

Pulse-Wave Analysis

Clinical Evaluation of a Noninvasive, Widely Applicable Method for Assessing Endothelial Function

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Abstract—Current methods for assessing vasomotor endothelial function are impractical for use in large studies. We tested the hypothesis that pulse-wave analysis (PWA) combined with provocative pharmacological testing might provide an alternative method. Radial artery waveforms were recorded and augmentation index (AIx) was calculated from derived aortic waveforms. Thirteen subjects received sublingual nitroglycerin (NTG), inhaled albuterol, or placebo. Twelve subjects received NTG, albuterol, and placebo separately during an infusion of N^G -monomethyl-L-arginine (LNMMA) or norepinephrine. Twenty-seven hypercholesterolemic subjects and 27 controls received NTG followed by albuterol. Endothelial function was assessed by PWA and forearm blood flow in 27 subjects. Albuterol and NTG both significantly and repeatably reduced AIx ($P < 0.001$). Only the response to albuterol was inhibited by LNMMA ($-9.8 \pm 5.5\%$ vs $-4.7 \pm 2.7\%$; $P = 0.02$). Baseline AIx was higher in the hypercholesterolemic subjects, who exhibited a reduced response to albuterol ($P = 0.02$) but not to NTG when compared with matched controls. The responses to albuterol and acetylcholine were correlated ($r = 0.5$, $P = 0.02$). Consistent with an endothelium-dependent effect, the response to albuterol was substantially inhibited by LNMMA. Importantly, the response to albuterol was reduced in subjects with hypercholesterolemia and was correlated to that of intra-arterial acetylcholine. This methodology provides a simple, repeatable, noninvasive means of assessing endothelial function in vivo. (*Arterioscler Thromb Vasc Biol.* 2002;22:147-152.)

Key Words: hypercholesterolemia ■ nitric oxide ■ pulse-wave analysis ■ augmentation index ■ endothelial function

The vascular endothelium releases a number of biologically active mediators, including nitric oxide (NO), that regulate vessel tone and prevent the development of atheroma.¹ Endothelial dysfunction is associated with a range of risk factors for cardiovascular disease, including hypercholesterolemia,^{2,3} and some therapies that improve clinical outcome also reverse endothelial dysfunction.⁴ Vasomotor responses in the peripheral and coronary circulations are correlated,⁵ and coronary endothelial dysfunction is associated with cardiovascular risk.⁶ However, no direct link between improved endothelial function and reduced risk has been made, and the prognostic significance of endothelial dysfunction has not been assessed in a major observational study.

Established methods for assessing vasomotor endothelial function center on measuring the response to an endothelium-dependent, NO-mediated stimulus such as acetylcholine (ACh)^{2,3} or reactive hyperemia, and a direct (endothelium-independent) nitrovasodilator, like sodium nitroprusside

(SNP) or nitroglycerin (NTG). However, current methods are not suitable for inclusion in large-scale trials. Therefore, new and relatively simple, noninvasive techniques are required to assess the predictive value of endothelial dysfunction.⁷

Arterial stiffness depends, in part, on smooth muscle tone.⁸ The shape of the arterial pressure waveform provides a measure of systemic arterial stiffness⁹ and can be assessed noninvasively by using the technique of pulse-wave analysis (PWA).¹⁰ We hypothesized that PWA might provide a simple method for assessing endothelial vasomotor function. Chwienzyk et al¹¹ demonstrated that albuterol, a β_2 -agonist, in part reduces wave reflection by activation of the L-arginine-NO pathway and suggested that such methodology might be applied to the assessment of endothelial function. The aim of this series of experiments was to provide robust validation of the noninvasive assessment of endothelial function by PWA combined with provocative administration of NTG and albuterol. In particular, we wanted to define the repeatability of the responses to albuterol and NTG; to

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determine whether albuterol acts via the L-arginine–NO pathway; to apply the technique to a large cohort of healthy controls and hypercholesterolemic subjects, the latter of whom are known to exhibit endothelial dysfunction²; and then to compare the technique with the “gold standard” of forearm venous occlusion plethysmography.³

Methods

All studies were conducted at the Clinical Pharmacology Unit, University of Edinburgh; the Clinical Pharmacology Unit, University of Cambridge, and the Wales Heart Research Institute, University of Wales, Cardiff. Healthy subjects were enrolled from community databases of volunteers, and patients were recruited from cardiovascular risk clinics and family practices local to all institutions. Approval for all studies was obtained from the respective local research ethics committees, and informed consent was obtained from each participant.

Hemodynamics

Blood pressure was recorded in duplicate in the dominant arm by using a validated oscillometric technique (HEM-705CP, Omron Corp). Cardiac index (CI) was assessed noninvasively by using a validated¹² transthoracic electrical bioimpedance technique (BoMed NCCOM3-R7), and peripheral vascular resistance (PVR) was calculated as mean arterial pressure (MAP) divided by CI.

Radial artery waveforms were recorded with a high-fidelity micromanometer (SPC-301, Millar Instruments) from the wrist of the dominant arm. PWA (SCOR 6.1, PWV) was then used to generate a corresponding central waveform by using a validated transfer function,^{13–15} as previously described.¹⁶ From this, augmentation index (AIx) and heart rate were determined by using the integral software. AIx, a measure of systemic arterial stiffness,⁹ was calculated as the difference between the second and first systolic peaks, expressed as a percentage of the pulse pressure.

Venous Occlusion Plethysmography

The brachial artery of the nondominant arm was cannulated with a 27-gauge steel needle (Coopers Needle Works) under local anesthesia, and blood flow was measured simultaneously in both arms by venous occlusion plethysmography, with temperature-compensated, indium/gallium-in-silicone elastomer strain gauges, as previously described.¹⁷ Blood flows were recorded during the final 3 minutes of each infusion period, and the mean of the final 5 measurements was used for analysis.

Plasma Albuterol Assay

Venous blood (10 mL) was taken from the antecubital fossa into lithium-heparin tubes and centrifuged immediately at 4°C (2000g for 10 minutes). The plasma was then removed and stored at –80°C for later analysis by a chiral liquid chromatography/tandem mass spectrometry technique.¹⁸ The lower limit of detection for the assay was 0.05 ng · mL⁻¹, and the coefficient of variation was <10%.

Drugs

Albuterol (Allen and Hanbury's) was given by inhalation with a spacer device (2 × 200 µg). A 500-µg tablet of NTG (Cox) was placed under the tongue for 3 minutes and then removed. Norepinephrine (NE, Abbott Laboratories) and N^G-monomethyl-L-arginine (LNMMMA, Clinalfa) were prepared aseptically with 0.9% saline as a diluent and infused intravenously at 1 mL · min⁻¹. LNMMMA was given as a bolus of 3 mg · kg⁻¹ (5 minutes) and then continuously at 3 mg · kg⁻¹ · h⁻¹ for 45 min. NE was infused at 50 ng · kg⁻¹ · h⁻¹ for 45 min as a control constrictor; the dose was chosen from pilot studies and published literature to produce an elevation of MAP and AIx similar to that effected by LNMMMA.^{19,20} SNP (David Bull Laboratories) at 3 and 10 µg · min⁻¹ and ACh (Novartis) at 7.5 and 15 µg · min⁻¹ were infused intra-arterially, each for 6 minutes.

Protocol

Subjects were studied in the morning after an overnight fast. Subjects with an elevated blood pressure at screening (>160/100 mm Hg), diabetes mellitus, or a clinical history of cardiovascular disease were excluded, as were individuals receiving medication. Studies were conducted in a double-blind fashion (where appropriate) in a quiet, temperature-controlled room (22 ± 2°C). All hemodynamic recordings were made in duplicate. Three sets of recordings were made during a 45-minute period of supine rest, and the last was taken as a baseline. Recordings were made 3, 5, 10, 15, and 20 minutes after NTG administration and 5, 10, 15, and 20 minutes after albuterol or aerosol placebo. Pilot studies had confirmed that 20 minutes was sufficient for the hemodynamic changes after NTG to return to baseline but that a longer period was required for albuterol. Therefore, NTG was always administered first, followed by albuterol 25 minutes later.

Study 1: Repeatability

Thirteen healthy subjects (11 male) were studied on 3 occasions, separated by 1 week. After baseline recordings of heart rate, blood pressure, CI, and radial artery waveforms were made, subjects received sublingual NTG. This was followed by albuterol (on 2 occasions) or matching placebo (once) in random order. Blood was taken for estimation of plasma albuterol at baseline and at 5-minute intervals thereafter.

Study 2: Inhibition of NO Synthase

Twelve healthy males were studied on 6 occasions, separated by 1 week. An 18-gauge cannula was sited in the nondominant arm and saline infused for 30 minutes. After baseline recordings of heart rate, blood pressure, CI, and radial artery waveforms were made, LNMMMA was then infused for 15 minutes alone, on 3 occasions, followed by administration of NTG, albuterol, or matching aerosol placebo. On the other 3 visits, NE was infused in place of LNMMMA. The order of administration of all drugs was fully randomized.

Study 3: Hypercholesterolemia

Twenty-seven hypercholesterolemic subjects (total cholesterol >6.0 mmol · L⁻¹) and 27 matched normocholesterolemic (<6.0 mmol · L⁻¹) were studied. Heart rate, blood pressure, and radial artery waveforms were assessed at baseline and after NTG and albuterol administration. Twenty minutes after albuterol was given, blood was taken for determination of cholesterol (total, LDL, and HDL), glucose, and albuterol concentrations.

Study 4: Comparison With Venous Occlusion Plethysmography

Twenty-seven subjects with a range of serum cholesterol values (3 to 8.6 mmol · L⁻¹; 12 subjects >6.0) but no other cardiovascular risk factors were studied on a single occasion. Endothelial function was first assessed invasively by venous occlusion plethysmography coupled with an intra-arterial infusion of SNP and ACh (after 30 minutes of saline infusion and a 20-minute washout between drugs). After a further 30 minutes, endothelial function was then assessed by PWA coupled with administration of NTG and albuterol.

Data Analysis

The response to albuterol, placebo, or NTG was defined as the maximum change in each parameter after drug administration (an a priori summary measure). Forearm blood flow was calculated as mL · min⁻¹ · 100 mL⁻¹ tissue, and the ratio of blood flow in the infused to the noninfused arm was used to reduce blood flow variability in response to extraneous stimuli such as temperature fluctuations.²¹ Again, the maximum responses to albuterol and to ACh were used as a priori summary measures. Data were analyzed by SPSS software (version 9.0) and unpaired or paired, 2-tailed Student's *t* tests or ANOVA, as appropriate. Multiple regression analysis was conducted by using the “enter method.” Repeatability was assessed by constructing Bland-Altman plots and reported as the mean ± SD of the differences between samples.²² All values represent mean ± SD, and a *P* value <0.05 was considered significant. All changes cited represent absolute differences, rather than percentage changes.

TABLE 1. Hemodynamic Changes in Study 1

	Albuterol (1)	NTG (1)	Albuterol (2)	NTG (2)	Placebo	NTG (3)
AIx, %	-9.3±3.8‡	-12.8±4.4‡	-11.6±3.8‡	-13.0±7.0†	-0.2±5.4	-11.1±5.9‡
MAP, mm Hg	-1±3	-2±8	-1±4	-3±5	-1±7	2±4
CI, L · min ⁻¹ · m ⁻²	0.5±0.6*§	-0.1±0.3	0.7±0.6*	-0.2±0.4	-0±0.3	-0.2±0.2
PVR, AU	-2.9±4.7*	-0.2±3.4	1.0±4.3*	-0.7±4.6	-0.6±4.8	0.3±2.7
HR, beats · min ⁻¹	3±5†	+6±7*	+5±6*	1±6	0±5	0±4

Changes in AIx, MAP, CI, PVR, and heart rate (HR) after albuterol, matching placebo, or NTG. Numbers in parentheses refer to visit number. AU indicates arbitrary units.

Values represent means±SD, and significant changes from baseline are indicated by **P*<0.05, †*P*<0.01, ‡*P*<0.001. Significant differences in the response between albuterol and placebo are indicated in column by §*P*<0.05, ||*P*<0.001; n=13.

Results

Study 1

The mean age of the subjects was 37 years (range, 25 to 56). There were no significant differences in baseline values between the 3 visits. The hemodynamic changes are shown in Table 1. Only AIx (*P*<0.001) and CI (*P*=0.01) changed significantly after albuterol. There was no difference in the mean response to albuterol compared with NTG for any parameter except CI (*P*=0.02). The response to albuterol or NTG did not differ between visits. The mean difference in the AIx response between visits was -2.3±3.0% and -0.2±6.5% for albuterol and NTG, respectively.

Plasma albuterol was below the level of detection at baseline and after administration of placebo. Plasma albuterol increased after administration of the active drug (*P*<0.001 on both occasions, Figure 1), and there was no difference in the peak albuterol concentration between visits (mean difference of -0.12±0.55 ng · mL⁻¹, *P*=0.9).

Study 2

The mean age of the subjects was 32 years (range, 22 to 52). There were no significant differences in baseline values across the 6 visits. Hemodynamic changes are summarized in Table 2. The effects of NE and LNMMA did not differ significantly between visits. Both drugs had similar effects on AIx, MAP, and heart rate, but there was a greater change in CI (*P*<0.001) and PVR (*P*<0.001) with LNMMA. The responses to NTG and placebo with LNMMA did not differ significantly from those during coinfusion of NE. However, albuterol had less effect on AIx during infusion of LNMMA (*P*=0.02, Figure 2A), despite causing a greater reduction in MAP (*P*<0.001) and PVR (*P*<0.001).

Study 3

The baseline characteristics of the subjects are given in Table 3. There was no significant change in MAP or heart rate after NTG

or albuterol in either group. AIx fell significantly after administration of albuterol, but the response was significantly reduced in hypercholesterolemics compared with controls (-7.4±5.7% vs -11.9±7.7%, respectively; *P*=0.02). Despite this disparity, the time to maximum change (12±3 vs 11±3 minutes, *P*=0.2) and peak plasma albuterol concentrations (1.2±0.6 vs 1.1±0.5 ng · mL⁻¹, *P*=0.5) were similar in both groups. Moreover, the maximum change in AIx after NTG (-14.8±8.4% vs -10.2±9.0%, *P*=0.1) did not differ significantly.

To investigate further the factors influencing the response to albuterol, a multiple linear regression model was constructed, with change in AIx as the dependent variable. Age, sex, weight, smoking status, baseline MAP and AIx, LDL and HDL cholesterol, triglycerides, glucose, and change in MAP and heart rate were entered into the model. Variables with a significance >0.25 were then removed and the analysis repeated. The final model (Table 4) explained ≈60% of the variability in the response to albuterol. Change in AIx was negatively associated with plasma LDL and glucose and positively correlated with plasma albuterol concentration and fall in heart rate.

Study 4

Resting forearm blood flow did not differ between the 2 arms, and there was no change in blood flow in the noninfused arm during the study (data not shown). As expected, blood flow increased significantly during infusion of SNP and ACh (*P*<0.001 for both, ANOVA). However, the response to ACh (7.5 μg · min⁻¹: 1.6±1.0 vs 3.4±1.0 mL · min⁻¹ · 100 mL⁻¹, *P*=0.003; and 15.0 μg · min⁻¹: 2.0±0.3 vs 5.8±0.3 mL · min⁻¹ · 100 mL⁻¹, *P*=0.04) but not to SNP was reduced in subjects with a cholesterol level >6.0 mmol · L⁻¹. NTG and albuterol both significantly reduced AIx (*P*<0.001), and the response to albuterol (*r*=0.5, *P*=0.02) was related to serum cholesterol. There was a significant, linear relationship between the absolute change in AIx after albuterol and the change in the forearm blood flow ratio during infusion of ACh at 15 μg · min⁻¹ (Figure 2B). There was no relationship between the response to SNP and to NTG.

Discussion

This study describes the effects of albuterol and NTG on the aortic pressure waveform. AIx, a quantitative index of systemic arterial stiffness,⁹ was calculated from aortic waveforms generated by PWA.¹⁰ Our main novel findings are that albuterol and NTG produce qualitatively and quantitatively similar and repeatable effects on AIx, that the effect of albuterol but not of NTG is inhibited by LNMMA and reduced in hypercholesterolemic subjects, and that the response to albuterol is correlated with the

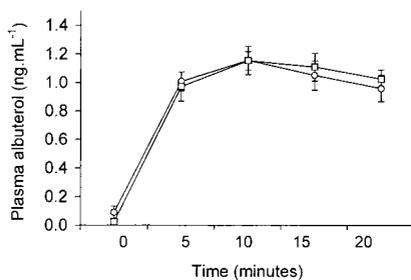


Figure 1. Changes in plasma albuterol concentration after visits 1 (○) and 2 (□); n=13.

TABLE 2. Hemodynamic Changes in Study 2

	NE				LNMMA			
	NE	Albuterol	NTG	Placebo	LNMMA	Albuterol	NTG	Placebo
AIx, %	10.7±10.5‡	-9.8±5.5‡	-12.5±11.1‡	0.4±6.2	6.1±9.7‡	-4.7±2.7‡	-13.4±2.4‡	-1.2±5.5
MAP, mm Hg	7±8‡	1±3‡	5±4‡	4±6*	6±6‡	-4±6*	4±4*	-2±6
CI, L · min ⁻¹ · m ⁻²	-0.1±0.4*	0.8±0.4‡	0.1±0.4	-0.1±0.3	-0.6±0.6‡	0.6±0.4‡	0.1±0.2	0.1±0.3
PVR, AU	3.2±4.3‡	-2.5±5.8‡	-1.1±3.1	0.5±2.2	7.3±4.8‡	-6.4±4.0‡	-2.5±3.3*	-1.6±3.4
HR, beats · min ⁻¹	-5±6‡	9±5‡	4±4*	2±7	-6±6‡	5±4‡	2±4	1±6

Changes (means±SD) in AIx, MAP, CI, PVR, and heart rate (HR) during study 2. NE or LNMMA was infused, and then albuterol, matching placebo, or NTG was administered. AU indicates arbitrary unit.

* $P<0.05$, † $P<0.01$, ‡ $P<0.001$.

effect of ACh in the forearm vascular bed. These data indicate that the effect of albuterol is, in part, NO and endothelium dependent and are consistent with the presence of endothelial dysfunction in hypercholesterolemic subjects. Moreover, they suggest that PWA and administration of albuterol and NTG provide a simple, reliable, noninvasive method for assessing endothelial function, as we and others have previously hypothesized.^{23,24}

As expected, inhalation of albuterol at the dose used reduced AIx without any accompanying alteration in heart rate or MAP, compared with placebo, which is important because AIx is influenced by both.^{20,25} The magnitude of the response to both drugs was comparable, and the repeatability, high. Indeed, the repeatability is similar to what we previously reported for PWA¹⁶ and to values quoted for other techniques of assessing endothelial function, such as intra-arterial infusion of ACh²⁶ and flow-mediated dilatation.²⁷

Previous studies have shown similar changes in the arterial pressure waveform after NTG,^{28,29} but the effect of endothe-

lium-dependent NO dilators has not been reported. Nevertheless, comparable changes in the volume waveform after infusion of ACh into rabbits³⁰ and of NTG and albuterol into humans¹¹ have been described. However, unlike Chowienzyk et al¹¹ and relevant to wider application of the present technique, we included a suitable placebo and showed that the responses to albuterol and NTG are repeatable. We also measured plasma albuterol concentrations, which were relatively stable between 5 and 20 minutes (Figure 1). This pharmacokinetic profile is in keeping with the pharmacodynamic response to albuterol. Peak plasma levels did not differ significantly between visits, confirming that inhalation of albuterol with a spacer device provides a repeatable method of drug delivery. As might be expected, the results of the multiple regression analysis from study 3 (Table 4) demonstrated a significant relationship between plasma albuterol and the maximum change in AIx. Therefore, some of the variability in the response to albuterol between subjects may have been due to differences in drug absorption and peak plasma concentrations. Such an effect may be important when making comparisons between subjects groups, especially when relatively small numbers of subjects are studied or smokers are included, when plasma albuterol values may improve the reliability of data interpretation.

To investigate the NO dependence of albuterol, we infused LNMMA, a specific substrate-analogue inhibitor of NO synthase. However, because systemic LNMMA infusion

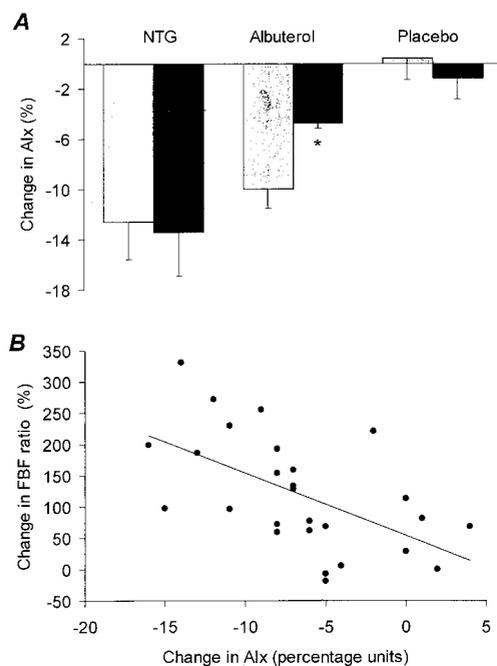


Figure 2. A, Effect of inhibition of NO synthase on the response to NTG, albuterol, and placebo. Changes in AIx after albuterol, NTG, and placebo during infusion of NE (□) and *N*^G-monomethyl-L-arginine (■); n=12, * $P<0.05$. B, Relationship between the response to albuterol and ACh. Change in AIx after albuterol and change in forearm blood flow (FBF) after ACh. A regression line is shown, for which $r=0.5$, $P=0.02$, slope=-9.8, n=27.

TABLE 3. Subject Characteristics in Study 3

	Controls	Hypercholesterolemic	Significance, P
Age, y	47±11	48±11	0.8
Male/female, n/n	21/6	22/5	1.0
Smokers, n	5	5	1.0
Height, m	1.73±0.06	1.68±0.09	0.3
Weight, kg	74.8±13.8	78.8±13.6	0.2
Cholesterol, mmol · L ⁻¹	5.1±0.6	6.6±0.5	<0.001
LDL, mmol · L ⁻¹	2.9±0.8	4.5±0.9	<0.001
HDL, mmol · L ⁻¹	1.3±0.4	1.3±0.3	0.6
Triglycerides, mmol · L ⁻¹	2.0±1.6	2.2±1.1	0.7
Glucose, mmol · L ⁻¹	5.0±0.5	5.3±1.7	0.3
MAP, mm Hg	84±9	90±12	0.04
Heart rate, beats · min ⁻¹	66±9	65±7	0.5
AIx, %	15±7	21±9	0.04

Values represent means±SD.

TABLE 4. Results of the Regression Analysis for Study 3, With Change in AIx After Albuterol as the Dependent Variable

Variable	Units	Regression Coefficient \pm SD	P
Glucose	mmol \cdot L ⁻¹	-6.4 \pm 1.7	0.001
LDL cholesterol	mmol \cdot L ⁻¹	-2.1 \pm 0.8	0.012
Albuterol	ng \cdot mL ⁻¹	3.9 \pm 2.2	0.021
Change in heart rate	beats \cdot min ⁻¹	-0.4 \pm 0.1	<0.001
Smoking	...	-3.5 \pm 2.2	0.13

Biochemical parameters were derived from plasma samples, and the R^2 for the entire group was 0.61, $P=0.001$, $n=54$.

alters MAP and heart rate,¹⁹ we used NE as a control vasoconstrictor to produce comparable hemodynamic changes.²⁰ As expected, but not previously reported, we observed a significant increase in AIx with administration of LNMMA. However, both drugs had similar effects on AIx, heart rate, and MAP, but the response to albuterol was significantly attenuated by LNMMA, despite a greater reduction in MAP and PVR. Moreover, the responses to NTG and placebo were not affected by LNMMA. This indicates that NO mediates a significant part of the response to inhaled albuterol. Furthermore, the degree of inhibition produced by LNMMA, $\approx 50\%$, is consistent with data from the forearm vascular bed³¹ and systemic studies on the volume waveform.¹¹ However, in the present study, we included a suitable placebo aerosol and control vasoconstrictor to investigate the potential confounding effect of baseline changes in heart rate, MAP, and AIx after LNMMA.

Hypercholesterolemia is the condition most consistently associated with endothelial dysfunction.^{2,3,32} Therefore, we investigated the response to albuterol and NTG in a group of 27 hypercholesterolemic and matched controls and, in a separate cohort, compared endothelial function as assessed by PWA with that measured by intra-arterial infusion of ACh and SNP in the forearm. Baseline AIx was significantly higher in the hypercholesterolemic subjects, indicating increased arterial stiffness. This is the first time that increased aortic AIx has been reported in hypercholesterolemic, but increased stiffness has been previously demonstrated by several other techniques,^{33,34} although this is not a universal finding.³⁵ However, the higher AIx may have been due to the slightly higher MAP²⁰ in the hypercholesterolemic, arterial stiffening per se, or a combination of both.^{34,36} AIx fell less after albuterol in the hypercholesterolemic than in controls, despite their being well matched. Moreover, mean plasma albuterol concentration was similar in both groups, as were the effects of albuterol and NTG on other hemodynamic variables, suggesting blunting of a direct effect of albuterol on AIx in the hypercholesterolemic subjects, rather than, for example, an indirect mechanism through changes in heart rate or MAP. Moreover, in the multiple regression model, which included known and potential confounding variables, LDL cholesterol was inversely and independently correlated with the change in AIx. Interestingly, although endothelial function declines with age, we were unable to show any relationship between age and the albuterol response in our multiple regression model. However, this is likely due to the relatively narrow age range of the study subjects and the small sample size.

The inverse association between the response to albuterol and plasma glucose is of considerable interest. Endothelial dysfunction is associated with type 1³⁷ and type 2 diabetes³⁸

and with acute hyperglycemia in normal subjects.³⁹ However, any relationship between insulin sensitivity and endothelial function in nondiabetic subjects is controversial.^{40,41} Moreover, data concerning cardiovascular risk and plasma glucose in normal subjects are divided.⁴² Nevertheless, glycosylated hemoglobin is positively associated with the risk of future coronary heart disease risk in a linear, stepwise manner,⁴³ suggesting a continuum of risk across the glycemic range, which may be explained by the inverse relationship between endothelial function and plasma glucose in the present study.

To compare our novel methodology for noninvasive assessment of endothelial function with a more established one, we assessed endothelial function by both PWA and forearm blood flow in 27 individuals with a wide range of serum cholesterol values. As previously noted,³ we observed a significant blunting of the vasodilator effect of ACh, but not of SNP, in subjects with a serum cholesterol value >6 mmol \cdot L⁻¹. Moreover, as in study 3, the response to albuterol but not to NTG was related to serum cholesterol. The main novel finding, however, was that there was a significant and linear correlation between the effect of albuterol on AIx and of ACh on forearm blood flow (Figure 2). This suggests that differences in the response to albuterol are likely to be caused by differences in endothelial function and that PWA provides a reasonable means of assessing endothelial function noninvasively. Although the correlation between the 2 methods in the present study was not absolute, the strength of the relationship is greater than that reported previously when endothelial function was compared in different vascular beds.⁵ Thus, PWA may be suitable for use in large studies investigating the predictive value of endothelial function.

Based on our previous data,^{20,25} it is unlikely that the small alterations in MAP and heart rate observed in the present study could account for the effect of the 2 drugs on AIx. Moreover, in study 2 the changes in MAP and heart rate with albuterol were greater during infusion of NE, which would tend to reduce the observed difference between LNMMA and NE. Furthermore, in study 3 the changes in MAP and heart rate were similar between the 2 groups. Therefore, it seems likely that NTG and albuterol altered the waveform, in part by a direct effect of NO on large-artery mechanics.

In summary, albuterol and NTG produce repeatable changes in the arterial waveform. The response to albuterol but not to NTG can be substantially inhibited by LNMMA, indicating that albuterol reduces AIx in part through generation of NO. Therefore, albuterol can be considered an endothelium-dependent, NO-mediated vasodilator and NTG, endothelium independent. Moreover, hypercholesterolemic exhibit a reduced response to albuterol but not to NTG, consistent with the presence of endothelial dysfunction. Finally, the response to albuterol was correlated with the response to ACh in the forearm, suggesting that there is good agreement between the 2 methods of assessing endothelial function. These data support the view that PWA coupled with administration of albuterol and NTG provides a simple, noninvasive, repeatable method for assessing endothelial function. We believe that this technique provides a suitable means for assessing endothelial function in large numbers of patients and thus, answers the important question of the predictive value of endothelial function. PWA has already been included in substudies of the ASCOT, SEARCH, and FIELD investigations. This will address the importance of stiffness as a

predictor of risk, but we now need to include the noninvasive assessment of endothelial function by PWA in such studies.

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