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Investigation of Endothelial Hyperreactivity in the Obese Zucker Rat In-situ: Reversal by Vitamin E

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Abstract

The obese Zucker rat, a popular model of insulin resistance allied with oxidant stress, is associated with either normal or paradoxically enhanced endothelial vasodilator function compared with its lean litter mate. We have investigated hindquarter endothelium-dependent vasodilation in the obese Zucker rat in-situ and have examined its relationship with oxidant stress.

In perfused hindquarter preparations equivalently preconstricted with phenylephrine, vasodilator responses to the endothelium-dependent agent acetylcholine (0·03–1000 pmol) were greater in obese ($pD_2 = 11{\cdot}03 \pm 0{\cdot}19$) compared with lean ($pD_2 = 10{\cdot}53 \pm 0{\cdot}13$) animals ($P < 0{\cdot}01$, two-way analysis of variance). In contrast, maximal vasodilation to the nitric oxide (NO) donor sodium nitroprusside (100 nmol) was similar in obese ($59{\cdot}6 \pm 9{\cdot}8\%$) and lean ($51{\cdot}9 \pm 2{\cdot}6\%$) preparations ($P > 0{\cdot}05$). However, this exaggerated vasodilator reactivity to acetylcholine in obese animals was abolished following four-week dietary supplementation with the lipophilic antioxidant vitamin E (obese $pD_2 = 10{\cdot}74 \pm 0{\cdot}18$; lean $pD_2 = 10{\cdot}74 \pm 0{\cdot}08$). This antioxidant-mediated effect was associated with a reduction ($P < 0{\cdot}02$, two-way analysis of variance) and an enhancement ($P < 0{\cdot}01$, two-way analysis of variance) in endothelium-dependent vasodilator responses in obese and lean hindlimb preparations, respectively.

Our data therefore now point to a differential modulation of hindquarter endothelium-dependent vasodilation in the obese and lean Zucker rat by the prevailing oxidant tone, resulting in an agonist-stimulated endothelial vasodilator hyperreactivity in obese animals.

Oxidant stress is associated with endothelial dysfunction in a number of conditions including atherosclerosis and type II diabetes mellitus (for review see Laight et al 1999a). However, although the obese Zucker rat exhibits features of insulin resistance syndrome and type II diabetes mellitus (Reaven 1995; Laight et al 1999b), including oxidant stress determined in-vivo (Laight et al 1999c), there is curiously little evidence of impaired endothelial vasodilator function in this animal. Possibly as a result of this, the animal remains largely free of cardiovascular complications (Bohlen & Lash 1995; Laight et al 1999a). Indeed, endothelium-dependent vasodilation has been consistently shown to be either normal or paradoxically enhanced in both conductance vessels and resistance vascular beds of the obese Zucker rat compared with its lean, insulin-sensitive litter mate (Auguet et al 1989; Cox & Kikta 1992; Sexl et al 1995; Turner & White 1996; Laight et al 1998a; Kaw et al 1999).

We have previously observed augmented vasodilator responses to acetylcholine, but not sodium nitroprusside, in the perfused hindquarters of the obese Zucker rat in-situ (Andrews et al 1998). Therefore, our aim in this study was to begin to examine the relationship between enhanced endothelial vasodilator function and oxidant stress using this preparation.

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Materials and Methods

The experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 (Home Office, London, UK).

Dietary protocol
Male, 9-week old Zucker rats (Harlan, Blackthorn, Bicester, UK) were maintained for four weeks on a standard chow diet (Special Diet Services, Witham, Essex, UK) with or without supplementation with vitamin E (as (±)-α-tocopherol acetate added to the chow at 0·5% w/w). This dietary antioxidant regimen has been shown to attenuate elevated plasma levels of a lipid peroxidation marker in the obese Zucker rat in-vivo (Laight et al 1999c). Water and food were freely available.

Hindquarter perfusion in-situ
Animals were anaesthetised with sodium pentobarbitone (60 mg kg^{-1}, i.p.) and killed by exsanguination after the administration of heparin (200 int. units kg^{-1}, i.v.). The abdominal aorta and vena cava were cannulated near the bifurcation point of the iliac arteries for perfusion inflow and outflow, respectively. The hindquarters were perfused at constant volume (7 mL min^{-1}) in open circuit with physiological salt solution of the following composition (mM): NaCl 133, KCl 4·7, NaH_2PO_4 1·35, $NaHCO_3$ 16·3, $MgSO_4$ 0·61, $CaCl_2$ 2·52, and D-glucose 7·8) gassed with carbogen and warmed to 37°C (Andrews et al 1998). Animal rectal temperature was also maintained at 37°C by a homeothermic blanket. Perfusion pressure, which reflected vascular resistance, was measured by a pressure transducer and continuously recorded using a MacLab system (Hastings, Sussex, UK).

Assessment of hindquarter endothelial function
Following stabilization (15min), perfusion pressure was submaximally elevated with a continuous infusion of phenylephrine (100 nmol min^{-1}) to allow the assessment of endothelium-dependent vasodilation to bolus dose acetylcholine (0·03–1000 pmol). Maximal vasodilation to a standard bolus dose of the nitric oxide (NO) donor sodium nitroprusside (100 nmol) was then assessed to provide a simple measure of endothelium-independent vasodilator reactivity in each preparation. We have previously observed vasodilation to sodium nitroprusside to be similar over a full dose–response range (0·001–100 nmol) in the perfused hindquarter in-situ of the obese and lean Zucker rat (Andrews et al 1998).

Drugs
All drugs were obtained from Sigma Chemical Co. (Poole, Dorset, UK).

Data analysis
Data are presented as means ± s.e.m. Two-way analysis of variance was used to examine the effects of rat strain and antioxidant treatment on the parameters measured, including the differences in the magnitude of vasodilator responses between dose–response curves. $P < 0.05$ was considered significant.

Results

Animal weights
The body weight of 13-week old obese rats (413·0 ± 15·2 g, n = 5) was significantly greater compared with lean animals (280·2 ± 4·2 g, n = 5) ($P < 0.01$). Body weights were not affected by dietary vitamin E (obese: 428·2 ± 8·6 g, n = 6; lean: 280·6 ± 6·3 g, n = 5).

Hindquarter perfusion pressure
Basal perfusion pressure in obese preparations (28·5 ± 6·1 mmHg, n = 5) was comparable with that in lean preparations (38·1 ± 5·8 mmHg, n = 5) and was not affected by dietary vitamin E (obese: 29·9 ± 5·4 mmHg, n = 6; lean: 25·5 ± 2·3 mmHg, n = 5) ($P > 0.05$). Similarly, phenylephrine-elevated perfusion pressure in obese preparations (144·2 ± 20·5 mmHg, n = 5) was comparable with that in lean preparations (129·6 ± 6·0 mmHg, n = 5) ($P > 0.05$) and was not significantly altered by dietary vitamin E (obese: 144·8 ± 13·0, n = 6; lean: 141·6 ± 13·3, n = 5) ($P > 0.05$).

Endothelial vasodilator function
Vasodilator responses to acetylcholine (0·03–1000 pmol) were significantly augmented ($P < 0.01$) in obese compared with lean animals (Figure 1), while maximal vasodilation to sodium nitroprusside (100 nmol) was similar (obese: 59·6 ± 9·8%, n = 5; lean 51·9 ± 2·6%, n = 5) ($P > 0.05$). This was accompanied by a slight (3-fold) increase in the sensitivity of vasodilation to acetylcholine, assessed as the negative log of the concentration eliciting the half-maximal response (pD_2), in obese ($pD_2 = 11.03 \pm 0.19$, n = 5) compared with lean ($pD_2 = 10.53 \pm 0.13$) hindquarter preparations ($P = 0.09$). However, differences in hindquarter endothelium-dependent vasodilation between Zucker strains, was abolished following four-week dietary vitamin E (Figure 1) (obese $pD_2 = 10.74 \pm 0.18$, n = 6; lean $pD_2 = 10.74 \pm 0.08$, n = 5) ($P > 0.05$). This effect was associated with

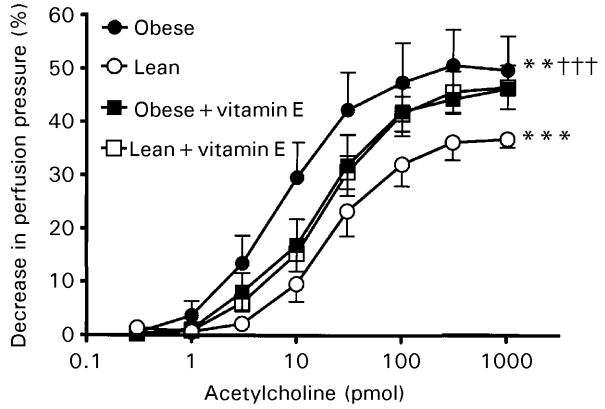

Figure 1. Decrease in perfusion pressure in response to a bolus dose of acetylcholine in the phenylephrine-preconstricted, perfused hindquarter in-situ of the 13-week old lean or obese Zucker rat. Vitamin E (as (±)-α-tocopherol acetate added to the chow at 0·5% w/w) was administered from the age of 9 weeks. Values are means ± s.e.m., n = 5–6. ** $P < 0{\cdot}02$, *** $P < 0{\cdot}01$ compared with vitamin E treatment in corresponding group; ††† $P < 0{\cdot}01$ compared with lean control group.

an attenuation and augmentation in the magnitude of vasodilator responses to acetylcholine (0·03–1000 pmol) in obese ($P < 0{\cdot}02$) and lean ($P < 0{\cdot}01$) hindquarter preparations, respectively (Figure 1). In contrast to endothelium-dependent responses, maximal vasodilation to sodium nitroprusside was similarly augmented ($P < 0{\cdot}02$) following four-week dietary vitamin E in obese (77·5 ± 8·0%, n = 6) and lean (71·5 ± 5·4%, n = 5) hindquarter preparations. Similar results were obtained following four-week dietary supplementation with the aqueous antioxidant vitamin C, administered as a 0·1 % w/v solution in the drinking water, or a combination of vitamins E and C at the doses used above (data not shown, n = 4–5).

Discussion

Endothelium-dependent vasodilation, but not vasodilation to an endothelium-independent vasodilator, was augmented in the perfused hindquarter in-situ of the obese Zucker rat. This therefore suggests that enhanced vasodilation to acetylcholine did not simply reflect a general increase in vascular smooth muscle vasodilator reactivity in obese animals, but instead represents a selective enhancement in endothelial vasodilator function. The notion of an endothelial hyperreactivity in the obese compared with the lean Zucker rat, is in fact well supported by other studies both in-vitro (Auguet et al 1989; Cox & Kikta 1992; Sexl et al 1995; Turner & White 1996; Kaw et al 1999) and recently, in-vivo (Laight et al 1998a). While the majority of these studies has exclusively employed cholinergic agonists as pharmacological tools for the assessment of endothelial function, at least the demonstration by Sexl et al (1995) that vasodilation to the calcium ionophore A23187 was similarly augmented along with responses to acetylcholine in isolated vascular smooth muscle of the obese Zucker rat, suggests that endothelial hyperreactivity is not simply a result of altered muscarinic or cholinesterase activity. However, the precise role of endothelium-derived relaxing factors such as NO, prostacyclin and hyperpolarizing factor(s) in this hyperreactivity, remains to be established.

Dietary vitamin E improved endothelium-dependent vasodilation in lean hindquarter preparations and in addition, enhanced vasodilation to an NO donor. This antioxidant action on endothelial function is therefore consistent with the protection of endothelium-derived NO from inactivation by reactive oxygen species (Gryglewski et al 1986; Laight et al 1998b). In contrast, endothelium-dependent vasodilation in obese hindquarter preparations was depressed by vitamin E treatment, despite an augmentation in vasodilation to an NO donor. This now suggests that agonist-stimulated endothelial vasodilator function is differentially modulated by oxidant tone in lean and obese Zucker rats. Hence, reactive oxygen species appear to lead to a net upregulation in agonist-stimulated endothelium-dependent vasodilation in the obese Zucker rat in the face of reduced vasodilation to NO itself.

Such endothelial hyperreactivity is initially paradoxical in the light of in-vivo evidence of elevated lipid peroxidation and oxidant stress in the obese Zucker rat (Laight et al 1999c), which would be expected to impair endothelium-dependent vasodilation and in addition, promote macroangiopathy, especially within the context of insulin resistance syndrome (Laight et al 1999a). However, Graier et al (1997a) have previously reported a mechanism for a net reactive oxygen species-mediated enhancement in agonist-stimulated endothelial function, involving altered calcium signalling in the endothelium. This has been suggested to account for the anomaly of endothelial hyperreactivity associated with early type I diabetes (Wascher et al 1994; Graier et al 1997a). It is therefore conceivable that a similar scenario in the obese Zucker rat may account, at least in part, for endothelial hyperreactivity in this animal and also for its experimental reversibility or 'down-regulation' by dietary antioxidants (Graier et al 1997b), demonstrated in this study. Indeed, such a preserved or 'compensated' endothelial function in the obese Zucker rat, also documented in the

Zucker diabetic fatty rat, may protect these insulin resistant animals from the development of significant macrovascular disease (Bohlen & Lash 1995; Laight et al 1999a).

Since vasodilation to an NO donor was equivalently sensitive to enhancement by an antioxidant in the lean and obese perfused hindquarter, implying a similar level of inactivation by reactive oxygen species in-situ, we suggest that the primary signal for endothelial hyperreactivity concerns in-vivo oxidant stress in the obese Zucker rat (Laight et al 1998a, 1999c). While an upregulation in the synthesis/release of endothelium-derived relaxing factors such as prostacyclin or NO cannot be ruled out in any adaptive response to this elevated oxidant tone, it is reasonable to speculate on a role for a non-prostanoid, non-NO component of vasodilation, possibly a hyperpolarizing factor, recently described in the isolated aorta of the obese Zucker rat (Kaw et al 1999).

In conclusion, the efficacy of an antioxidant, vitamin E, to reverse hindquarter endothelial vasodilator hyperreactivity in the obese Zucker rat observed in-situ, now suggests that this phenomenon is related to differences in the regulation of agonist-stimulated endothelium-dependent vasodilation by oxidant tone in lean and obese animals in-vivo.

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