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Effects of chylomicrons and chylomicron remnants on endothelium-dependent relaxation of rat aorta

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Abstract

The effects of chylomicrons and chylomicron remnants on endothelium-dependent relaxation of rat aorta were studied in vitro. Chylomicrons and chylomicron remnants were prepared in vivo. Aortic rings were incubated with the lipoproteins for 45 min before the vessels were constricted with phenylephrine and concentration relaxation response curves constructed to carbachol, ATP, A23187 and *S*-nitroso-*N*-acetylpenicillamine. Maximum % relaxations to carbachol were significantly reduced by both chylomicrons and chylomicron remnants but responses to ATP and *S*-nitroso-*N*-acetylpenicillamine were unaffected. In addition, chylomicrons significantly inhibited A23187-induced relaxation, causing an increase in the EC_{50} value. Chylomicron remnants cause selective inhibition of carbachol-induced relaxation suggesting an action at the receptor or G protein-coupled component of the receptor-mediated activation of the L-arginine-nitric oxide pathway. Chylomicrons appear to be less selective in their inhibition of the endothelium-dependent relaxation. This study demonstrates that lipoprotein particles of dietary origin may cause endothelial cell dysfunction. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Chylomicron; Chylomicron remnant; Endothelium; Aorta; (Rat); Nitric oxide (NO)

1. Introduction

It has been known for some time that atherosclerosis and hypercholesterolaemia are associated with impaired endothelium-dependent vascular relaxation (Freiman et al., 1986; Verbeuren et al., 1986; Shimokawa and Vanhoutte, 1989). This is thought to be due to cholesterol from plasma lipoproteins accumulating within the artery wall (Stender and Zilversmit, 1981). Indeed, low density lipoprotein (LDL), which is strongly atherogenic due to its small size and high cholesterol content, has been shown to accumulate in the rabbit aorta both in vitro (Bjornheden et al., 1996) and in vivo (Mamo and Wheeler, 1994). LDL has also been shown to be taken up by the rat aorta in vivo (Cardona-Sanclemente et al., 1994) and to inhibit endothelium-dependent relaxation in vitro in rabbit aorta (Andrews et al., 1987), rabbit femoral artery (Galle et al., 1991) and porcine coronary artery (Hayashi et al., 1991) by interfering with the L-arginine-nitric oxide pathway (Myers et al., 1994).

Oxidative modification of LDL is known to occur in the artery wall (Steinbrecher et al., 1984) and it has been suggested that this is responsible for the inhibitory effects of the lipoprotein on endothelium-dependent relaxation (Simon et al., 1990; Tanner et al., 1991). Oxidation of LDL causes a change in its structure and composition such that it is no longer taken up by the LDL receptor, which is down-regulated when intracellular cholesterol levels rise, but binds to scavenger receptors on macrophages and endothelial cells which are unregulated (Steinbrecher et al., 1984). The lipid peroxides formed by oxidation are cytotoxic to endothelial cells and chemotactic to monocytes leading to accumulation of inflammatory cells, local free radical production and increased degradation of nitric oxide (Keaney et al., 1993).

Multiple mechanisms are thought to exist by which LDL exerts its inhibitory effect on endothelium-dependent relaxation. Much research has indicated that one of these mechanisms may be due to disruption of the L-arginine-nitric oxide pathway. Atherosclerotic vessels have been

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shown to exhibit decreased responsiveness to some vasodilators that act through endothelial cell surface receptors, whereas responsiveness to those that bypass receptor-dependent membrane regulation are preserved (Bossaller et al., 1987). This suggests that LDL may interfere with an intramembrane regulatory pathway shared by the different receptor mechanisms. Lysophosphatidylcholine, which is present in oxidised LDL, has been shown to inhibit endothelium-dependent arterial relaxation (Kugiyama et al., 1990) and to modify G protein-dependent signalling (Flavahan, 1993). Lysophosphatidylcholine can also be removed from oxidised LDL by high density lipoprotein, a known antiatherogenic factor (Matsuda et al., 1993).

Until recently, it was hypothesised that large lipoproteins such as chylomicrons and chylomicron remnants were excluded from the artery wall (Stender and Zilversmit, 1982; Nordestgaard and Zilversmit, 1988), but there is now increasing evidence that chylomicron remnants may be involved in the atherogenic process. Mamo et al. (1994) showed that chylomicron remnants are rapidly metabolised by macrophages and patients with Type III hyperlipoproteinaemia, characterised by a defect in apolipoprotein E receptors, have been reported to have an increased incidence of vascular disease (Mahley et al., 1991). Furthermore, Mamo and Wheeler (1994) have demonstrated that chylomicron remnants accumulate in the thoracic aortae of conscious rabbits and that this uptake by the artery wall is non-specific, its rate being dependent on plasma concentration. In addition, at high concentrations chylomicron remnants have been shown to be cytotoxic to arterial smooth muscle cells in vitro which may be a possible cause of cell death in atherosclerotic plaques (Yu and Mamo, 1996). Several other lines of evidence support a contribution of chylomicron remnants to atheroma development. Firstly, postprandial plasma chylomicron remnant concentrations have been shown to be significantly increased in patients with coronary artery disease (Groot et al., 1991) when compared to subjects with normal coronary vessels. Secondly, octogenarian subjects without any evidence of vascular disease exhibit low postprandial levels of chylomicron remnants (Weintraub et al., 1992). Thirdly, patients with familial hyperchylomicronaemia, previously thought to be resistant to atheroma development because of low circulating LDL concentrations, do develop premature atherosclerosis (Benlian et al., 1996). Fourthly, chylomicron remnants, but not LDL, have been found to be taken up by the atherosclerotic rabbit aorta in vivo (Proctor and Mamo, 1996). Despite the increasing implication of dietary lipid particles in atherosclerosis, as far as we are aware no studies have investigated the effect of chylomicrons or chylomicron remnants on endothelial function in vitro.

The aim of this study was to determine whether chylomicrons and chylomicron remnants cause inhibition of endothelium-dependent relaxation and, by looking at different endothelium-dependent vasodilators, to investigate the underlying mechanisms involved.

2. Materials and methods

Male Wistar rats (300–350 g) were used throughout this study. They were housed under constant climatic conditions (temperature 21–22°C, relative air humidity 50 ± 5%, constant 12 h daylength) with free access to food and water.

2.1. Preparation of chylomicrons and chylomicron remnants

Chylomicrons and chylomicron remnants were prepared as described by Lambert et al. (1996). Rats were tube-fed with 1.5 ml corn oil, supplemented with α-tocopherol (4 mg ml^{-1}) as an antioxidant. After approximately 1 h, anaesthesia was induced (sodium pentobarbitone; 60 mg kg^{-1} body weight i.p.), the thoracic duct was cannulated with polyethylene tubing (external diameter 1.52 mm) and the chyle was collected overnight into a tube containing 2 mg ampicillin as a preservative. Following collection, the chyle was layered under NaCl solution (d 1.006 g ml^{-1}) and centrifuged in a fixed-angle rotor for $6 \times 10^5 g \cdot$ min at 12°C. The top fraction containing large chylomicrons (diameter > 100 nm) was harvested and ampicillin in 0.9% saline was added (final concentration 100 μg ml^{-1}). For the in vivo preparation of chylomicron remnants, rats were anaesthetised (sodium pentobarbitone; 60 mg kg^{-1} body weight i.p.) and a functional hepatectomy was carried out by ligation of all vessels supplying the liver. Chylomicrons containing 40 μmol triacylglycerol and 50 mg glucose were administered into the ileolumbar vein and after 45 min blood was withdrawn from the bifurcation of the aorta and allowed to clot. The serum was obtained by centrifugation, and the chylomicron remnants were isolated by ultracentrifugation for $6 \times 10^7 g \cdot$ min at 12°C. The top 1–1.5 ml was then layered under NaCl (d 1.006 g ml^{-1}) and centrifuged for $3 \times 10^7 g \cdot$ min at 12°C. The top 1–1.5 ml from this centrifugation containing the purified chylomicron remnants was harvested and 100 mg ml^{-1} ampicillin was added. The preparations were then stored at 4°C until required. Free and total cholesterol and triacylglycerol concentrations of the chylomicrons and chylomicron remnants were determined using a commercially available kit (Boehringer Mannheim, Mannheim, Germany).

2.2. Isolated vessel studies

Rats were killed with a sodium pentobarbitone overdose (200 mg kg^{-1} body weight i.p.) and the entire aorta rapidly removed and placed in modified Krebs–Henseleit solution (KHS; composition (mM): NaCl 118, KCl 4.57, $CaCl_2$ 1.27, KH_2PO_4 1.19, $MgSO_4$ 1.19, $NaHCO_3$ 25 and

glucose 5.55) at 37°C. The vessel was then cleared of any surrounding connective tissue and the thoracic portion cut into 3 mm ring segments, taking care not to damage the endothelium. The rings were then placed in organ bath chambers containing 10 ml KHS, gassed with 95% O_2:5% CO_2 and suspended between two parallel stainless steel wires, for isometric tension recording (HSE 30 isometric force transducer). The aortic rings were held under 1 g resting tension (preliminary studies determined this to be the optimum resting tension) and allowed to equilibrate for 1 h. Vessel segments were then contracted by exchanging the KHS for a depolarising KHS, where the NaCl had been replaced with 118 mM KCl. After 15 min, the depolarising KHS was replaced with KHS and resting tension re-established before chylomicrons or chylomicron remnants were added to the bathing solution to produce a concentration of 16 μM cholesterol in the organ bath (preliminary studies demonstrated that this concentration produced the maximal effect). The vessels were incubated with the lipoprotein for 45 min before the effects of the drugs were tested. Control vessel segments were treated in an identical fashion except that the same volume of 100 μg ml^{-1} ampicillin was added instead of the lipoprotein. No change in the basal tone of the vessel was observed during the incubation period. Thiobarbituric acid reactive substances were measured to confirm that the incubation period did not cause any significant oxidation of the lipoprotein (see below). Phenylephrine (0.3 μM) was then added to the bathing solution to constrict the vessel segments. Preliminary studies showed that this concentration caused 90% of the maximal response to phenylephrine in both control and treated vessels. When a stable increase in tone had been reached, cumulative concentration response curves were constructed to the relaxants carbachol (10 nM to 0.1 mM), ATP (10 nM to 1 mM), Ca^{2+} ionophore A23187 (1 nM to 10 μM) and *S*-nitroso-*N*-acetylpenicillamine (0.1 nM to 0.1 mM). In a further series of experiments, cumulative concentration relaxation response curves were constructed to ATP (10 nM to 1 mM) in the presence of N^{ω}-nitro-L-arginine (0.1 mM), and to bradykinin (1 nM to 10 μM).

2.3. Measurement of lipoprotein oxidation

Lipoprotein oxidation was measured by the thiobarbituric acid reactive substances assay as described by Steinbrecher et al. (1984). Tetraethoxypropane, which yields malondialdehyde was used as a standard and results were expressed as nmol malondialdehyde/ml lipoprotein.

2.4. Drugs and reagents

Carbamylcholine chloride, ATP (sodium salt), Ca^{2+} ionophore A23187 (sodium salt), L-phenylephrine hydrochloride, N^{ω}-nitro-L-arginine and bradykinin acetate were all purchased from Sigma Chemical (Poole, UK). *S*-nitroso-*N*-acetylpenicillamine was purchased from Research Biochemicals International (Natick, USA). All drugs, with the exception of A23187, were initially dissolved in deionised water (at 10 mM) and diluted in 0.9% w/v saline. A23187 was initially dissolved in ethanol (at 10 mM) and diluted in 0.9% saline. All solutions were freshly prepared on the day of the experiment. Concentrations are expressed as the final concentration of each drug in the organ bath. Ethylenediaminetetra-acetic acid (disodium salt) and trichloroacetic acid were purchased from BDH Laboratory Supplies (Poole, UK) and 4–6-dihydroxypyrimidine-2-thiol (2-thiobarbituric acid) and 1,1,3,3-tetraethoxypropane from Sigma. Bovine serum albumin fraction V powder and ampicillin were purchased from Sigma and corn oil and α-tocopherol from domestic suppliers.

2.5. Statistical analysis of data

The decrease in tension which resulted from the vasodilator drugs was expressed as a percentage of the initial phenylephrine-induced tone, and these data were plotted against log agonist concentration. Cumulative concentration response curves for each vessel segment were fitted to the single site logistic equation:

$$\text{Increase in tension} = E_{max} \cdot A^n / (A^n + EC_{50}^n)$$

by a modified Marquart procedure using Multifit® (Day Computing, Cambridge, England) running on a Macintosh 475. E_{max} represents the maximum response, A is the concentration of drug used and n is the Hill slope of the concentration response curve. The best fit values for EC_{50}, E_{max} and Hill slope for each vessel segment were used to calculate the means ± S.E.M. A paired Student's *t*-test ($P < 0.05$ was taken to be significant) was used to compare EC_{50}, maximum response and Hill slope values between rings incubated with lipoprotein and control vessels obtained from the same animal.

3. Results

3.1. Agonist-induced relaxation after incubation with chylomicron remnants

The effects of chylomicron remnants on relaxations to carbachol, A23187, ATP and *S*-nitroso-*N*-acetylpenicillamine are shown in Fig. 1a–d. The mean best fit dose response curve parameters derived from these curves are presented in Table 1. Chylomicron remnants inhibited the response to carbachol, resulting in a reduction in the maximum % relaxation whereas they had no significant effect on responses to ATP, A23187 or *S*-nitroso-*N*-acetylpenicillamine. In all these experiments the level of pre-contraction induced by 0.3 μM phenylephrine in chylomicron remnant-treated vessel rings was found to be significantly different from control vessel rings (0.43 ±

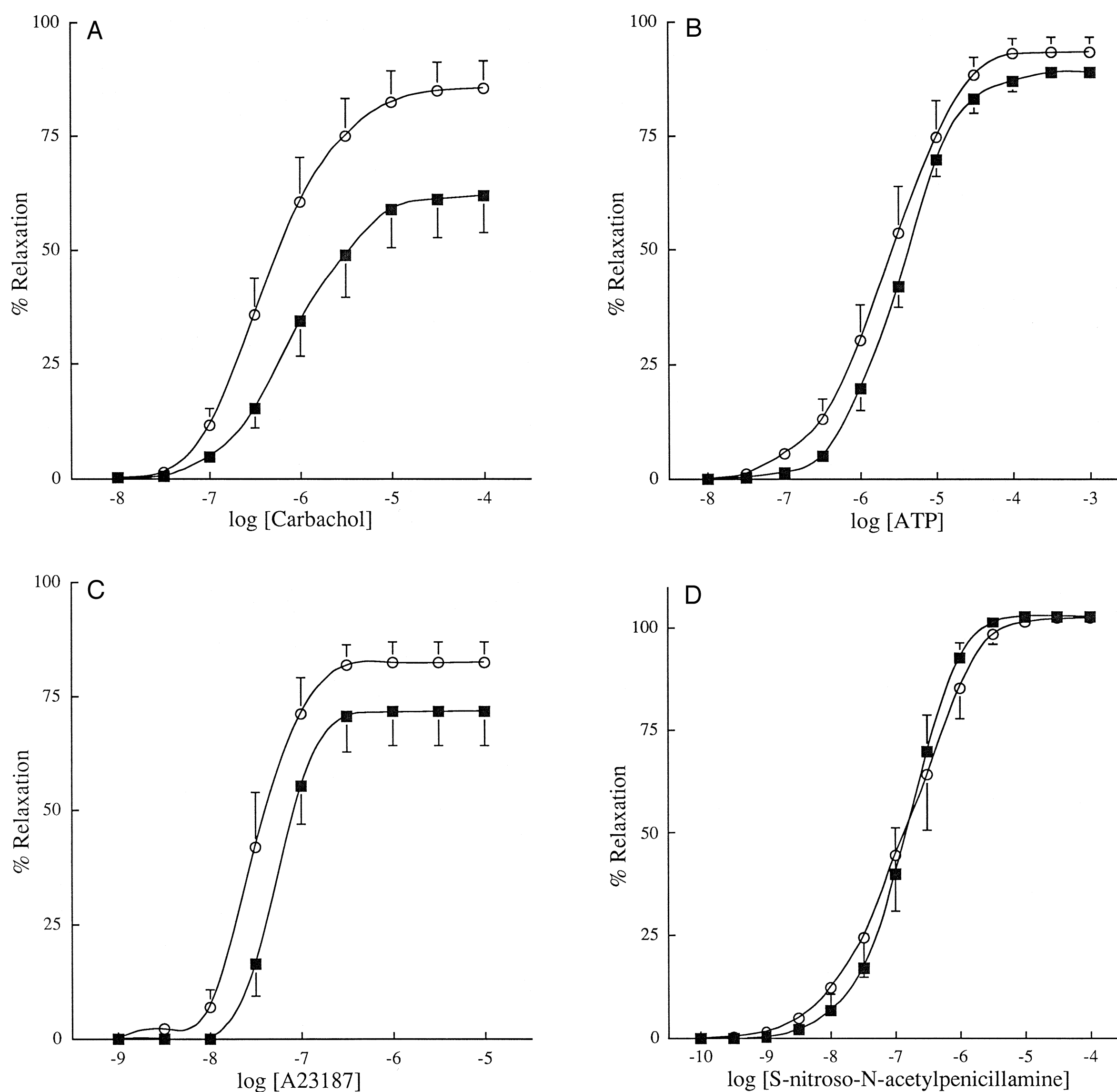

Fig. 1. Concentration relaxation response curves to (a) carbachol, (b) ATP, (c) A23187 and (d) *S*-nitroso-*N*-acetylpenicillamine in endothelium-intact rat aortic rings. The vessel segments were incubated with chylomicron remnants containing 100 μg ml^{-1} ampicillin ($n=6$, ■) at an organ bath concentration of 16 μM cholesterol or with the same volume of 100 μg ml^{-1} ampicillin ($n=6$, ○) for 45 min before they were contracted with 0.3 μM phenylephrine. The ordinate scale represents the relaxation expressed as a percentage of the contraction induced by 0.3 μM phenylephrine. Each data point represents the mean ± S.E.M.; the S.E.M. values are only included when they exceed the size of the symbol. The best fit values derived from these concentration response curves are shown in Table 1.

Table 1
Best fit values for concentration relaxation response curves ± chylomicron remnants

Agonist	Condition	EC_{50} (M)	% Maximum relaxation	Hill slope
Carbachol	+Remnants	$1.27 \times 10^{-6} \pm 0.39$ ($P=0.19$)	62.5 ± 7.7 ($P=0.02$)	1.19 ± 0.10 ($P=0.15$)
	Control	$6.59 \times 10^{-7} \pm 2.32$	85.2 ± 5.7	1.31 ± 0.10
ATP	+Remnants	$3.46 \times 10^{-6} \pm 0.47$ ($P=0.93$)	89.1 ± 1.8 ($P=0.21$)	1.22 ± 0.11 ($P=0.34$)
	Control	$3.34 \times 10^{-6} \pm 1.19$	94.3 ± 3.4	1.13 ± 0.07
A23187	+Remnants	$6.15 \times 10^{-8} \pm 1.51$ ($P=0.15$)	71.1 ± 7.7 ($P=0.25$)	4.63 ± 1.43 ($P=0.99$)
	Control	$4.30 \times 10^{-8} \pm 1.48$	82.9 ± 4.8	4.65 ± 1.68
S-nitroso-*N*-acetylpenicillamine	+Remnants	$2.04 \times 10^{-7} \pm 0.61$ ($P=0.56$)	103.5 ± 0.9 ($P=0.93$)	1.39 ± 0.06 ($P=0.08$)
	Control	$2.76 \times 10^{-7} \pm 1.26$	103.4 ± 0.8	1.14 ± 0.12

Rat aortic rings were incubated with chylomicron remnants containing 100 mg ml^{-1} ampicillin ($n=6$) at an organ bath concentration of 16 μM cholesterol or with the same volume of 100 mg ml^{-1} ampicillin ($n=6$) before they were contracted with 0.3 μM phenylephrine.
Concentration relaxation response curves were then constructed to carbachol, ATP, A23187 and *S*-nitroso-*N*-acetylpenicillamine.
Each value represents the mean ± S.E.M. for six experiments. *P* values are shown in parentheses.

0.02 vs. 0.35 ± 0.02 g/mg tissue, $n = 24$, $P < 0.05$; paired Student's *t*-test).

3.2. Agonist-induced relaxation after incubation with chylomicrons

The effects of chylomicrons on relaxations to carbachol, A23187, ATP and *S*-nitroso-*N*-acetylpenicillamine are shown in Fig. 2a–d. The mean best fit dose response curve parameters derived from these curves are presented in Table 2. Chylomicrons inhibited responses to carbachol and A23187, resulting in a reduction in the maximum % relaxation to carbachol and the vessel sensitivity to A23187 whereas they had no significant effect on responses to ATP or *S*-nitroso-*N*-acetylpenicillamine. In all these experiments the level of pre-contraction induced by 0.3 μM phenylephrine was found to be not significantly different between chylomicron-treated and control vessel rings (0.38 ± 0.03 vs. 0.35 ± 0.03 g/mg tissue, $n = 24$).

3.3. Further studies on ATP and bradykinin-induced relaxations

In the presence of 0.1 mM N^{ω}-nitro-L-arginine relaxations to ATP (at concentrations of 0.1 mM and below) were completely inhibited. Relaxations to ATP at 0.3 and

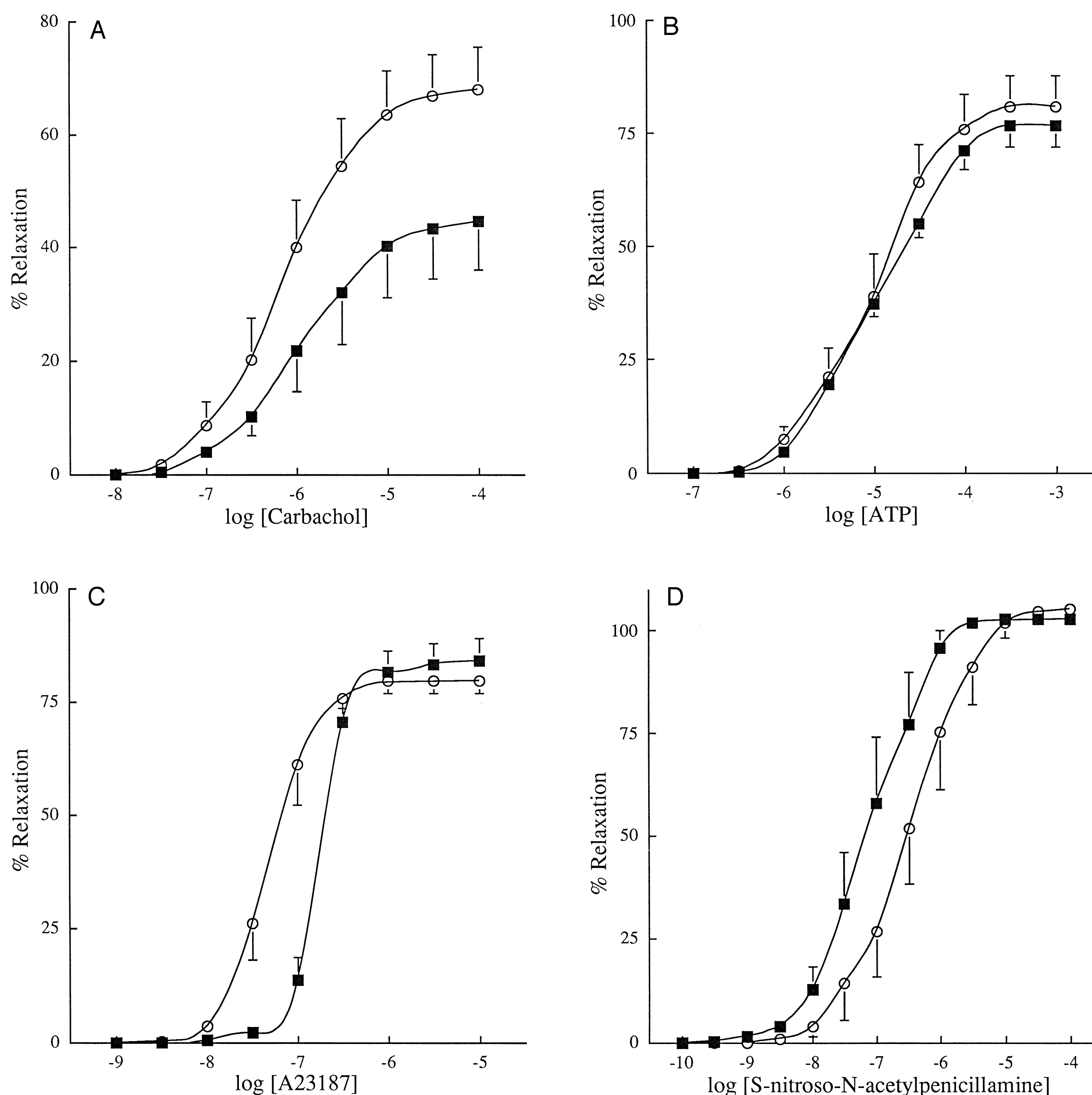

Fig. 2. Concentration relaxation response curves to (a) carbachol, (b) ATP, (c) A23187 and (d) *S*-nitroso-*N*-acetylpenicillamine in endothelium-intact rat aortic rings. The vessel segments were incubated with chylomicrons containing 100 μg ml^{-1} ampicillin ($n = 6$, ■) at an organ bath concentration of 16 μM cholesterol or with the same volume of 100 μg ml^{-1} ampicillin ($n = 6$, ○) for 45 min before they were contracted with 0.3 μM phenylephrine. The ordinate scale represents the relaxation expressed as a percentage of the contraction induced by 0.3 μM phenylephrine. Each data point represents the mean ± S.E.M.; the S.E.M.s are only included when they exceed the size of the symbol. The best fit values derived from these concentration response curves are shown in Table 2.

Table 2
Best fit values for concentration relaxation response curves ± chylomicrons

Agonist	Condition	EC_{50} (M)	% Maximum relaxation	Hill slope
Carbachol	+Chylomicrons	$2.12 \times 10^{-6} \pm 0.71$ ($P = 0.15$)	45.4 ± 8.4 ($P = 0.03$)	1.04 ± 0.11 ($P = 0.31$)
	Control	$9.07 \times 10^{-7} \pm 1.56$	68.1 ± 7.2	1.11 ± 0.09
ATP	+Chylomicrons	$1.19 \times 10^{-5} \pm 0.24$ ($P = 0.99$)	78.5 ± 5.3 ($P = 0.52$)	1.04 ± 0.04 ($P = 0.32$)
	Control	$1.18 \times 10^{-5} \pm 0.29$	81.4 ± 6.5	1.38 ± 0.28
A23187	+Chylomicrons	$1.73 \times 10^{-7} \pm 0.18$ ($P = 0.01$)	83.4 ± 4.7 ($P = 0.54$)	3.51 ± 0.52 ($P = 0.19$)
	Control	$6.35 \times 10^{-8} \pm 2.42$	79.7 ± 2.7	2.54 ± 0.16
S-nitroso-*N*-acetylpenicillamine	+Chylomicrons	$1.61 \times 10^{-7} \pm 0.82$ ($P = 0.20$)	103.3 ± 2.1 ($P = 0.35$)	1.52 ± 0.13 ($P = 0.46$)
	Control	$7.87 \times 10^{-7} \pm 5.06$	105.3 ± 2.6	1.42 ± 0.18

Rat aortic rings were incubated with chylomicrons containing 100 mg ml^{-1} ampicillin ($n = 6$) at an organ bath concentration of 16 μM cholesterol or with the same volume of 100 mg ml^{-1} ampicillin ($n = 6$) before they were contracted with 0.3 μM phenylephrine.
Concentration relaxation response curves were then constructed to carbachol, ATP, A23187 and *S*-nitroso-*N*-acetylpenicillamine.
Each value represents the mean ± S.E.M. for six experiments. *P* values are shown in parentheses.

1 mM which were resistant to N^{ω}-nitro-L-arginine (% relaxation 32.6 ± 18.0 and 73.3 ± 13.1, respectively, $n = 4$) were observed. Further experiments demonstrated that removal of the endothelium by rubbing the vessels failed to prevent relaxation to ATP at 0.3 and 1 mM. Bradykinin failed to cause relaxation of the rat aorta which could be measured accurately with the maximum % relaxation recorded at 10 μM bradykinin being 11.0 ± 3.4 ($n = 4$).

3.4. Measurement of lipoprotein oxidation

Organ bath incubation for a period of 45 min resulted in thiobarbituric acid reactive substances values for chylomicrons and chylomicron remnants of 2.44 ± 0.41 and 1.96 ± 0.34 nmol malondialdehyde/ml, respectively, which did not differ significantly (by an unpaired Student's *t* test, $n = 3$) from lipoprotein preparations which had been freshly prepared (thiobarbituric acid reactive substances values 1.98 ± 0.28 and 1.63 ± 0.60 nmol malondialdehyde/ml, respectively).

4. Discussion

In the present study, we found that both chylomicrons and chylomicron remnants significantly inhibited relaxations to carbachol but had no significant effect on relaxations to ATP and *S*-nitroso-*N*-acetylpenicillamine. Chylomicrons also significantly decreased vessel sensitivity to A23187. Although, it is well established that atherosclerosis and hypercholesterolaemia are associated with impaired endothelium-dependent relaxation (Woodman, 1995), with both atherosclerotic vessels (Freiman et al., 1986; Bossaller et al., 1987) and those from hypercholesterolaemic experimental animals (Verbeuren et al., 1986; Keaney et al., 1993) exhibiting impaired acetylcholine-induced relaxation, as far as we are aware, all previous in vitro studies have focused on the effects of native and oxidised LDL and none have investigated the actions of chylomicrons or chylomicron remnants on endothelium-dependent relaxation (Cox and Cohen, 1996).

Both the chylomicron and chylomicron remnant particles used in the present study were formed in vivo and under conditions which ensure incorporation of α-tocopherol into the particle. Indeed, thiobarbituric acid reactive substances assays showed that no significant oxidation of the lipoprotein occurred in the 45 min incubation period in the organ bath. It would appear, therefore, that we were dealing with an effect of the native lipoprotein particles rather than oxidised forms. This contrasts with the situation reported for LDL, where the majority of studies have shown that lipoprotein modification by oxidation was required before any effect on endothelium-dependent relaxation was seen. Oxidised LDL has been shown to attenuate relaxations in response to carbachol in rat aorta (McPherson et al., 1995) and acetylcholine in rabbit aorta (Jacobs et al., 1990; Yokoyama et al., 1990). The few studies that have shown native LDL to inhibit endothelium-dependent relaxation (Andrews et al., 1987; Galle et al., 1991; Hayashi et al., 1991) did not measure thiobarbituric acid reactive substances, and as no antioxidant was present in the LDL particles used in these studies it is possible that the lipoprotein may have undergone oxidative modification during the incubation period in the organ bath.

The results from the present study suggest that chylomicrons and chylomicron remnants exert their effect by inhibition at some stage of the receptor-activated L-arginine-nitric oxide pathway of the endothelial cell. The relaxation of aortic rings induced by the nitric oxide donor *S*-nitroso-*N*-acetylpenicillamine was not affected by chylomicrons or chylomicron remnants, demonstrating that these lipoprotein particles had no effect on the vasodilator action of nitric oxide at the level of the vascular smooth muscle cell. Previous workers have shown that acetylcholine-induced relaxation of rat aorta was both endothelium-dependent (Bullock et al., 1986) and completely inhibited by L-N^{G}-nitroarginine methyl ester (Martin et al.,

1992). Studies in our own laboratory have confirmed relaxations of rat aorta to carbachol are completely inhibited by 0.1 mM N^{ω}-nitro-L-arginine, suggesting that activation of the L-arginine-nitric oxide pathway mediates the carbachol-induced relaxation (data not shown).

In a previous study, we found chylomicron remnants, but not chylomicrons to potentiate contraction to phenylephrine in an endothelium-dependent manner (Grieve et al., 1997a). Indeed, in the present study the level of pre-contraction used to study the relaxant effects of carbachol did not differ significantly between control and chylomicron-treated tissues, but did differ significantly between control and chylomicron remnant-treated groups. However, we do not feel that this small but significant difference explains the effect of chylomicron remnants on the relaxant actions of carbachol for the following reasons. Firstly, there was no significant difference in the level of pre-contraction for vessels incubated with chylomicrons and a significant inhibition of carbachol-induced relaxation was seen, indicating that similar lipoproteins can produce an inhibitory effect on carbachol relaxation without a difference in the level of pre-contraction. Secondly, in a previous study (Grieve et al., 1997b) using control tissue, we showed that the same concentration response curves were obtained to carbachol whether vessel tone had been raised with 0.3 or 3 μM phenylephrine (maximum % relaxation $85.2 \pm 5.7\%$ and $88.7 \pm 2.6\%$, respectively). Thus, the concentration of phenylephrine chosen to pre-contract the vessels does not appear to significantly affect the maximum relaxation to carbachol (0.3 μM phenylephrine gives a submaximal response whereas 3 μM phenylephrine gives a supramaximal response). Finally, the same difference in pre-contraction between control and chylomicron remnant-treated vessels applied to the other agonists studied and no significant effect was found on these dose response curves. As the effector pathway of *S*-nitroso-*N*-acetylpenicillamine is identical to that of carbachol at the smooth muscle cell and both ATP and A23187 cause nitric oxide production by the endothelial cells in a similar manner to carbachol, it would appear that the effect of chylomicron remnants on carbachol-induced relaxations occurred independently of the difference in the level of phenylephrine-induced pre-contraction. The mechanism by which chylomicron remnants potentiate the contractile effect of phenylephrine remains to be determined and is the subject of further studies, as discussed below.

Neither chylomicrons nor chylomicron remnants had any significant effect on relaxation to ATP in our experiments. There have been relatively few studies examining the mechanism of ATP-induced relaxation in rat aorta. Both native (Andrews et al., 1987) and oxidised LDL (Mangin et al., 1993) have been found to impair ATP-induced relaxation of rabbit aorta, although Flavahan (1993) found lysophosphatidylcholine to have no significant effect in porcine coronary artery. Hypercholesterolaemia inhibited relaxations of porcine vessels to ADP (Shimokawa and Vanhoutte, 1989) and rabbit vessels to ATP (Verbeuren et al., 1986) whereas no inhibition of ATP-induced relaxation occurred in atherosclerotic vessels from primates (Sellke et al., 1990). A possible explanation for the observed lack of inhibition of ATP-induced relaxation by the lipoproteins studied is that ATP was able to stimulate an alternative endothelium-dependent relaxant pathway which compensated for inhibition of nitric oxide production. In the present study, we have demonstrated that relaxations to concentrations of ATP of 0.1 mM and below were endothelium-dependent and completely inhibited by N^{ω}-nitro-L-arginine, indicating that activation of the L-arginine-nitric oxide pathway is of major importance in the relaxant effects of ATP. These findings confirm those reported by Dominiczak et al. (1991). We did show that ATP, at higher concentrations, caused relaxation in the absence of endothelium. This was probably due to an effect of AMP, generated by the breakdown of ATP (Furchgott, 1983), acting on P_1 receptors located on the vascular smooth muscle cells of rat aorta (Burnstock and Kennedy, 1986). Thus, it would appear that chylomicrons and chylomicron remnants selectively inhibited carbachol-induced activation of nitric oxide production without affecting ATP-induced activation.

The mechanism by which chylomicrons and chylomicron remnants caused selective inhibition of carbachol-induced relaxations of rat aorta remains to be determined. Oxidised LDL used in vitro and short term hypercholesterolaemia in vivo have been shown to cause selective impairment of G_i protein coupled receptor pathways leading to nitric oxide production, with G_q protein coupled receptor pathways (including ATP) appearing less sensitive (Flavahan, 1992). The receptor types and their G protein coupling for carbachol and ATP have not been characterised in rat aortic endothelial cells. It seems likely that carbachol produced its effects via the muscarinic M_3 receptor (Jaiswal et al., 1991) and that ATP acted via a P_{2Y} purinoceptor (Burnstock and Kennedy, 1986). The nature of the G protein receptor coupling may vary between different endothelial cell types (Flavahan and Vanhoutte, 1995) and so this could be the basis for the differential effects of the chylomicrons and chylomicron remnants on carbachol-induced vascular relaxation. However, both muscarinic M_3 receptors and P_{2Y} purinoceptors are most likely to be G_q protein-linked (Flavahan, 1992; Brauner-Osborne and Brann, 1996) which, if true for rat aortic endothelial cells, would not support the G protein as the site of selective action of the dietary lipoproteins. Further work is necessary to clarify the situation.

Bradykinin is an endothelium-dependent relaxant which is thought to act through a G_q protein-dependent pathway (Flavahan, 1992) whose relaxant effects seem to be relatively resistant to oxidised LDL (Tanner et al., 1991; Flavahan, 1993). In the present study, we tried to construct concentration relaxation response curves to bradykinin but found the rat aorta to be unresponsive. This would suggest

that rat aortic endothelial cells lack functional receptors for bradykinin.

In the present study we also examined the Ca^{2+} ionophore A23187 which stimulates nitric oxide production by endothelial cells by inducing a receptor-independent Ca^{2+} influx (Cohen et al., 1988). We found that chylomicrons reduced the tissue sensitivity to A23187. There was also a tendency for chylomicron remnants to reduce the sensitivity of the tissue to A23187, but this effect was not statistically significant at the lipoprotein concentration examined. Had a larger number of animals been used, the effects of chylomicron remnants on A23187-induced relaxation might have reached statistical significance. However, as far as we are aware there have been no previous reports of a disturbance in Ca^{2+} ionophore mediated activation of endothelial nitric oxide synthase with normal receptor mediated activation of the same pathway. A possible explanation for this finding is that the lipid component of chylomicrons and their remnants is amphiphillic and so may integrate within the cell membrane and cause changes which interfere with the incorporation of the Ca^{2+} ionophore into the cell membrane. Indeed, Matsuda et al. (1993) demonstrated that lysophosphatidylcholine from oxidised LDL was incorporated into the plasma membrane of endothelial cells. Such phospholipid incorporation has been shown to alter membrane functions such as transmembrane ion transport (Sedlis et al., 1983), activity of membrane-bound enzymes (Shier et al., 1976; Owens et al., 1982) and ligand-receptor coupling (Briggs and Lefkowitz, 1980).

Previous studies have produced conflicting findings on the effect of LDL and hypercholesterolaemia on A23187-induced relaxation. Inhibition of relaxation to A23187 by oxidised LDL has been demonstrated in rabbit aorta (Jacobs et al., 1990; Mangin et al., 1993) although Tanner et al. (1991) found no significant effect in porcine coronary artery. Studies using vessels from hypercholesterolaemic animals have found reduced relaxations to A23187 (Shimokawa and Vanhoutte, 1989; Keaney et al., 1993) whereas others have shown no inhibition of A23187-induced relaxation (Cohen et al., 1988; Hayashi et al., 1991). The explanation for the differences reported in these studies may be that the effects of lipoproteins on A23187-mediated relaxation are dependent upon the incubation or exposure time and the concentration of lipoprotein used.

It has been shown that oxidised LDL causes the release of contractile factors such as endothelin (Boulanger et al., 1992) and superoxide (Cox and Cohen, 1996). As mentioned earlier, in a previous study we found chylomicron remnants to potentiate contraction to phenylephrine in an endothelium-dependent manner (Grieve et al., 1997a). This effect was further potentiated when nitric oxide release was completely blocked by N^{ω}-nitro-L-arginine which rules out any possible effect on basal nitric oxide production. These data suggest that chylomicron remnants potentiate the contraction to phenylephrine by stimulating the release of a contractile factor from the endothelium. However, as discussed earlier, the hypothesis that the lipoproteins studied may cause the release of a contractile factor cannot account for the selective impairment of carbachol and A23187-induced endothelium-dependent relaxation seen. If this were the case then ATP and *S*-nitroso-*N*-acetylpenicillamine would have been inhibited to a similar extent and a more non-selective effect would have been observed.

In conclusion, we have shown that both chylomicrons and chylomicron remnants inhibit relaxation of the rat aorta to carbachol but not ATP or *S*-nitroso-*N*-acetylpenicillamine, while chylomicrons inhibit relaxations to A23187. This is the first study to demonstrate that lipoprotein particles of dietary origin interfere with agonist-induced nitric oxide production by endothelial cells. The effects of these dietary lipoproteins on both carbachol and A23187-induced relaxation could be due to a mechanism involving lipid incorporation into the endothelial cell membrane, although further experiments are required to elucidate their exact mechanism of action. These results have important implications for the pathogenesis of atherosclerosis and support the hypothesis that dietary lipoprotein particles, in addition to LDL, are involved in the aetiology of this disease.

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