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RESEARCH PAPER

Differential effects of glucose on agonist-induced relaxations in human mesenteric and subcutaneous arteries

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Background and purpose: Acute periods of hyperglycaemia are strongly associated with vascular disorder, yet the specific effects of high glucose on human blood vessel function are not fully understood. In this study we (1) characterized the endothelial-dependent relaxation of two similarly sized but anatomically distinct human arteries to two different agonists and (2) determined how these responses are modified by acute exposure to high glucose.
Experimental approach: Ring segments of human mesenteric and subcutaneous arteries were mounted in a wire myograph. Relaxations to acetylcholine and bradykinin were determined in a control (5 mM) and high glucose (20 mM) environment over a 2 and 6 h incubation period.
Key results: Bradykinin-induced relaxation in both sets of vessels was mediated entirely by EDHF whilst that generated by acetylcholine, though principally generated by EDHF, also had contribution from prostacyclin and possibly nitric oxide in mesenteric and subcutaneous vessels, respectively. A 2-h incubation of high glucose impaired bradykinin-induced relaxation of subcutaneous vessels whilst, in contrast, the relaxation generated by bradykinin in mesenteric vessels was enhanced at the same time point. High glucose significantly augmented the relaxation generated by acetylcholine in mesenteric and subcutaneous vessels at a 2 and 6 h incubation point, respectively.
Conclusions and implications: Short periods of high glucose exert a variable influence on endothelial function in human isolated blood vessels that is dependent on factors of time, agonist-used and vessel studied. This has implications for how we view the effects of acute hyperglycaemia found in patients with diabetes mellitus as well as other conditions.
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Keywords: glucose; human arteries; endothelium; EDHF; nitric oxide; vasodilatation

Abbreviations: EDHF, endothelium-derived hyperpolarizing factor; GTN, glyceryl trinitrate; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide

Introduction

Periods of high plasma glucose are a common consequence of diabetes mellitus and are likely to be an underlying cause of many significant diabetic complications such as blood vessel disorder. Abnormalities are found in both the macro- and microvascular circulations and as a result people with diabetes are 2–4 times more likely to die of heart disease or stroke than non-diabetics (Panzram, 1987). Intensive control of plasma glucose levels in diabetic patients significantly reduces cardiovascular mortality (UKPDS 33, 1998), suggesting that hyperglycaemia is itself an independent risk factor for vascular disorder (Wilson *et al.*, 1991). Furthermore, several studies have demonstrated that the transient rises in plasma glucose levels that commonly occur during the post-prandial state in diabetic subjects are more accurate predictors of mortality than fasting glucose levels (DECODE study group, 1999). Similar findings have been obtained for mildly high glucose excursions of (non-diabetic) glucose-intolerant subjects (Balkau *et al.*, 1998).

The endothelium plays an essential role in the control of vascular smooth muscle cell tone by the synthesis and release of several vasoactive substances, including the vasodilators nitric oxide (NO), prostacyclin and endothelium-derived hyperpolarizing factors (EDHFs). There is an abundance of evidence demonstrating that hyperglycaemia has an inhibitory effect on endothelial function (Taylor and Poston, 1994; Dorigo *et al.*, 1997; Jin and Bohlen, 1997; Williams *et al.*, 1998). However, this is not a universal finding, with

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some studies showing that high glucose mediates no change (Houben *et al*., 1996; Brands and Fitzgerald, 1998; Charkoudian *et al*., 2002; Reed *et al*., 2004) in endothelial function or even enhances vascular vasodilatation (Graier *et al*., 1993; Sieber *et al*., 1993; Cipolla *et al*., 1997). The inconsistencies in these findings are likely to be exacerbated by the fact that the individual contributions made by NO, prostacyclin and EDHF to overall endothelial-dependent relaxation are often difficult to characterize, since the individual importance of each factor may vary between species, anatomical origin and size of blood vessel investigated and the agonist used to stimulate the endothelium. In the latter category for example, the functional characteristics of endothelial-dependent relaxation of arteries supplying human skeletal muscle were also shown to be critically dependent on the agonist used that is, EDHF was responsible for up to 50% of ACh-mediated relaxation but at least 70% of bradykinin-mediated relaxation with the remainder being due to NO (Schrage *et al*., 2005).

Therefore, while the evidence clearly indicates that hyperglycaemia exerts a global detrimental influence on the vascular system, it appears that its effects on endothelial function may not be universal throughout all blood vessels. To investigate this further, the aims of our study were to (1) characterize the endothelial-dependent relaxation of two similarly sized but anatomically distinct human arteries to two different endothelial agonists and (2) determine how these responses are modified by an acute exposure to high glucose.

Methods

Glasgow Royal Infirmary Local Research Ethics Committee gave ethical consent to this study. All human tissue samples were obtained from the BioBank at Glasgow Royal Infirmary and informed consent was obtained from all patients involved in the study. Patient age, sex and plasma glucose were recorded.

Patient exclusion criteria

Patients were excluded from the study if they were diagnosed diabetic by consulting medical staff or if their fasting plasma glucose concentration exceeded 7.0 mmol l^{-1} (American Diabetes Association, 2007).

Samples

Mesenteric and subcutaneous arteries were obtained from patients undergoing elective abdominal or breast reduction surgery at the Glasgow Royal Infirmary. Tissue was excised and placed into ice-cold Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 5. The samples were transported to the laboratory for dissection within 2 h of surgery. Vessel segments (length 2 mm; intraluminal diameter: mesenteric 295–730 μm, $n = 30$, mean 470 ± 35 μm; subcutaneous 165–875 μm, 616 ± 18 μm, $n = 52$) were mounted on a Danish Myotechnology M610 wire myograph containing Krebs solution at 37 °C, gassed with 95% O_2/5% CO_2, pH 7.4. Vessels were stretched to give a transmural pressure equivalent to approximately 60 mm Hg and then equilibrated for 30 min.

Experimental protocols

Characterization of vascular relaxations. Cumulative concentration–response curves to the thromboxane analogue U46619 (0.1 nM–1 μM) were constructed. Vessels were washed and allowed to restabilize. Endothelium-dependent relaxations induced by bradykinin (0.1 nM–1 μM) and ACh (1 nM–10 μM) were then assessed in vessels pre-contracted with U46619 ($EC_{60–80}$). To characterize the influence of the various endothelium-derived relaxing factors, vessels were then incubated in either 5 mM glucose solution (control), N^G-nitro-L-arginine methyl ester (L-NAME; 100 μM; inhibitor of NOS), indomethacin (10 μM, inhibitor of cyclooxygenase) or apamin plus charybdotoxin (both 100 nM, EDHF blockers; Waldron and Garland, 1994; Zygmunt and Högestätt, 1996). Endothelium-dependent relaxations induced by bradykinin (0.1 nM–1 μM) and ACh (1 nM–10 μM) were then reassessed in vessels pre-contracted with U46619 ($EC_{60–80}$).

Effect of incubation with 20 mM glucose on vascular reactivity. Cumulative concentration–response curves to the thromboxane analogue U46619 (0.1 nM–1 μM) were constructed. Vessels were washed and allowed to restabilize. Endothelium-dependent relaxations induced by bradykinin (0.1 nM–1 μM) and ACh (1 nM–10 μM) and endothelial-independent relaxations induced by glyceryl trinitrate (GTN; 0.1 nM–1 μM) were then assessed in vessels pre-contracted with U46619 ($EC_{60–80}$). To investigate the effect of high concentrations of glucose, vessels were then incubated in either control glucose solution (5 mM) or high glucose solution (20 mM) for periods of either 2 or 6 h. Endothelium-dependent relaxations induced by bradykinin (0.1 nM–1 μM) and ACh (1 nM–10 μM) and endothelial-independent relaxations induced by GTN (0.1 nM–1 μM) were then reassessed in these vessels pre-contracted with U46619 ($EC_{60–80}$).

Materials

Charybdotoxin was obtained from Latoxan (Valence, France). GTN was obtained from Merck (Germany). All other agents were obtained from Sigma-Aldrich (Poole, Dorset, UK).

All drugs were dissolved and diluted in 0.9% saline except indomethacin (0.01 M stock), which was dissolved in Na_2CO_3 (0.4 mg ml^{-1}) and U46619 (1 mM), which was dissolved in 50% ethanol.

Statistical analysis

Data are expressed as mean ± s.e. mean; n values represent the number of vessel segments, minimum of three patients per protocol with no more than two rings per protocol per patient. Relaxations are expressed as a percentage of the vasoconstrictor tone generated by U46619 ($EC_{60–80}$). Graphs were drawn and statistical comparisons made using one- or

two-way ANOVA with Bonferroni's post-test with the aid of a computer programme, Prism (GraphPad, San Diego, CA, USA). $P<0.05$ was considered significant.

Results

Patient characteristics

A total of 27 individuals were included in the study. A summary of patient age and plasma glucose is presented in Table 1. All subcutaneous vessels were obtained from the female patients. Mesenteric vessels were obtained from either sex (5 females and 10 males).

Characterization of endothelium-dependent relaxations

Bradykinin (0.1 nM–1 μM), ACh (1 nM–10 μM) and GTN (0.1 nM–1 μM) all induced concentration-dependent relaxation in both mesenteric and subcutaneous human arteries pre-constricted with the thromboxane mimetic U46619 (EC_{60-80}).

In subcutaneous arteries, the endothelium-dependent relaxation induced by bradykinin (0.1 nM–1 μM) was not significantly affected by either L-NAME (100 μM) or indomethacin (10 μM). However, it was significantly impaired following incubation with apamin and charybdotoxin (both at 100 nM; $P<0.001$; Table 2). Relaxation induced by ACh (1 nM–10 μM) in subcutaneous arteries was unaffected following incubation with indomethacin (10 μM), was depressed following incubation with L-NAME (100 μM, did not achieve statistical significance) but was significantly inhibited following incubation with apamin and charybdotoxin (both at 100 nM; $P<0.05$; Table 2).

Similarly, in mesenteric arteries the endothelium-dependent relaxation induced by bradykinin (0.1 nM–1 μM) was not significantly affected by either L-NAME (100 μM) or indomethacin (10 μM). However, it was significantly impaired following incubation with apamin and charybdotoxin (both at 100 nM; $P<0.001$; Table 2). Similar to bradykinin, yet in contrast to the observation in the subcutaneous vessel segments, the relaxation induced by ACh (1 nM–10 μM) in mesenteric arteries was unaffected following incubation with L-NAME (100 μM), but was significantly inhibited following incubation with indomethacin (10 μM) or apamin and charybdotoxin (both at 100 nM; $P<0.05$; Table 2).

Table 1 Summary of patient age and fasting plasma glucose

	Mean ± s.e.mean (n)	*Range*
Age (years)	50 ± 4 (27)	18–79
FPG (mmol l^{-1})	3.5 ± 0.3 (27)	1.2–6.7

Abbreviation: FPG, fasting plasma glucose.

Effect of incubation with 20 mM glucose on vascular reactivity

Bradykinin-induced relaxation. Incubation in high glucose (20 mM compared to 5 mM control) for 2 h resulted in a small but significant inhibition of bradykinin (0.1 nM–1 μM)-induced relaxation in subcutaneous arteries (Figure 1a; $P<0.05$; two-way ANOVA). In contrast, a longer incubation period in 20 mM glucose of 6 h had no significant effect on bradykinin-induced relaxation (Figure 1b).

In contrast, in mesenteric vessel segments, incubation in high glucose (20 mM compared to 5 mM control) for 2 h resulted in a clear enhancement of bradykinin-induced relaxation (Figure 1c; $P<0.01$; two-way ANOVA). Again, a longer incubation period in 20 mM glucose of 6 h had no significant effect on bradykinin-induced relaxation (Figure 1d).

ACh-induced relaxation. Incubation in high glucose (20 mM compared to 5 mM control) for 2 h had no effect on ACh (1 nM–10 μM)-induced relaxation in subcutaneous arteries (Figure 2a). In contrast, a longer incubation period in 20 mM glucose of 6 h resulted in a significant enhancement of ACh-induced relaxation (Figure 2b; $P<0.05$; two-way ANOVA).

However, in mesenteric vessel segments incubation in high glucose (20 mM compared to 5 mM control) for 2 h resulted in a clear enhancement of ACh-induced relaxation (Figure 2c; $P=0.01$; two-way ANOVA). A longer incubation period of 6 h in 20 mM glucose, had no significant effect on ACh-induced relaxation (Figure 2d). The responses to ACh in the mesenteric vessels at the 6 h time point were to an extent variable compared to those observed at earlier time points in mesenteric vessels or those observed in subcutaneous vessels at 6 h.

Table 2 Maximum agonist-induced relaxation to bradykinin (1 μM) or ACh (10 μM) in human subcutaneous and mesenteric arteries following incubation (20 min) in either 5 mM glucose solution (control); L-NAME (100 μM; inhibitor of NOS); indomethacin (10 μM, inhibitor of COX) or apamin plus charybdotoxin (both 100 nM, EDHF blockers)

	Control	*L-NAME*	*Indomethacin*	*Apamin + charybdotoxin*
Subcutaneous arteries				
Bradykinin	86.5 ± 4.5 (5)	73.2 ± 13.8 (5)	87.1 ± 10.8 (5)	21.4 ± 12.0 (5)**
ACh	82.1 ± 9.9 (5)	57.5 ± 17.4 (5)	84.0 ± 11.4 (5)	30.9 ± 13.6 (5)*
Mesenteric arteries				
Bradykinin	93.7 ± 2.5 (7)	80.9 ± 6.2 (7)	86.1 ± 4.7 (7)	32.7 ± 13.5 (7)***
ACh	62.8 ± 8.6 (6)	56.9 ± 13.4 (6)	15.1 ± 9.2 (5)*	19 ± 11.8 (6)*

Abbreviations: COX, cyclooxygenase; EDHF, endothelium-derived hyperpolarizing factor; GTN, glyceryl trinitrate; L-NAME, N^G-nitro-L-arginine methyl ester. Data are mean ± s.e. mean (*n*). *$P<0.05$; **$P<0.01$; ***$P<0.001$ one-way ANOVA compared to control.

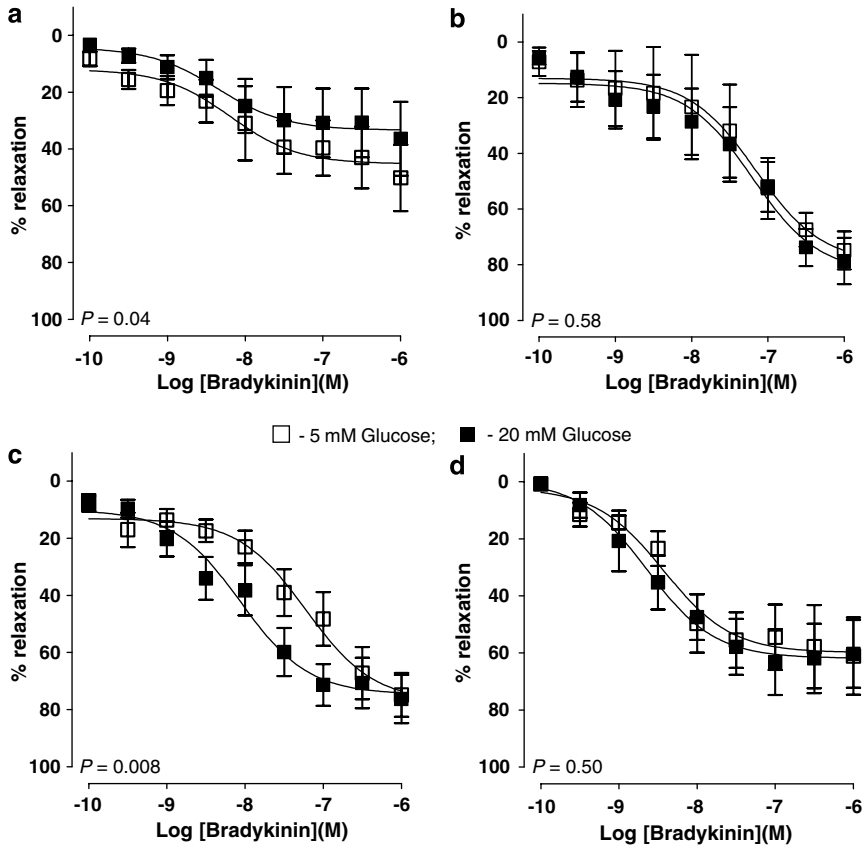

Figure 1 (**a**) Cumulative log concentration–response curves showing endothelium-dependent vasorelaxation to bradykinin (0.1 nM–1 μM) in human subcutaneous (**a** and **b**) and mesenteric (**c** and **d**) arteries following incubation in high glucose (20 mM) or under time-matched control conditions (5 mM glucose). Incubation times are either 2 (**a** and **c**) or 6 h (**b** and **d**). Data are mean ± s.e. mean of 4–11 observations. Individual *P*-values represent two-way ANOVA, showing effect of treatment.

GTN-induced relaxation. Incubation in high glucose (20 mM compared to 5 mM control) for 2 or 6 h had no significant effect on GTN (0.1 nM–1 μM)-induced relaxation in either subcutaneous or mesenteric arteries (Figure 3).

Discussion

Although not universal, in the majority of studies investigating the influence of high levels of glucose on endothelial-dependent relaxation, a clear inhibitory effect was observed (Creager *et al*., 2003; Gerich, 2003; Endemann and Schiffrin, 2004). However, in this study, a much more complex picture is revealed, in that high glucose exerts a differential influence on human vascular activity that is dependent on variables of time, endothelial agonist used and vessel studied. Indeed, this is the first time that a high glucose environment has been shown to both inhibit and augment EDHF activity in similarly sized but anatomically distinct human blood vessels.

Characterization of human arterial function

Relaxant responses produced by both bradykinin and ACh in subcutaneous vessels were substantially impaired following treatment with apamin and charybdotoxin, but were unaffected by L-NAME or indomethacin, suggesting that an EDHF is the principal relaxant generated by both agonists. However, it is known that prostacyclin, NO and EDHF can work in parallel and that each pathway may be able to compensate for the loss of one of the other components (for review, see Félétou and Vanhoutte, 2006). Thus, we cannot exclude a possible role for prostacyclin or NO in the relaxation. In fact, although it is not statistically significant, there was a noticeable depression in ACh-induced relaxation in the presence of L-NAME, which may suggest a small NO component alongside the dominant role of EDHF. Indeed, such a finding has been obtained previously in human subcutaneous vessels in which EDHF was shown to be the major (~80%) component of ACh-induced vasorelaxation, with the remainder being mediated by NO (Coats *et al*., 2001). In contrast to our findings showing no NO component in the relaxation induced by bradykinin, the bradykinin-mediated relaxation of similarly sized subcutaneous vessels from pregnant women was found to be mediated equally by EDHF and NO (Luksha *et al*., 2004). This discrepancy may be due to the elevated levels of plasma oestrogens found in pregnant women; these are known to both activate and promote NO activity (Sack *et al*., 1994; Chen *et al*., 1999).

As with our findings in subcutaneous vessels, we have demonstrated that the endothelial-dependent relaxation

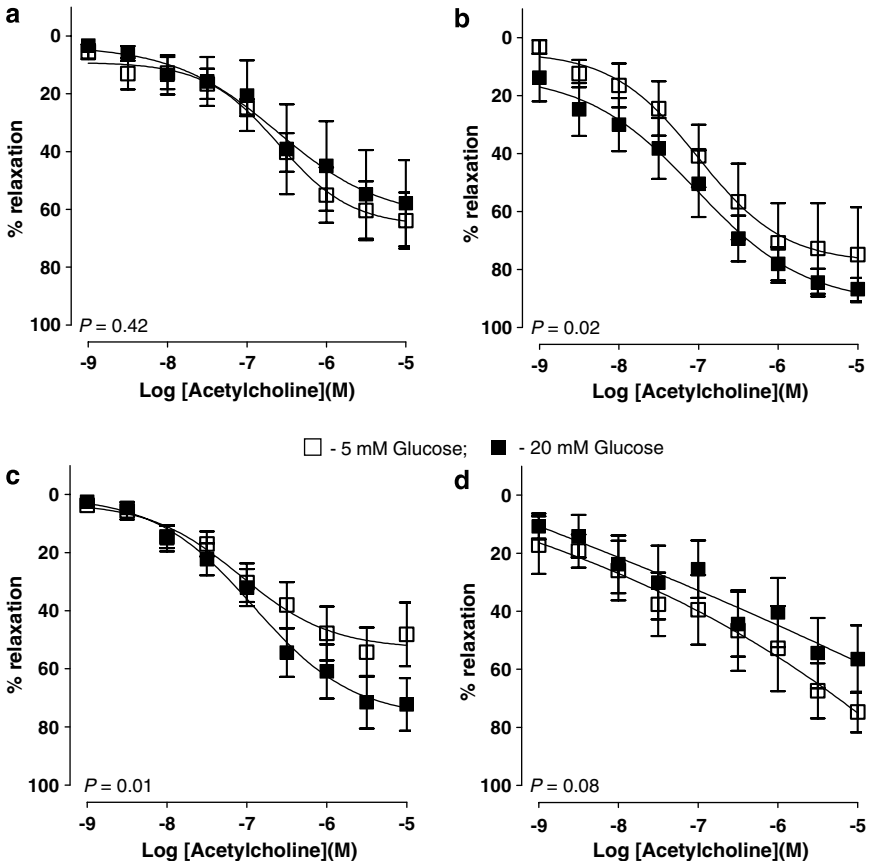

Figure 2 (**a**) Cumulative log concentration–response curves showing endothelium-dependent vasorelaxation to acetylcholine (1 nM–10 μM) in human subcutaneous (**a** and **b**) and mesenteric (**c** and **d**) arteries following incubation in high glucose (20 mM) or under time-matched control conditions (5 mM glucose). Incubation times are either 2 (**a** and **c**) or 6 h (**b** and **d**). Data are mean ± s.e. mean of 4–11 observations. Individual *P*-values represent two-way ANOVA, showing effect of treatment.

generated by bradykinin in mesenteric arteries was mediated entirely by EDHF with no clear role being found for NO or prostacyclin. The relaxation produced by ACh in mesenteric vessels was generated principally by EDHF but with a significant contribution made by prostacyclin. Similar findings demonstrating that bradykinin-mediated relaxation of human isolated mesenteric vessels is mediated entirely by EDHF have been obtained previously (Urakami-Harasawa *et al*., 1997). However, unlike our findings, in this study, it was further shown that the relaxation induced by ACh was mediated entirely by EDHF with no prostacyclin component demonstrated. Interestingly, although this group did find, as we did, that the control relaxation produced by ACh in mesenteric vessels in the absence of any inhibitors was somewhat blunted compared with that generated by bradykinin. We found no such disparity in the magnitude of relaxation produce by ACh and bradykinin in subcutaneous vessels in these characterization experiments.

Our findings are in general agreement with those from a number of studies that suggest that EDHF is the principal relaxing factor in small human arteries (McGuire *et al*., 2001). However, we demonstrated that the contributions played by NO or prostacyclin are variable and clearly agonist dependent. Such agonist-specific disparities have been observed previously in arteries supplying human skeletal muscle, where EDHF is responsible for up to 50% of ACh-mediated relaxation and for at least 70% of bradykinin-mediated relaxation, with the remainder being due to NO (Schrage *et al*., 2005). One factor that may contribute to the differences between the vessels is that the subcutaneous arteries are all from female patients, whereas the mesenteric are from either sex. It is recognized in the literature that there are subtle differences in vascular function between the sexes (for review, see Denton and Bayliss, 2007).

Influence of high glucose on human artery function

Our results give no indication that incubation of either vessel type with high glucose impairs the ability of NO to induce relaxation. Firstly, our data suggest that ACh may induce a small NO component to the relaxation generated in subcutaneous arteries, yet this relaxation is unchanged following a 2-h incubation in high glucose and is actually enhanced following 6 h of incubation. Secondly, high glucose, in either vessel, does not inhibit the relaxation mediated by GTN, which results from the exogenous production of NO. Therefore, 20 mM glucose does not impair the NO activity in human isolated vessels over the time frame studied. These glucose concentrations reflect post-prandial plasma glucose concentrations in Type II diabetic patients (Kipnes *et al*., 2003), and have been shown to impair relaxation in isolated vessels from animals (Taylor and

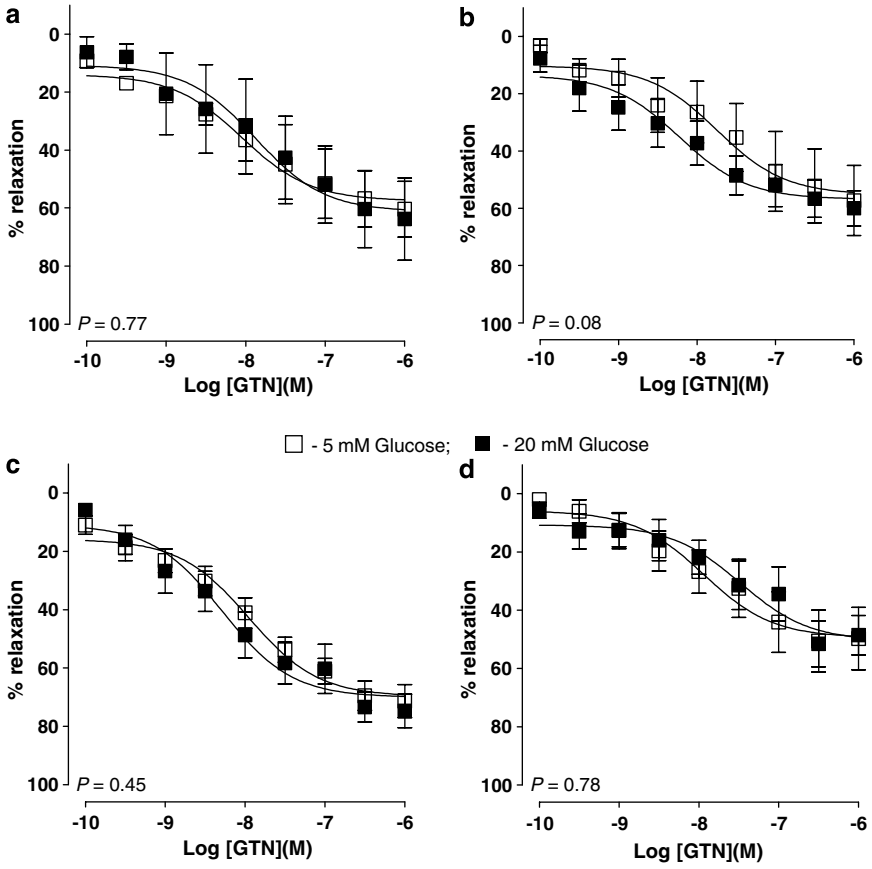

Figure 3 (**a**) Cumulative log concentration–response curves showing endothelium-independent vasorelaxation to GTN (0.1 nM–1 μM) in human subcutaneous (**a** and **b**) and mesenteric (**c** and **d**) arteries following incubation in high glucose (20 mM) or under time-matched control conditions (5 mM glucose). Incubation times are either 2 (**a** and **c**) or 6 h (**b** and **d**). Data are mean ± s.e. mean of 4–11 observations. Individual *P*-values represent two-way ANOVA, showing effect of treatment. GTN, glyceryl trinitrate.

Poston, 1994; Gomes *et al*., 2005; Ozkan and Uma, 2005). In this context, our findings suggest that healthy humans have sufficient defense mechanisms in place to protect NO activity against the effects of high glucose. However, this supposition is not supported by the findings of Williams *et al*. (1998) that a 6-h infusion of around 17 mM glucose impaired metacholine-induced relaxation in brachial artery in healthy humans. However, it is possible that the effects of hyperglycaemia *in vivo* could be exacerbated by other hormonal or paracrine factors that would be absent in the isolated vessels, such as those used in our study. It is possible that the change in osmolarity may have contributed to the effects of high glucose. However, this is unlikely as the total change is small (<5%) and previous studies show no effect of osmotic controls, such as mannitol, on endothelium-dependent relaxation (Williams *et al*., 1998; Ozkan and Uma, 2005; Beckman *et al*., 2007).

Nevertheless, in the present study, it is clear that high glucose does influence the activity of endothelial function in both the sets of vessels. Our results demonstrate that the relaxation generated by bradykinin in both subcutaneous and mesenteric vessels was mediated entirely by EDHF yet, interestingly, there are substantial differences between these vessels with respect to how high glucose modifies the bradykinin-induced relaxation. Specifically, high glucose impaired bradykinin-induced relaxation of subcutaneous vessels, whereas in contrast, the relaxation generated by bradykinin in mesenteric vessels was significantly enhanced at the same 2-h time point. Yet in both the vessel groups the high-glucose-mediated alteration of relaxation was lost after 6 h of incubation; at this time point, the relaxation responses were no different from controls. This suggests that high glucose has a differential influence on EDHF activity in different vessel types. The impairment of EDHF relaxations by a hyperglycaemic environment has been observed previously, albeit in mesenteric vessels of streptozotocin-induced diabetic rats, and in this study, the mechanism was attributed to increased phosphodiesterase activity (Matsumoto *et al*., 2003). In contrast, EDHF activity was found to be enhanced in the thoracic aorta of early-stage diabetic mice that exhibit hyperglycaemia (Shen *et al*., 2003).

We also found that high glucose significantly enhanced ACh-induced relaxation in mesenteric and subcutaneous vessels at a 2- and 6-h incubation point, respectively. As EDHF is the predominant factor generated by ACh in these vessels, it seems likely that the enhancement is due to an increase in its activity. However, a potential role for NO and prostacyclin in the augmented relaxation in subcutaneous and mesenteric vessels, respectively, cannot be excluded.

In the present study, it was demonstrated that a high glucose environment can either enhance or inhibit EDHF activity depending on the vessel studied or agonist used.

Yet the nature of the enhanced EDHF-mediated relaxation to bradykinin observed in mesenteric vessels is different from that produced by ACh in subcutaneous and mesenteric preparations. In the former, the augmented relaxation occurred only at the mid-range concentrations of bradykinin with no change in maximal relaxation observed. This suggests that either the mechanism by which glucose mediates enhancement of bradykinin-mediated relaxation in mesenteric vessels is effective only when EDHF levels are low or that there is more than one EDHF released by bradykinin, each with a differential sensitivity to high glucose. It is widely accepted that there are multiple EDHF identities and mechanisms (for review, see Félétou and Vanhoutte, 2006). Furthermore, such a phenomenon of multiple EDHFs produced by bradykinin might be extrapolated to explain why high glucose impairs relaxation to bradykinin in subcutaneous vessels, yet enhances that in the mesenteric. Indeed, bradykinin has been found to mediate an EDHF-induced relaxation, which comprised two components, in the renal artery of the rat (Wang *et al*., 2003). Furthermore, it was suggested that the first component is an endothelial hyperpolarization transmitted to the smooth muscle by myoendothelial coupling and that the second component is due to cytochrome-P450-dependent production of epoxyeicosatrienoic acids. Further studies are needed to clarify the specific nature of EDHF in human mesenteric arteries.

A final observation from our study relates to the effect of time on vessel reactivity. In subcutaneous arteries, relaxations to both agonists appeared to be reduced at the 2-h time point when compared to the 6-h time point and, also, compared to the responses observed in the separate characterization experiments (equivalent to approximately 1-h time point). Time-dependent changes are also apparent in the mesenteric vessels, but speculation into the underlying causes of these effects is beyond the scope of this study.

In conclusion, we demonstrated that short periods of high glucose exert a variable influence on endothelial function in human isolated blood vessels, which is dependent on factors of time, agonist-used and vessel studied. As such, the results from this study promote the concept that exposure to high glucose does not have a uniform influence throughout the vascular system. This has major implications in how we view the effects of acute hyperglycaemia found in patients with diabetes mellitus as well as other conditions.

Acknowledgements

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Conflict of interest

The authors state no conflict of interest.

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