced vasorelaxation and plasma levels of either glucose or insulin, and stepwise multiple variant analyses similarly showed no significant effect attributable to these parameters. Neither was there any influence of NEFA (Table 3).

DISCUSSION

This study has yielded two novel and important findings. Firstly, rats with dietary-induced obesity – like the widely studied genetic models – show markedly impaired arterial relaxation, mainly due to endothelial dysfunction. Secondly, these defects occurred in the absence of significant insulin resistance, as shown by unchanged plasma glucose and insulin concentrations and by normal M-values in the parallel hyperinsulinaemic–glucose clamp study. Our findings therefore throw new light on the causes of arterial disease in obesity, and also directly challenge the notion that vascular dysfunction in obesity is caused primarily by insulin resistance [3,13].

The vascular dysfunction induced by dietary obesity was severe and multifactorial. Impaired relaxation in response to ACh was comparable in magnitude to that in *fa/fa* Zucker [17], JCR-LA *cp* [16] and ZDF [17] rats, in which morbid obesity and severe resistance to leptin and insulin action are due to mutations in the leptin receptor [18,19,24]. Furthermore, despite a larger increase in total body weight of obese male rats, attenuation of ACh-induced vasodilatation was more pronounced in obese female rats than in obese male rats, indicating a gender-dependent variation in vascular reactivity. Similar observations have also been reported previously implying an effect of gender on vascular contractility [25–27].

Dietary obesity was associated with impairment of relaxation induced by four different endothelium-dependent stimuli: ACh, CCh, A23187 and insulin. All

Table 3 Correlations between vasorelaxation and physiological and metabolic characteristics in dietary-obese male and female rats

Vasorelaxation was defined as % reduction in maximal ACh-induced vasorelaxation.

	Males ($n = 18$)				Females ($n = 13$)			
	Univariate correlation coefficient		Partial correlation coefficient		Univariate correlation coefficient		Partial correlation coefficient	
Parameter	r^2	P value	r^2	P value	r^2	P value	r^2	P value
Body weight	0.504	0.002	0.506	0.003	0.159	0.11	0.148	0.13
Gonadal + perineal fat-pad mass	0.371	0.012	0.365	0.017	0.326	0.02	0.315	0.02
Plasma TG	0.513	0.002	0.506	0.003	0.302	0.02	0.298	0.02
Plasma NEFA	0.167	0.116	0.158	0.315	0.040	0.12	0.036	0.12
Insulin	0.017	0.641	0.015	0.515	0.002	0.85	0.002	0.82
Leptin	0.347	0.021	0.345	0.022	0.179	0.09	0.170	0.10

induce vasorelaxation by stimulating endothelial NO production through endothelial NO synthase [5,6]; blunted vasodilatation in response to all these agents points to a defect in the NO synthase or in NO production, rather than to specific abnormalities, such as in the muscarinic cholinergic receptor or in the action of insulin (as has been suggested in the *fa/fa* Zucker rat [23]). Comparable responses to the stable ACh analogue, CCh, argue against the attenuated ACh-induced vasorelaxation being due to excessive degradation of ACh by cholinesterase.

Endothelium-independent mechanisms are also involved, as SNP-induced vasorelaxation was also attenuated, but to a lesser extent than the upstream endothelial-mediated mechanisms. This additional defect could reflect reduced responsiveness of vascular smooth muscle and/or enhanced clearance of NO, and indicates that dietary obesity induces distinct abnormalities in both endothelium and vascular smooth muscle.

Analogous vascular dysfunction has been reported in states that show the common feature of insulin resistance. Endothelium-dependent vasorelaxation is impaired in morbidly obese and massively insulin-resistant *fa/fa* Zucker and JCR:LA-*cp* rats [16,23,28], and also in humans with Type II diabetes [9,23,29] and obesity [10,30]. Attenuated SNP-induced vasorelaxation has also been described in subjects with Type II diabetes [8,31], although others have reported no abnormalities in the response to SNP in either humans with Type II diabetes or genetically obese animals [29,32].

It has been proposed that insulin resistance is the primary defect that underlies the features of 'syndrome X' [13], and it has been assumed to play a role in the vascular dysfunction characteristic of obesity and Type II diabetes [6,13,14,17,23]. This view has been supported by studies in rats with genetic obesity that is due to defects in leptin or its receptors, and which display severe metabolic insulin resistance. As well as showing a general impairment of NO-dependent vasorelaxation, the vasodilatory properties of insulin are also attenuated in these rats [23]. However, it is not clear whether impaired vasorelaxation represents 'resistance' to insulin's vasodilatory effects, or whether it is functionally related to other consequences of metabolic insulin resistance. In the present study we found that moderate diet-induced obesity caused endothelial dysfunction comparable with that in genetically obese rats, but in the absence of insulin resistance. This indicates that other metabolic abnormalities must be responsible.

In our study, both sexes showed a significant negative correlation between fat-pad mass and attenuation of ACh-induced vasorelaxation. Particularly strong negative correlations were seen between terminal plasma TG levels and ACh-induced vasorelaxation, again in both sexes; this relationship suggests that hypertriglyceridaemia may induce endothelial dysfunction. This is consistent with the findings in patients with Type II diabetes that impaired vasodilatory responses to ACh were significantly correlated with hypertriglyceridaemia [1,2], although proof of causality will require further investigation. Deleterious effects on endothelial function could contribute to the atherogenic potential of hypertriglyceridaemia in normal and diabetic subjects independently of cholesterol [1,2]. Dietary-induced obesity also caused proportionate rises in plasma leptin levels. The endothelium is known to express functional leptin receptor [33], and leptin has been shown to induce direct endothelium-dependent vasorelaxation in addition to its central effect of enhancing sympathetic vascular tone [34]. However, although the plasma leptin level was correlated with impaired ACh-induced vasorelaxation in male rats, it was not an independent determinant of endothelial impairment in either sex. High NEFA levels have been implicated in endothelial dysfunction in humans [35], but we found no significant correlation between NEFA levels and attenuation of ACh-induced vasorelaxation.

In conclusion, we have demonstrated that moderate dietary-induced obesity markedly impairs vasorelaxation, primarily at the endothelial level, but also in smooth muscle. This vascular dysfunction is comparable in magnitude with that reported in insulin-resistant states, but occurred in the absence of insulin resistance. These defects may contribute to the hypertension and atheroma formation that accompany obesity, independently of any influence of insulin resistance. Hypertriglyceridaemia may provide a link between expanded fat-pad mass and endothelial dysfunction.

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REFERENCES

1 Tka, I., Kimball, B. P., Lewis, G., Uffelman, K. and Steiner, G. (1997) The severity of coronary atherosclerosis in type 2 diabetes mellitus is related to the number of circulating triglyceride-rich lipoprotein particles. Arterioscler. Thromb. Vasc. Biol. **17**, 3633–3638
2 Lewis, T. V., Dart, A. M. and Chin-Dusting, J. P. (1999) Endothelium-dependent relaxation by acetylcholine is impaired in hypertriglyceridemic humans with normal levels of plasma LDL cholesterol. J. Am. Coll. Cardiol. **33**, 805–812
3 Pinkney, J. H., Stehouwer, C. D. A., Coppack, S. W. and Yudkin, J. S. (1997) Endothelial dysfunction: cause of the insulin resistance syndrome. Diabetes, **46**, S9–S13
4 Shimokawa, H. (1999) Primary endothelial dysfunction: atherosclerosis. J. Mol. Cell. Cardiol. **31**, 23–37

5 Drexler, H and Hornig, B. (1999) Endothelial dysfunction in human disease. J. Mol. Cell. Cardiol. **31**, 51–60
6 Mombouli, J.-V. and Vanhoutte, P. M. (1999) Endothelial dysfunction: from physiology to therapy. J. Mol. Cell. Cardiol. **31**, 61–74
7 Cook, J. P. and Dzau, V. J. (1997) Nitric oxide synthase: role in the genesis of vascular disease. Annu. Rev. Med. **48**, 489–509
8 Creager, M. A., Cooke, J. P., Mendelsohn, M. E. et al. (1990) Impaired vasodilatation of forearm resistance vessels in hypercholesterolemic humans. J. Clin. Invest. **86**, 228–234
9 McVeigh, G. E., Brennan, G. M., Johnston, G. D. et al. (1992) Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia **35**, 771–776
10 Steinberg, H. O., Chacker, H., Leaming, R., Johnson, A., Brechtel, G. and Baron, A. D. (1996) Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. J. Clin. Invest. **97**, 2601–2610
11 Shimokawa, H. (1999) Primary endothelial dysfunction: atherosclerosis. J. Mol. Cell. Cardiol. **31**, 23–37
12 Boulanger, C. M. (1999) Secondary endothelial dysfunction: Hypertension and heart failure. J. Mol. Cell. Cardiol. **31**, 39–49
13 Reaven, G. M. (1988) Role of insulin resistance in human disease. Diabetes **37**, 1595–1607
14 Ferrannini, E., Buzzigoli, G., Bonadonna, R. et al. (1987) Insulin resistance in essential hypertension. N. Engl. J. Med. **317**, 350–357
15 Hsueh, W. A., Quinones, M. and Creager, M. A. (1997) Endothelium in insulin resistance and diabetes. Diabetes Rev. **5**, 343–352
16 Russell, J. C., Dolphin, P. J., Graham, S. E., Amy, R. M. and Brindley, D. N. (1998) Improvement of insulin sensitivity and cardiovascular outcomes in the JCR:LA-cp rat by D-fenfluramine. Diabetologia **41**, 380–389
17 Jin, J. S. and Bohlen, H. G. (1997) Non-insulin-dependent diabetes and hyperglycemia impair rat intestinal flow-mediated regulation. Am. J. Physiol. **272**, H728–H734
18 Phillips, M. S., Liu, Q., Hammond, H. A. et al. (1996) Leptin receptor missense in the fatty Zucker rat. Nat. Genet. **13**, 18–19
19 Russell, J. C. (1995) The atherosclerosis-prone JCR:LA-corpulent rats. In Atherosclerosis X: Proceedings of the 10th International Symposium on Atherosclerosis (Woodford, F. P., Davignon, J. and Sniderman, A., eds.), pp. 121–125, Elsevier, Amesterdam
20 Widdowson, P. S., Upton, R., Buckingham, R. E., Arch, J. and Williams, G. (1997) Inhibition of food response to intracerebroventricular injection of leptin is attenuated in rats with diet-induced obesity. Diabetes **46**, 1782–1785
21 Terrettaz, J. and Jeanrenaud, B. (1983) In vivo hepatic and peripheral insulin resistance in genetically obese (fa/fa) rats. Endocrinology **112**, 1346–1351
22 Naderali, E. K., Doyle, P. and Williams, G. (2000) Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs. Clin. Sci. **98**, 537–543
23 Walker, A. B., Dores, J., Buckingham, R. E., Savage, M. W. and Williams, G. (1997) Impaired insulin-induced attenuation of noradrenaline-mediated vasoconstriction in insulin-resistant obese Zucker rats. Clin. Sci. **93**, 235–241
24 Zanchi, A., Delacretaz, E., Taleb, V. et al. (1995) Endothelial function of the mesenteric arteriole and mechanical behaviour of the carotid artery in rats with insulin resistance and hypercholesterolemia. J. Hypertens. **12**, 1463–1470
25 Jain, V., Vedernikov, Y. P., Saade, G. R., Chwalisz, K. and Garfield, R. E. (1998) Effects of gestational age on in vitro responses of pregnant rat aorta. Hum. Reprod. **13**, 214–219
26 Naderali, E. K., Doyle, P. and Williams, G. (2000) Resveratrol induces vasorelaxation of mesenteric and uterine arteries from female guinea-pigs. Clin. Sci. **98**, 537–543
27 Naderali, E. K., Smith S. L., Doyle, P. and Williams, G. (2001) Vasorelaxant effects of oestradiols on guinea-pigs: a role for gender differences. Eur. J. Clin. Invest. **31**, 215–220
28 McNamee, C. J., Kappagoda, C. T., Kunjara, R. and Russell, J. C. (1994) Defective endothelium-dependent relaxation in the JCR:LA-corpulent rat. Circ. Res. **74**, 1126–1132
29 Enderle, M. D., Benda, N., Schmuelling, R. M., Hearing, H. U. and Pfhol, M. (1998) Preserved endothelial function in IDDM patients, but not in NIDDM patients, compared with healthy subjects. Diabetes Care **21**, 271–277
30 Zeiher, A. M., Drexler, H., Wollschlager, H. and Just, H. (1991) Modulation of coronary vasomotor tone in humans. Progressive endothelial dysfunction with different early stages of coronary atherosclerosis. Circulation **83**, 391–401
31 Williams, S. B., Cusco, J. A., Roddy, M. A., Johnstone, M. T. and Creager, M. A. (1996) Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J. Am. Coll. Cardiol. **27**, 567–574
32 Lebuffe, G., Boullier, A., Tailleux, A. et al. (1997) Endothelial derived vasorelaxation is impaired in human APO A-1 transgenic rabbits. Biochem. Biophys. Res. Commun. **241**, 205–211
33 Maruyama, I., Nakata, M. and Yamaji, K. (2000) Effects of leptin in platelet and endothelial cells. Obesity and arterial Thrombosis. Ann. N. Y. Acad. Sci. **902**, 315–319
34 Gardiner, S. M., Kemp, P. A., March, J. E. and Bennett, T. (1998) Recombinant human, but not murine, leptin causes hindquarters vasoconstriction in conscious rats. Br. J. Pharmacol. **125**, p. 91 (Abstract)
35 Steinberg, H. O., Tarshoby, M., Monestel, R. et al. (1997) Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. J. Clin. Invest. **100**, 1230–1239

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