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INHIBITION OF PHOSPHODIESTERASE TYPE 5 IN CARDIOVASCULAR DISEASE

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PUBLICATIONS

The following publications are relevant to the work described in this thesis. Papers included in the appendix, for which permission to reproduce in this thesis was obtained from both the publishers and co-authors, are indicated.

OLIVER, J.J. Diabetic neuropathy - a further indication for phosphodiesterase type 5 inhibitors? *Int J Clin Pract*, **60,** 1026-7.

OLIVER, J.J., MELVILLE, V.P. & WEBB, D.J (2006). Effect of regular phosphodiesterase type 5 inhibition in hypertension. *Hypertension*, **48,** 622-7. Included in the appendix.

OLIVER, J.J., BOWLER, A., BEUDEKER, Q., TEN CATE, T. & WEBB, D.J. (2005). Dose-response relationship of sublingual nitroglycerin with brachial artery dilatation and change in central and peripheral augmentation index. *Clin Pharmacol Ther*, **77,** 337-8. Included in the Appendix.

DEANFIELD, J., DONALD, A., FERRI, C., GIANNATTASIO, C., HALCOX, J., HALLIGAN, S., LERMAN, A., MANCIA, G., OLIVER, J.J., PESSINA, A.C., RIZZONI, D., ROSSI, G.P., SALVETTI, A., SCHIFFRIN, E.L., TADDEI, S. & WEBB, D.J. (2005). Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens*, **23,** 7-17.

BRUNNER, H., COCKCROFT, J.R., DEANFIELD, J., DONALD, A., FERRANNINI, E., HALCOX, J., KIOWSKI, W., LUSCHER, T.F., MANCIA, G., NATALI, A., OLIVER, J.J., PESSINA, A.C., RIZZONI, D., ROSSI, G.P., SALVETTI, A., SPIEKER, L.E., TADDEI, S. & WEBB, D.J. (2005). Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens*, 23, 233-246.

OLIVER, J.J. & WEBB, D.J. (2003). Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol*, 23, 554-566.

OLIVER, J.J. & WEBB, D.J. (2003). Prehypertension and high normal blood pressure – a paradigm shift in the management of cardiovascular risk? *J R Coll Physicians Edinb*, 33, 239-47. Also reproduced online in Behind the Medical Headlines (http://www.behindthemedicalheadlines.com/articles/prehypertension.shtml).

OLIVER, J. & WEBB, D.J. (2002). Sildenafil for "blue babies". Such unlicensed drug use might be justified as last resort. *BMJ*, 325, 1174.

PRESENTATIONS

The following presentations are relevant to the work described in this thesis.

HUGHES, V.E.C., OLIVER, J.J., VINKEN, M. & WEBB, D.J. Investigation of combined phosphodiesterase 5 inhibitor and isosorbide mononitrate for the treatment of resistant hypertension. Poster presentation at the British Pharmacological Society 2006 winter meeting, Oxford. BPS undergraduate student prize.

OLIVER, J.J. & WEBB, D.J. Time dependent interactions of the hypotensive effects of sildenafil citrate and sublingual GTN in men with stable angina pectoris. European Society of Cardiology/World Congress of Cardiology, Barcelona, September 2006.

OLIVER, J.J. & WEBB, D.J. Time dependent interactions of the hypotensive effects of sildenafil and sublingual GTN in men with stable angina. Oral presentation at the British Pharmacological Society 2005 winter meeting, London.

HUMPHREY, V., OLIVER, J.J., WEBB, D.J. Salbutamol administration and pulse wave analysis as an assessment of endothelial function in subjects of different ages. Poster presentation at the British Pharmacological Society 2005 winter meeting, London. BPS undergraduate student prize.

OLIVER, J.J., BEUDEKER, Q.H., TEN CATE, T., BOWLER, A., WEBB, D.J. The dose-response relationship of sublingual GTN to brachial artery diameter and change in augmentation index. *Br J Clin Pharmacol* 2004;57:673. Oral presentation at the British Pharmacological Society 2004 winter meeting, Newcastle.

OLIVER, J.J. Phosphodiesterase 5 inhibition - a treatment for hypertension? Oral presentation at the 2004 meeting of Pfizer Fellows, Pfizer UK, Sandwich.

OLIVER, J.J., BELL, K., LECKIE, S.M., WEBB, D.J. Interaction between glyceryl trinitrate and sildenafil citrate (Viagra®) may last less than 4 hours. *Eur Heart J* 2003; 24 Suppl: 708. Oral presentation at the 2003 European Society of Cardiology annual meeting, Vienna.

OLIVER, J.J., BELL, K., LECKIE, S.M., WEBB, D.J. Interaction between glyceryl trinitrate and sildenafil citrate (Viagra®) may last less than 4 hours. Oral presentation at the 10th World Congress of the International Society for Sexual and Impotence Research, Montreal 2002.

DECLARATION

This thesis and the data presented within it are entirely the results of my own efforts, except where stated otherwise. This work contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution and, to the best of my knowledge, contains no material previously published or written by another person, except where stated in the text.

Dr James John Oliver

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ABBREVIATIONS

ABPM	Ambulatory blood pressure monitoring
ACCT	Anglo-Cardiff Collaborative Trial
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ADMA	Asymmetric dimethylarginine
AIx	Augmentation index
AIx@75	Augmentation index normalised to a heart rate of 75 beats per minute
ALLHAT	The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial
AP	Augmentation pressure
ARA	Angiotensin II receptor (type AT_1) antagonist
AU	Arbitrary units
AUC	Area under the curve
ß-blocker	ß-adrenoreceptor antagonist
BMI	Body mass index
BP	Blood pressure
bpm	Beats per minute
BHS	British Hypertension Society
CAD	Coronary artery disease
CCB	Calcium channel blocker
CF-PWV	Carotid-femoral pulse wave velocity
cGMP	Guanosine 3',5'-cyclic monophosphate
CI	Confidence interval
CRC	Clinical Research Centre
ECG	Electrocardiogram
EDRF	Endothelium-derived relaxing factor
FMD	Flow-mediated dilatation
GTF	Generalised transfer function
HDL	High density lipoprotein
HR	Heart rate
ISH	Isolated systolic hypertension
ISMN	Isosorbide mononitrate
LDL	Low density lipoprotein
L-NMMA	N^G monomethyl-L-arginine
LVH	Left ventricular hypertrophy
MAP	Mean arterial blood pressure
Max	Maximum
MI	Myocardial infarction
mmHg	Millimetres of mercury
NICE	National Institute for Health and Clinical Excellence
NO	Nitric oxide
NOS	Nitric oxide synthase
RAIx	Radial augmentation index
PDE	Phosphodiesterase
PED	Penile erectile dysfunction

PKG	cGMP-dependent protein kinase
PP	Pulse pressure
PAH	Pulmonary arterial hypertension
PWA	Pulse wave analysis
PWV	Pulse wave velocity
SD	Standard deviation
SEM	Standard error of the mean
sGC	Soluble guanylate cyclase
t-PA	Tissue plasminogen activator
TRH	Treatment-resistant hypertension
VTI	Velocity time integral
WGH	Western General Hospital

ABSTRACT

Nitric oxide is released from the endothelium and causes relaxation of vascular smooth muscle by stimulating guanylate cyclase to produce guanosine 3',5'-cyclic monophosphate (cGMP) which, in turn, is degraded by phosphodiesterase type 5 (PDE5). Inhibition of PDE5, with drugs like sildenafil citrate, promotes NO-stimulated relaxation of vascular smooth muscle. The overall aim of the work contained within this thesis was to further characterise the systemic vascular effects of PDE5 inhibition. Four clinical studies were performed.

The aims of the first study were to investigate in healthy men the effect of smoking on endothelium-dependent vasomotor function measured as the change in peripheral arterial wave reflection with inhaled salbutamol, and the effect of acute sildenafil 100 mg on this response. Smokers (n=12) exhibited a reduced response to inhaled salbutamol compared to non-smokers (n=11) [mean(standard deviation) area under the curve of the change in central augmentation index following salbutamol 400 μg: -29(143) AU in smokers *vs* -159(124) AU in non-smokers, P=0.03]. In the smokers, there was a trend to an improvement in the response to salbutamol following sildenafil [-96(266) AU *vs* -29(143) AU with matched placebo; P=0.2].

The co-administration of glyceryl trinitrate (GTN) and sildenafil is absolutely contraindicated because of the potential for profound hypotension. The aim of the second study was to characterise the time course of this interaction. Twenty men with stable angina, maintained on their usual medicines, were administered sublingual GTN 400 μg 1, 4, 6 and 8 hours after sildenafil 100 mg or matched placebo. Compared to the combination of GTN and placebo, the combination of GTN and sildenafil resulted in greater mean maximum reductions from baseline in sitting systolic blood pressure (BP) at 1, 4 and 8 hours, and in sitting diastolic BP at all time points (all P<0.05). Compared to placebo, sildenafil alone reduced systolic BP at 1, 4, 6 and 8 hours (P<0.01 at 1 hour and P<0.05 at 4, 6, and 8 hours) and diastolic BP at 4, 6, and 8 hours (all P<0.01). Analysis of the change in BP from the measures taken before each GTN challenge suggested that the interaction on BP

might be synergistic at 1 hour after sildenafil, but no more than additive at 6 and 8 hours after sildenafil. Symptoms consistent with hypotension occurred following GTN in 6 subjects at 1 hour and 3 subjects at 4 hours after sildenafil, but in no subjects at 6 and 8 hours after sildenafil or at any time after placebo.

In the third study, 25 otherwise untreated hypertensives were given sildenafil 50 mg or matched placebo three times daily for 16 days and the effects on ambulatory BP, clinic BP, arterial wave reflection, carotid-femoral pulse wave velocity and brachial artery flow-mediated dilatation were measured. Three subjects were withdrawn because of side effects and the data from the remaining 22 subjects were analysed. Sildenafil reduced ambulatory BP [change from baseline in average daytime BP: systolic -8(9) mmHg *vs* 2(9) mmHg with placebo, $P<0.01$; diastolic -6(5) mmHg *vs* 0(6) mmHg, $P<0.01$] and clinic BP [change from baseline to 1 hour after drug administration on day 16: systolic -5(11) mmHg *vs* 4(10) mmHg, $P<0.01$; diastolic -5(5) mmHg *vs* 2(7) mmHg, $P<0.01$]. Sildenafil, but not placebo, reduced arterial wave reflection [central augmentation index from 32(9)% at baseline to 30(10)% at 1 hour after administration on day 16, $P<0.05$; radial augmentation index from 88(13)% to 84(13)%, $P<0.01$], but the change in arterial wave reflection was not statistically significant compared to the change with placebo. Sildenafil did not affect pulse wave velocity or flow-mediated dilatation.

The fourth study investigated the potential of combined PDE5 inhibition and organic nitrate for the management of treatment-resistant hypertension (TRH). In 6 patients with TRH, maintained on their usual antihypertensives sildenafil 50 mg alone, isosorbide mononitrate (ISMN) 10 mg alone and co-administered sildenafil and ISMN all acutely reduced systolic BP and diastolic BP compared to placebo (quantified as the area under the curve of the change from baseline to 4 hours after drug administration; all $P\leq0.01$). The combination produced a greater reduction in systolic BP than did either sildenafil alone ($P=0.03$) or ISMN alone ($P=0.01$) and a greater reduction in diastolic BP than did sildenafil alone ($P=0.02$). Compared to placebo, from 1 to 3 hours after drug administration BP was on average 13/10 mmHg

lower with sildenafil alone, 18/14 mmHg lower with ISMN alone and 26/18 mmHg lower with the combination.

The following conclusions were made. (1) Smokers exhibit impaired vascular responsiveness to inhaled salbutamol, indicating systemic endothelial dysfunction, which may be improved by sildenafil. (2) In men with stable angina there is an interaction on BP reduction between sildenafil 100 mg and sublingual GTN 400 μg for at least 8 hours after sildenafil administration, but this interaction is no more than additive from 6 hours after sildenafil administration. (3) Regular sildenafil monotherapy reduces BP in hypertension. (4) In patients with TRH maintained on their usual antihypertensives sildenafil alone and ISMN alone both acutely reduce BP and there is additional BP reduction when these drugs are given in combination.

CHAPTER 1

INTRODUCTION

1.1 THE ENDOTHELIUM AND THE NITRIC OXIDE PATHWAY

1.1.1 The endothelium

The endothelium is a single layer of cells that lines the entire vascular system. Although once thought to be inert, it is now considered to be an 'organ' that has a range of important functions and is highly metabolically active (Aird, 2004; Galley *et al.*, 2004; Pasyk *et al.*, 2004). Its major functions include acting as a barrier between blood and underlying tissues, selective transport of essential molecules, control of vascular tone, and regulation of haemostasis and coagulation.

Endothelial control of vascular tone is effected by its production and release of a number of vasodilators, including nitric oxide (NO), prostacyclin, and endothelium-derived hyperpolarising factor, and vasoconstrictor substances, including endothelin-1 and metabolites of arachidonic acid. The discovery of NO as an important and potent vasodilator was a major milestone in the history of vascular biology. Initially, Furchgott and Zawadzki (1980) demonstrated that relaxation of rabbit thoracic aorta and other blood vessels by acetylcholine (ACh) required the presence of endothelial cells. This suggested that ACh acted on endothelial cell muscarinic receptors to stimulate the production of a substance or substances, initially termed endothelium-derived relaxing factor (EDRF), that caused vascular smooth muscle relaxation. EDRF was subsequently shown to be NO (Palmer *et al.*, 1987).

1.1.2 Endothelial NO

NO is synthesised from the substrates arginine, NADPH and oxygen by the enzyme nitric oxide synthase (NOS). Of the 3 known isoforms of NOS, neuronal NOS (nNOS, type I) and endothelial NOS (eNOS, type III) are constitutively expressed in the endothelium, platelets and some neurones, and are acutely activated by calcium/calmodulin. They are also activated or inhibited by phosphorylation via various protein kinases (Stuehr, 1999). Inducible NOS (iNOS, type II) is not constitutively expressed, but is induced by various cytokines such as interferon-γ, IL-1β and TNF-α, endotoxin, and oxidative stress. NO increases the V_{max} of soluble

guanylate cyclase (sGC) ~400 fold and this enzyme catalyses the cyclisation of guanosine 5'-triphosphate to guanosine 3',5'-cyclic monophosphate (cGMP; Stone *et al.*, 1995). In turn, cGMP regulates cellular function largely through the activation of cGMP-dependent protein kinases (PKGs). There are a number of substrates for PKG in smooth muscle, including the regulatory myosin-binding subunit of myosin phosphatase (Surks *et al.*, 1999) and calcium-activated maxi K^+ channels (Fukao *et al.*, 1999). Phosphorylation of these targets contributes to a reduction in intracellular calcium concentration, which leads to vascular smooth muscle relaxation (Hofmann *et al.*, 2000; Lincoln *et al.*, 2001).

A series of experiments confirmed the central role of NO in the control of vascular tone in humans. Infusion of N^G monomethyl-L-arginine (L-NMMA), an arginine analogue that inhibits NOS, into the brachial artery reduced blood flow into the forearm (Vallance *et al.*, 1989), indicating that NO is continuously released by resistance arterioles and tonically opposes vasoconstrictor mechanisms. L-NMMA also inhibited ACh-stimulated vasodilatation in this study. Subsequently, the role of NO in regulating systemic blood pressure (BP) was established when intravenous infusion of L-NMMA was shown to increase systemic vascular resistance and BP (Haynes *et al.*, 1993a; Haynes *et al.*, 1993b; Stamler *et al.*, 1994). Although the control of vascular tone is a major function of endothelium-derived NO, it also has a number of other important anti-thrombotic and anti-atherosclerotic properties, including inhibition of platelet activation and adhesion, leukocyte adhesion and migration into the subendothelial space, and vascular smooth muscle proliferation (Naseem, 2005).

1.1.3 Cyclic GMP-specific, cGMP-binding phosphodiesterase 5

The phosphodiesterases (PDEs) are enzymes that degrade cGMP and the related second messenger cyclic adenosine 3',5'-monophosphate. Of the 11 known mammalian PDE gene families, the cGMP-specific, cGMP-binding PDE5 is the major cGMP-hydrolysing PDE in arterial smooth muscle under basal (i.e. low calcium) conditions, although under activated, high calcium, conditions PDE1 is probably also important (Beavo, 1995; Matsumoto *et al.*, 2003; Rybalkin *et al.*,

2003b). PDE5 is also abundant in platelets and other tissues (Lin *et al.*, 2003; Wallis *et al.*, 1999).

Intracellular cGMP concentrations are tightly controlled by PDE5 through a range of negative feedback mechanisms. Catalytic activity is increased by mass action with elevated cGMP concentration and by binding of cGMP to allosteric sites. Binding of cGMP to the catalytic site also increases cGMP binding to the allosteric sites. Furthermore, activated PKG phosphorylates PDE5 and enhances both its catalytic activity and binding of cGMP at allosteric sites (Corbin *et al.*, 2003; Corbin *et al.*, 2000; Mullershausen *et al.*, 2001; Rybalkin *et al.*, 2002; Rybalkin *et al.*, 2003a).

1.2 INHIBITION OF PDE5

1.2.1 A treatment for penile erectile dysfunction

Inhibitors of PDE5 slow the breakdown of cGMP and thus increase its intracellular concentration, with the consequence that NO-mediated cellular responses, such as vascular smooth muscle relaxation, are promoted. Currently, the major clinical indication for PDE5 inhibitors is penile erectile dysfunction (PED). Normal penile erection is dependent upon relaxation of vascular and sinusoidal smooth muscle in the corpora cavernosa which leads to the penis becoming engorged with blood. The stimulus to this is provided by NO released from both neurones and the endothelium. As elsewhere in the vasculature, NO stimulates the production of cGMP, which is subsequently hydrolysed by PDE5. By stimulating vascular relaxation within the corpora cavernosa during sexual stimulation, inhibitors of PDE5 promote penile erection and have proved to be a highly successful treatment of PED (Carson *et al.*, 2005; Setter *et al.*, 2005).

1.2.2 The PDE5 inhibitors

Sildenafil is a potent inhibitor of PDE5 (IC_{50} of 3.9 nM) that is >1000-fold more selective for PDE5 than for PDE2, PDE3 and PDE4. It is moderately selective (>80-fold) over PDE1 but only around 10 times as potent for PDE5 as for PDE6.

Following oral administration, sildenafil is rapidly absorbed, reaching peak plasma concentrations within 1 hour (range 0.5 to 2 hours; Jackson *et al.*, 1999; Milligan *et al.*, 2002). Although 92% of sildenafil is absorbed the mean absolute bioavailability is around 38-41%, indicating substantial gut wall and hepatic first-pass metabolism (Milligan *et al.*, 2002; Nichols *et al.*, 2002; Walker *et al.*, 1999). For oral doses up to 200 mg the relationship between dose and systemic exposure is approximately linear, with increases in C_{max} and area under the curve (AUC) of concentration over time of 2.2 and 2.1 respectively with doubling of dose (Nichols *et al.*, 2002). Sildenafil is extensively metabolised, with no unchanged drug being detected in either urine or faeces. Following oral administration, metabolites are mainly excreted in faeces (73-88%) and to a lesser extent in urine (6-15%). Sildenafil is predominantly metabolised by the cytochrome P450 enzyme CYP3A4, although CYP2C9 also contributes. The main circulating metabolite, N-desmethyl sildenafil, reaches t_{max} 1.4 hours after oral administration, has 50% of the potency of sildenafil with regard to inhibition of PDE5 and accounts for around 20% of the pharmacological effects.

As would be expected, inhibitors of CYP3A4, including erythromycin (Muirhead *et al.*, 2002a) and the protease inhibitors ritonavir and saquinavir (Muirhead *et al.*, 2000) increase the AUC and C_{max} of sildenafil. Grapefruit juice, a further inhibitor of CYP3A4, increases the AUC but does not affect C_{max} (Jetter *et al.*, 2002). Co-administration of CYP2C9 inhibitors, including tolbutamide and warfarin, do not affect the pharmacokinetics of sildenafil (Gupta *et al.*, 2005). Clearance of sildenafil is reduced in the healthy elderly (≥65 years) and in subjects with liver or severe renal dysfunction (Muirhead *et al.*, 2002b).

In addition to sildenafil there are now two further licensed specific PDE5 inhibitors. Selectivity of vardenafil for PDE5 is >1000-fold over PDE2-4 and 7-10, >300-fold over PDE11, >130-fold over PDE1 and >15-fold over PDE6 (Gupta *et al.*, 2005). Selectivity of tadalafil for PDE5 is >9,000-fold over PDE1-4 and 7-10, >700-fold over PDE6 and >5-fold over PDE11 (Curran *et al.*, 2003). A comparison of the pharmacological properties of sildenafil, vardenafil and tadalafil is given in Table 1.1. With respect to the treatment of PED there are no clear differences in efficacy

between the 3 available PDE5 inhibitors, although the longer duration of action of tadalafil may facilitate greater spontaneity in sexual intercourse.

The main side effects of the PDE5 inhibitors are dyspepsia, vomiting, headache, flushing, dizziness, raised intra-ocular pressure, and nasal congestion. Disturbance of colour vision can also occur. This is probably due to inhibition of PDE6 in photoreceptors of the retina (Boolell *et al.*, 1996; Cheitlin *et al.*, 1999) and appears to occur more commonly with sildenafil (Setter *et al.*, 2005). Myalgia and back pain have also been reported as side effects of the PDE5 inhibitors, especially with tadalafil (Setter *et al.*, 2005).

Sildenafil, made and marketed by Pfizer Inc. as Viagra® (Figure 1.1), was the first widely available, convenient and effective treatment of PED. As a treatment for a disorder of sexual function it rapidly became known throughout the world and is established as an icon of the early 21st century. Following its introduction, PED was transformed from a condition that was barely spoken about to one that was widely acknowledged and treated pharmacologically. Viagra has been the subject of countless jokes and cartoons (Figure 1.1), and has even sparked debate on the potential consequences of medicalising sexual function (Potts *et al.*, 2004), and on the nature of so-called "lifestyle drugs" and how healthcare systems should deal with them (Flower, 2004).

Figure 1.1. The highly characteristic blue, diamond-shaped Viagra tablet and a Viagra joke from the print media.
Print image reproduced by kind permission of PRIVATE EYE magazine.

	Sildenafil	Vardenafil	Tadalafil
t_{max}, h (range)	1 (0.5-2)	0.7 (0.25-3)	2 (0.5-6)
$t_{1/2}$, h	3-5	4-5	17.5
Renal excretion, %	<1	1	<0.3
Active metabolite	N-desmethyl sildenafil - 50% potency - 20% contribution to activity	N-desethyl vardenafil - 28% activity - 7% contribution to activity	None
CYP isoenzymes	3A4 (79%) 2C9 (20%) 2C19 (<2%) 2D6 (<2%)	3A4 (major) 3A5 (minor) 2C9 (minor)	3A4
Food effect (high-fat meal)	t_{max} 1h↑ C_{max} 29%↓ AUC 11%↓	t_{max} 1h↑ C_{max} 18%↓ AUC ↔	No significant effect
Protein binding, %	96	93-95	94
IC_{50} for PDE5, nM	3.9	0.1-0.7	0.94

Table 1.1. Comparison of the pharmacological properties of the 3 commercially available PDE5 inhibitors.

1.3 HYPERTENSION AND THE EFFECT OF PDE5 INHIBITION ON BP

1.3.1 Hypertension

In middle and old age there is a direct relationship between increasing BP, whether systolic or diastolic, and the risk of death due to coronary artery disease (CAD), death due to stroke and overall mortality, with no evidence of a threshold down to at least 115/75 mmHg (Prospective Studies Collaboration, 2002). As a cause of global disease burden, high BP ranks 3rd, behind underweight and unsafe sex and ahead of tobacco, alcohol and unsafe water, sanitation and hygiene (Ezzati *et al.*, 2002). In the UK (North of England Hypertension Guideline Development Group, 2004; Williams *et al.*, 2004), Europe (European Society of Hypertension & European Society of Cardiology, 2003) and the USA (Chobanian *et al.*, 2003a) hypertension is defined as a systolic BP ≥140 mmHg or a diastolic BP ≥90 mmHg. BP can vary substantially within individuals and there is increasing evidence that values obtained during ambulatory recording of BP (ABPM), for example over 24 hours, better predict the risk of cardiovascular disease than do values obtained in the clinic (Clement *et al.*, 2003; Hansen *et al.*, 2005). ABPM values are often lower than clinic values and, as a result, lower BP thresholds should be used to diagnose hypertension.

A number of lifestyle interventions, including weight reduction, a diet that is rich in fruit and vegetables and low in saturated fat and salt, physical exercise and reduction in alcohol consumption, can reduce BP in hypertension (Williams *et al.*, 2004). Although these interventions should be widely recommended to patients with hypertension, antihypertensive drug therapy is often required to control BP. There are 5 major classes of antihypertensives: thiazide/thiazide-like diuretics, ß-adrenoreceptor antagonists (ß-blockers), calcium channel blockers (CCBs), angiotensin converting enzyme (ACE) inhibitors and angiotensin II receptor (type AT_1) antagonists (ARAs). In recent years there has been much interest in whether or not each of these drug classes are equivalent in their ability to reduce cardiovascular events when used to lower BP. A major meta-analysis from the Blood Pressure Lowering Trialists Collaboration (2003), which included data from 29 randomised trials involving 162,341 subjects, found no differences in total major cardiovascular

events between treatment regimes based on the major antihypertensive classes and also that larger reductions in BP produced larger reductions in risk. However, since then the waters have been somewhat muddied. First, a further meta-analysis suggested that ß-blockers may be less effective in preventing stroke than other agents (Lindholm *et al.*, 2005). Second, the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA) was published (Dahlöf *et al.*, 2005). In this study (n = 19,257), atenolol with bendroflumethiazide added as required was compared to amlodipine with perindopril as required. While there was no statistical difference between the 2 regimens in the primary endpoint of nonfatal myocardial infarction and fatal CAD, in the amlodipine/perindopril arm there were lower incidences of stroke, total cardiovascular events and procedures, and all-cause mortality. Interpretation of this study is difficult because average achieved BP was lower in the CCB/ACE inhibitor arm. While the authors suggested that the difference in BP control did not fully account for the difference in event rates (Poulter *et al.*, 2005), this has been disputed (Staessen *et al.*, 2005). ASCOT-BPLA also found that treatment with atenolol/bendroflumethiazide was associated with a much higher rate of new-onset diabetes and, at least for this reason, thiazide/ß-blocker combinations should probably be avoided wherever possible.

All major hypertension treatment guidelines give targets for BP control. In the UK, the British Hypertension Society (BHS) target is ≤140/85 mmHg (Williams *et al.*, 2004) and the National Institute for Health and Clinical Excellence (NICE) target is ≤140/90 mmHg (North of England Hypertension Guideline Development Group, 2004). Unfortunately, control of hypertension to these targets in the population is poor (Table 1.2) The reasons for this are numerous, and include poor concordance with treatment, the use of suboptimal drug doses and combinations, and poor clinician persistence. It is now recognised that achieving target BP will require a combination of 2 or more antihypertensive drugs in most patients (Black *et al.*, 2001; Cushman *et al.*, 2002).

		Aware (%)	Treated (%)	Controlled (%)	Treated hypertensives controlled (%)
160/95	**England**	58	52	38	73
	USA	88	78	66	84
140/90	**England**	36	25	10	40
	USA	69	53	29	55

Table 1.2. Rates of hypertension awareness, treatment and control in the population and control in treated hypertensives.
Values are shown for 2 different BP thresholds, 160/95 mmHg and 140/90 mmHg. Data from Wolf-Maier *et al* (2003).

1.3.2 PDE5 inhibitors and systemic BP

1.3.2.1 Effect of sildenafil on BP in healthy subjects

Given that NO-mediated vasodilatation contributes a tonic negative influence on systemic BP, enhancement of this endogenous effect of NO through PDE5 inhibition might be expected to reduce BP. The effect of single doses of PDE5 inhibitors, in particular sildenafil, on BP have been extensively investigated. Jackson *et al* (1999) investigated the haemodynamic effects of sildenafil in healthy men and found that sildenafil increased forearm blood flow when infused into the brachial artery, indicating that it is an arterial vasodilator in man. When given intravenously, sildenafil reduced systemic vascular resistance and BP. The mean reduction in BP with 80 mg sildenafil, measured at the end of the 40-minute infusion, was 9/7 mmHg compared to placebo. Following oral administration of single doses up to 200 mg the mean maximum reduction in BP was 10/7 mmHg, which was similar to the effect of 100 mg orally in a further study in healthy subjects (Zusman *et al*., 1999). In the latter study BP had returned to normal by 8 hours after administration. In both of these studies there was no clear relationship between the dose of sildenafil and the magnitude of the reduction in BP. In addition, sildenafil did not affect heart rate (HR) or the BP response to standing. However, an acute reduction in BP with sildenafil has not been consistently demonstrated in healthy people. In a study by Schalcher *et al* (2002), which was not placebo controlled, oral sildenafil 50 mg

increased BP by 3/2 mmHg and HR by 4 beats per minute (bpm). Systemic administration of sildenafil also increased forearm blood flow and reduced forearm vascular resistance in this study, suggesting that sildenafil-induced peripheral vasodilatation was compensated for by a baroreceptor-mediated increase in HR, thus increasing cardiac output and maintaining BP. No effect on BP or HR of oral sildenafil 50 mg was observed by Dishy *et al*, either in healthy smokers (2004) or in healthy non-smokers (2001).

1.3.2.2 Effect of sildenafil on BP in patients with hypertension and cardiovascular disease

In a single-blind study Mahmud *et al* (2001) investigated the effect of a single oral dose of sildenafil 50 mg on BP in 8 hypertensive patients with PED and well controlled BP. The mean maximum reduction in BP was 24/8 mmHg with sildenafil and 6/3 mmHg with placebo. At 75 minutes BP was 17/11 mmHg lower with sildenafil than with placebo. However, interpretation of this data in terms of the 'pure' effect of sildenafil on BP in hypertension is difficult. In addition to PED and hypertension, some subjects had other comorbid conditions including cerebrovascular disease, atrial fibrillation and diabetes. Furthermore, the subjects were taking up to 5 antihypertensives, such that interactions with other agents may have been important in mediating the substantial observed effect on BP.

Vardi *et al* (2002) investigated the effect of single dose sildenafil 100 mg on ambulatory BP measured over 6 hours, from 21:00 to 03:00. Compared to an equivalent control period, mean BP was reduced by 9/6 mmHg in hypertensives and by 4/4 mmHg in normotensives, although this apparent difference between the groups was not statistically significant. As with the above study, the hypertensives were receiving antihypertensive drugs and drug interactions may have partly accounted for the slightly greater effect in this group. In addition, the study was not blinded, randomised or placebo-controlled. There was a suggestion that BP reduction may have been greater in older subjects. In patients with hypertension taking amlodipine 5 mg or 10 mg monotherapy the mean maximum change in supine BP was -17/-8 mmHg with sildenafil 100 mg compared to -9/-2 mmHg with placebo

(Webb *et al.*, 1999). In this study the greatest decreases in BP with sildenafil tended to occur in those subjects with the highest baseline BP values, a phenomenon that is well characterised with other antihypertensives (Hulthen *et al.*, 1982; Shen *et al.*, 1975; Shepherd, 1988; Shepherd *et al.*, 1991).

Zusman *et al* (2000) examined the effect of sildenafil on BP in a post-hoc subanalysis of 5 prospective, randomised, double-blind, placebo-controlled studies in men with PED. BP was measured between 1 and 6 hours after sildenafil (25 to 200 mg) or placebo. Out of a total of 1685 men with PED, there were complete data for 608 men who were taking one or more antihypertensives. In these subjects the mean reduction in BP was -3.6/-1.9 mmHg with sildenafil and -0.8/-0.1 mmHg with placebo. In the subjects not taking any antihypertensives the mean reduction in BP was -2.2/2.0 mmHg with sildenafil and -0.1/0.4 mmHg with placebo.

Other studies have investigated the effects on BP of sildenafil in patients with CAD. In an open-label, non-placebo controlled study, in which 8 patients were withdrawn from antianginals, vasodilators and diuretics for 48 hours before dosing, intravenous sildenafil 40 mg reduced invasively measured BP by 9/8 mmHg (Jackson *et al.*, 1999). In 24 patients with CAD (12 also hypertensive), in whom all medicines were discontinued for at least 5 half-lives prior to study, oral sildenafil 100 mg reduced BP by 12/9 mmHg (Halcox *et al.*, 2002a). In subjects undergoing diagnostic coronary angiography, a majority of whom had angiographically proven CAD, neither oral sildenafil 50 mg (Manfroi *et al.*, 2003) or 100 mg (Halcox *et al.*, 2002a) had any effect on invasively measured BP. In 14 men with severe CAD, who were taking their normal medicines except for nitrates, invasively-measured aortic BP fell by 10/5 mmHg 1 hour after oral sildenafil 100 mg (Herrmann *et al.*, 2000).

1.3.2.3 Effects of vardenafil and tadalafil on BP

The effects of vardenafil and tadalafil on BP have been much less extensively investigated than have the effects of sildenafil. Vardenafil 10 mg reduced BP by 6/5 mmHg compared to placebo in men with stable CAD (Thadani *et al.*, 2002) and by up to 8/7 mmHg in men with PED (Pomara *et al.*, 2004). Compared to placebo,

single doses of tadalafil 20 mg had no effect on systolic BP but reduced diastolic BP by around 5 mmHg in healthy subjects (Kloner *et al.*, 2003b). There was no effect on BP after 26 weeks of once daily tadalafil 20 mg in healthy men or men with mild PED (Kloner *et al.*, 2003b). In patients with CAD tadalafil 10 mg reduced BP by 7/4 mmHg (Kloner *et al.*, 2003b). In subjects regularly taking a single antihypertensive drug it reduced BP by up to 8/4 mmHg compared to placebo (Kloner *et al.*, 2003c).

1.3.2.4 Summary of the data on the effects of PDE5 inhibitors on BP

Most of the work on the effect on BP of PDE5 inhibitors, predominantly sildenafil, in hypertensives, has largely arisen through concerns of potentially hazardous reductions in BP in men taking these drugs for PED, particularly when taken with other drugs that reduce BP. With the exception of the interaction with NO donor drugs (see section 1.3.3), in this regard the data are reassuring that the effects on BP are no more than moderate and are unlikely to be of significance in terms of clinical safety. However, the effect on BP of PDE5 inhibition *alone* for the *treatment* of hypertension has not been investigated, either in single-dose or in chronic dosing studies.

1.3.3 PDE5 inhibitors with organic nitrates and α-adrenoreceptor antagonists

Organic nitrates, such as glyceryl trinitrate (GTN) and isosorbide mononitrate (ISMN), are commonly used to treat angina and heart failure. These drugs dilate arteries and veins through their action as NO donors and, given alone, reduce systemic BP. Although PDE5 inhibitors have no more than moderate effects on BP when administered alone, the simultaneous provision of exogenous NO from organic nitrates and inhibition of cGMP breakdown with PDE5 inhibition can result in substantial BP reduction. This interaction has been demonstrated for sildenafil in healthy subjects with GTN (Webb *et al.*, 1999) and in men with angina with both GTN (Figure 1.2) and ISMN (Webb *et al.*, 1999; Webb *et al.*, 2000), and for tadalafil (Kloner *et al.*, 2003a) and vardenafil (Summary of product characteristics for Levitra, 2005) in healthy subjects with GTN. The combined use of organic nitrates and PDE5 inhibitors is absolutely contraindicated because of the potential for harm from hypotension. For sildenafil, the American College of Cardiology and the

American Heart Association have, in a consensus document, recommended that sildenafil and organic nitrates are not co-administered within 24 hours of one another (Cheitlin *et al.*, 1999).

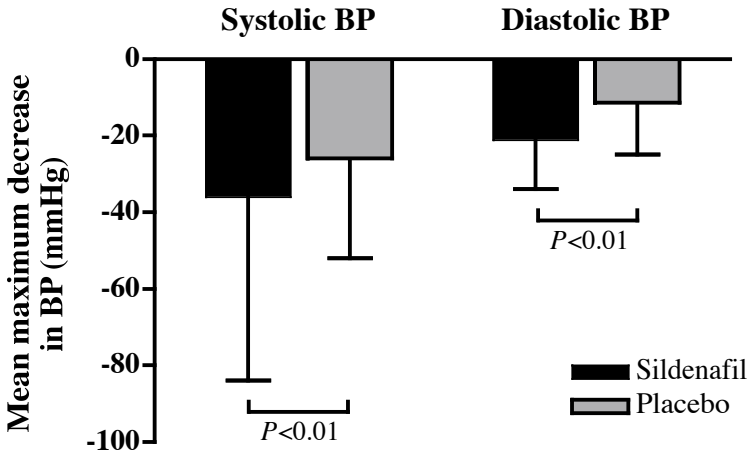


Figure 1.2. Effect of combined sildenafil and GTN on BP in men with stable angina.
Subjects were given GTN 500 μg sublingually 1 hour after sildenafil 50 mg or matched placebo. Sitting BP was measured at regular intervals for 6 hours after GTN and the maximum decrease in BP during this time was recorded. Error bars are lower limits of the 95% confidence intervals. Graph drawn using data from Webb *et al* (2000).

There is also evidence of an interaction on BP between PDE5 inhibitors and α-adrenoreceptor antagonists (Kloner, 2005). However, the effects on BP are not as potentially dramatic as with the combination of PDE5 inhibitors and organic nitrates. PDE5 inhibitors and α-adrenoreceptor antagonists are not absolutely contraindicated in combination but certain restrictions are advised.

1.4 ARTERIAL STIFFNESS, ARTERIAL WAVE REFLECTION AND THE EFFECT OF PDE5 INHIBITION

1.4.1 Arterial stiffness and arterial wave reflection

Data from the Framingham Heart Study have shown that diastolic BP increases until middle age and then tends to fall, whereas systolic BP and pulse pressure (PP; the difference between systolic BP and diastolic BP) increase continuously with age (Franklin *et al.*, 1997). These changes, especially from around 50 years of age, are largely the result of increasing stiffness of the large arteries, such as the aorta and its major branches. As a result, increased large artery stiffness manifests as raised systolic BP.

Traditionally, diastolic BP has been the major focus in the treatment of hypertension. However, over recent years systolic BP has become recognised as a stronger cardiovascular risk factor in older people, having, for example, greater predictive value than diastolic BP for CAD in people aged >60 years (Franklin *et al.*, 2001b; Kannel *et al.*, 1971). Isolated systolic hypertension (ISH; systolic BP ≥140 mmHg and DBP <90 mmHg) is the most common subtype of hypertension in middle-age and is overwhelmingly so in the elderly (Franklin *et al.*, 2001a). It is a major risk factor for stroke (Nielsen *et al.*, 1995), CAD (Franklin *et al.*, 2001b; Kannel *et al.*, 1971), and cardiovascular and total mortality (Alli *et al.*, 1999; Antikainen *et al.*, 1998). Furthermore, measurement of systolic BP alone identifies >90% of hypertensives, whereas diastolic BP alone identifies only ~20% (Lloyd-Jones *et al.*, 1999). The treatment of ISH with conventional antihypertensive drugs is of proven clinical benefit (SHEP Cooperative Research Group, 1991; Staessen *et al.*, 1997). However, whilst it is recognised that not enough hypertensives are controlled to target pressures (Wolf-Maier *et al.*, 2003), it is much more commonly systolic BP than diastolic BP that is not adequately controlled (Franklin *et al.*, 2001a; Lloyd-Jones *et al.*, 1999).

There is clearly a strong rationale for understanding the mechanisms of arterial stiffness to better treat ISH. In addition, other established cardiovascular risk factors

are also associated with increased arterial stiffness and there has been a great deal of interest in the extent to which more direct measures of arterial stiffness might improve cardiovascular risk stratification. Therapeutically targeting arterial stiffness might also be of benefit in the prevention and treatment of cardiovascular disease (Oliver *et al.*, 2003).

Windkessel theory treats the circulation as a central elastic reservoir (the large arteries), into which the heart pumps, and from which blood travels to the tissues through relatively non-elastic conduits (peripheral arteries). The elasticity of the proximal large arteries is the result of the high elastin to collagen ratio in their walls, which progressively declines towards the periphery. The increase in arterial stiffness that occurs with age (Hallock *et al.*, 1937) is largely the result of progressive elastic fibre degeneration (Avolio *et al.*, 1998). The elasticity of a given arterial segment is not constant, but depends upon its distending pressure (Greenfield *et al.*, 1962; Hallock *et al.*, 1937). As distending pressure increases there is greater recruitment of relatively inelastic collagen fibres (Apter, 1967; Bank *et al.*, 1996; Roach *et al.*, 1957) and, consequently, a reduction in elasticity. The background level of distending pressure in the circulation is determined by mean arterial BP (MAP). This is important because MAP must be taken into account whenever measurements of arterial stiffness are made, so that anticipated effects of distending pressure can be differentiated from real differences in the elasticity of the arterial wall.

Ejection of blood from the left ventricle during systole initiates an arterial pressure wave that travels towards the periphery. At points of impedance mismatch, chiefly at the high-resistance arterioles, wave reflection occurs (Nichols *et al.*, 1998). As a consequence of differing elastic qualities, and wave reflection, the shape of the arterial waveform varies throughout the arterial tree. Whereas MAP remains virtually unchanged (it declines slightly), in healthy young subjects systolic BP and PP are amplified in the peripheral circulation (Kroeker *et al.*, 1955). With increasing age this amplification is progressively reduced (Wilkinson *et al.*, 2001a). Although peripheral BP is most commonly measured clinically, the information contained within the waveform of the proximal aorta is of particular interest because it is the

BP profile at this site, rather than more peripherally, that determines left ventricular load and coronary blood flow. The effects of increased arterial stiffness on the central aortic waveform and BP are illustrated in Figure 1.3. The contour and amplitude of the pressure waveform are influenced by large artery pulse wave velocity (PWV), in that faster travelling pressure waves arrive at, and are reflected from, the peripheral circulation earlier. When arteries are relatively compliant and PWV is relatively slow, reflected waves return to the central aorta in diastole, augmenting diastolic BP and, therefore, coronary blood flow, which occurs predominantly during diastole. When arteries are stiffer and PWV is higher, reflected waves arrive earlier and augment central systolic BP, rather than diastolic BP, increasing left ventricular workload and compromising coronary blood flow (Bogren *et al*., 1989; Ohtsuka *et al*., 1994).

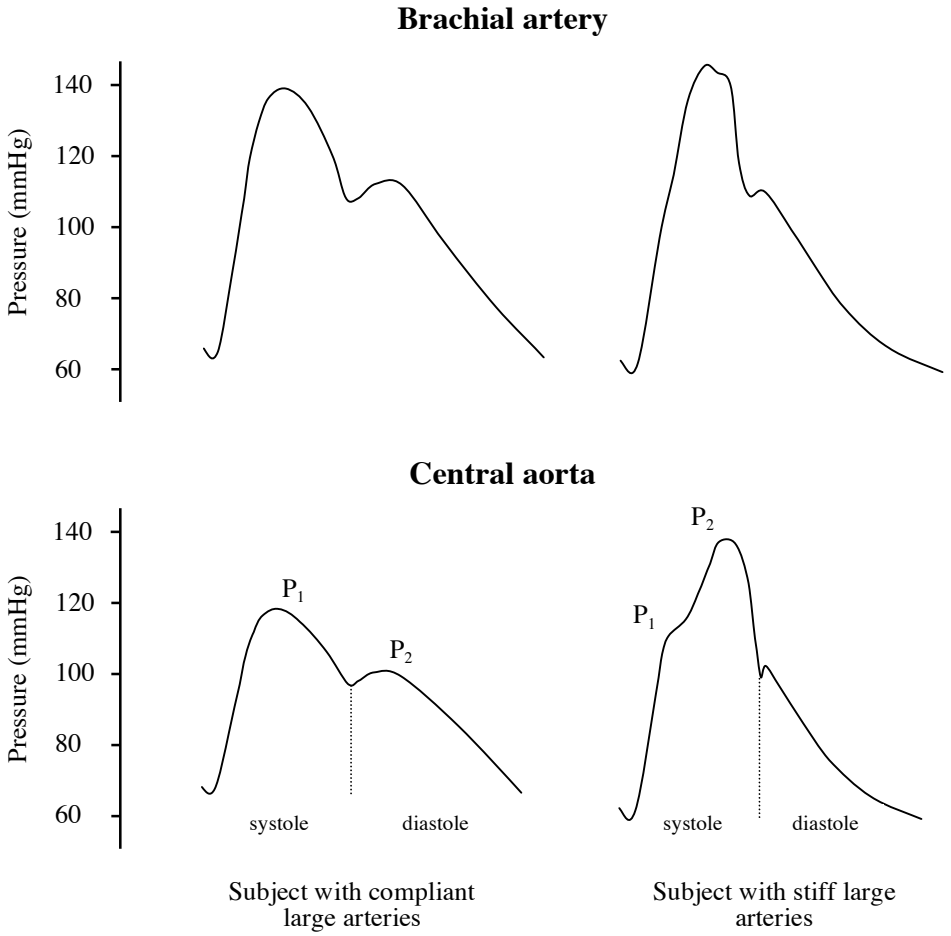


Figure 1.3. Schematic representation of pulse pressure amplification.
Typical pressure tracings from the brachial artery and central aorta. When the large arteries are compliant the arterial waveform is amplified as it travels towards the periphery. As the large arteries stiffen, for example with increasing age, diabetes or other cardiovascular risk factors, this amplification is reduced. The two subjects have similar BP at the brachial artery despite striking differences at the aorta, demonstrating the potential importance of assessing central BP. The effect of peripheral wave reflection on the central aortic waveform is illustrated in the lower tracings. When the large arteries are compliant the initial systolic pressure wave, P_1, travelling from the heart to the periphery, is responsible for peak systolic BP. Reflected pressure waves, P_2, arrive at the central aorta in diastole, augmenting diastolic BP and coronary artery filling. As large arteries stiffen, wave reflection occurs earlier so that systolic BP is augmented and diastolic BP falls.

1.4.2 Measures of arterial stiffness and wave reflection

Many methodologies, both invasive and non-invasive, have been applied to the assessment of arterial elasticity *in vivo*. Non-invasive measures fall into three broad groups: 1) measuring PWV, 2) relating change in diameter (or area) of an artery to distending pressure, and 3) assessing arterial pressure waveforms. Two of these, PWV and pressure waveform analysis, are used in the studies presented in this thesis.

1.4.2.1 PWV

Interest in, and measurement of, the velocity of arterial wave propagation as an index of vascular stiffness and vascular health, dates back to the early part of the last century (Bramwell *et al.*, 1922). PWV increases with stiffness and is defined by the Moens-Korteweg equation,

$$PWV = \sqrt{\frac{Eh}{2\rho R}}$$

where E is Young's modulus of the arterial wall, h is wall thickness, R is arterial radius at the end of diastole and ρ is blood density. There are a number of different ways to measure PWV and these are generally simple to perform. The arterial pulse wave is recorded at a proximal artery, such as the common carotid, as well as at a more distal artery, such as the femoral. The superficial location of the carotid and femoral arteries means that their pulse waveforms are readily measured non-invasively, and between these 2 sites the pulse wave has to travel through most of the aorta, an artery particularly prone to the development of atherosclerosis. The time delay between the arrival of a predefined part of the pulse wave, such as the foot, at these 2 points is obtained either by simultaneous measurement, or by gating to the peak of the R-wave of the ECG. The distance travelled by the pulse wave is measured over the body surface and PWV is then calculated as distance/time (m/s). The measured distance is an estimate of the true distance travelled and depends to some extent on body habitus. Furthermore, the abdominal aorta tends to become more tortuous with age (Wenn *et al.*, 1990), potentially leading to an underestimation of PWV. Increases in distending pressure increase PWV (Bramwell *et al.*, 1922).

Therefore, account should be taken of the level of BP in studies that utilise PWV as a marker of cardiovascular risk or as a measure of the effects on arterial stiffness of interventions that reduce BP. HR has also been reported to influence PWV. In one study an increase in HR of 40 bpm increased PWV by >1 m/s (Lantelme *et al.*, 2002), a difference that may be relevant to the assessment of cardiovascular risk. However, it has been suggested this finding may be an artefact of the methodology used (Hayward *et al.*, 2002a).

Raised PWV occurs with a range of established cardiovascular risk factors (Lehmann *et al.*, 1998), including age (Bramwell *et al.*, 1923; Vaitkevicius *et al.*, 1993), hypercholesterolaemia (Lehmann *et al.*, 1992b), type II diabetes (Lehmann *et al.*, 1992a), and sedentary lifestyle (Vaitkevicius *et al.*, 1993). In hypertension, carotid-femoral PWV (CF-PWV) is an independent predictor of both cardiovascular and all-cause mortality (Laurent *et al.*, 2001). The odds ratio for a 5 m/s increment in PWV was 1.34 for all-cause mortality and 1.51 for cardiovascular mortality. It should be noted that 5 m/s is a relatively large change in PWV. In this study PWV ranged from 9-13 m/s, while recently quoted values of CF-PWV in healthy individuals, with average ages of 24-62 years, ranged from around 6-10 m/s (O'Rourke *et al.*, 2002). Differences between studies regarding the method used to calculate the distance travelled between the carotid and femoral sites probably explains some of the variation in these normal values.

In hypertensives without a history of overt cardiovascular disease PWV predicts both coronary events (Boutouyrie *et al.*, 2002) and stroke (Laurent *et al.*, 2003) independently of classical risk factors. Blacher *et al* (1999a) found that an aortic PWV >13 m/s was a particularly strong predictor of cardiovascular mortality in hypertension. Effective treatment of hypertension appears to retard the rate of increase in PWV. Thus, CF-PWV was found to increase at a faster rate in treated hypertensives than in normotensive controls, but where BP was well controlled PWV progression was attenuated (Benetos *et al.*, 2002). Aortic PWV, assessed using Doppler flow recordings, also independently predicts mortality in patients with end-stage renal failure, a population with a particularly high rate of cardiovascular

disease (Blacher *et al*., 1999b; Pannier *et al*., 2005; Safar *et al*., 2002). The benefit associated with BP control in end-stage renal failure, either by adjustment of dry weight or the use of antihypertensives, was independently related to change in aortic PWV, such that a reduction in PWV of 1 m/s was associated with a relative risk of 0.71 for all-cause mortality (Guerin *et al*., 2001). Aortic PWV has been shown to independently predict cardiovascular outcome in a number of further studies and these are summarised in Table 1.3. Also included in this table are 3 studies that have investigated the value of other measures of arterial stiffness in predicting cardiovascular outcome.

Table 1.3. Summary of prospective studies on the value of measures of arterial stiffness in predicting cardiovascular events.

Study	Details
Aortic PWV	
Blacher *et al* (1999b)	Aortic PWV independently predicted cardiovascular and all-cause mortality in patients with end-stage renal disease
Laurent *et al* (2001)	Aortic PWV independently predicted cardiovascular and all-cause mortality in patients with essential hypertension
Meaume *et al* (2001)	Aortic PWV independently predicted cardiovascular mortality in subjects aged 70 to 100 years
Shoji *et al* (2001)	Aortic PWV independently predicted cardiovascular and all-cause mortality in patients with end-stage renal disease with and without diabetes
Boutouyrie *et al* (2002)	Aortic PWV independently predicted coronary events in patients with essential hypertension
Cruickshank *et al* (2002)	Aortic PWV independently predicted mortality in diabetics and patients undergoing a glucose tolerance test
Laurent *et al* (2003)	Aortic PWV independently predicted fatal stroke in patients with essential hypertension

Continued on next page

Table 1.3
Continued from previous page

Study	Details
Aortic PWV	
Sutton-Tyrrell *et al* (2005)	Aortic PWV independently predicted cardiovascular events and mortality in generally healthy adults
Shokawa *et al* (2005)	Aortic PWV independently predicted cardiovascular mortality in a general population
Willum Hansen *et al* (2006)	Aortic PWV independently predicted a composite of cardiovascular outcomes in a general population
Mattace-Raso *et al* (2006)	Aortic PWV independently predicted CAD and stroke in a general population
Ascending aorta	
Stefanadis *et al* (2000)	Increased stiffness of the ascending aorta independently predicted cardiovascular events in patients undergoing cardiac catheterisation for CAD
Carotid distensibility	
Blacher *et al* (1998)	The carotid artery incremental modulus of elasticity independently predicted all-cause mortality in patients with end-stage renal disease
Barenbrock *et al* (2002)	Carotid artery distensibility independently predicted cardiovascular events in renal transplant recipients

1.4.2.2 Arterial pressure waveform analysis

Peripheral artery pressure waveforms can be acquired non-invasively using applanation tonometry, in which a highly sensitive pressure sensor, commonly contained within a hand-held tonometer probe, is placed on the skin overlying an artery (Figure 1.4). Applying sufficient pressure to slightly compress the artery against firm underlying structures, such as bone, produces a signal that is very similar to the intravascular pressure waveform inside the artery. When measured at the radial artery the waveform is calibrated to conventionally-measured brachial BP.

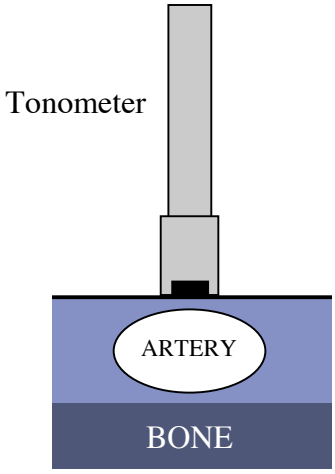


Figure 1.4. Applanation tonometry.

A transfer function can be applied to a waveform from a peripheral artery, most commonly the radial, to derive a corresponding central aortic waveform, from which central BP values and the central aortic augmentation index (aortic AIx; Figure 1.5) can be calculated. This technique has been termed pulse wave analysis (PWA). Aortic AIx is the proportion of central PP that results from arterial wave reflection and is a commonly used measure of arterial stiffness. Whilst the timing of the arrival of the reflected wave at the proximal aorta is largely determined by large artery PWV, Aortic AIx is not simply a surrogate measure of PWV. It is influenced by vasoactive drugs independently of PWV (Kelly *et al.*, 2001), suggesting that it is also determined by the intensity of wave reflection, which, in turn, is determined by the diameter and elasticity of small arteries and arterioles. Aortic AIx increases linearly with MAP, an increase of 5 mmHg being associated with an absolute increase in aortic AIx of around 4% (Wilkinson *et al.*, 2001b), is inversely linearly related to HR, an increase of 10 bpm being associated with an absolute reduction in aortic AIx of around 4% (Gatzka *et al.*, 2001; Wilkinson *et al.*, 2000a), and is also inversely related to body height (Smulyan *et al.*, 1998). PWA is simple and rapid to perform and has potential for use in the clinical as well as research setting.

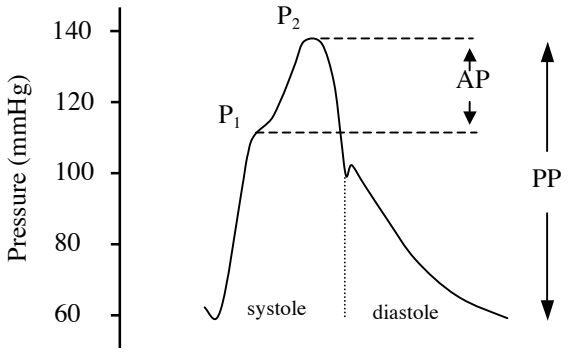


Figure 1.5. Central aortic augmentation index (aortic AIx).
Aortic AIx is calculated as the difference between the second (P_2) and first (P_1) systolic peaks (the augmentation pressure, AP) as a percentage of the pulse pressure (PP). Thus, aortic AIx is negative in healthy young people, but with aging or increasing cardiovascular risk arteries stiffen and aortic AIx becomes increasingly positive. Because aortic AIx can be zero or near zero it should always be presented in absolute terms.

PWA is most commonly performed using the SphygmoCor® apparatus which is made by Atcor Medical (http://www.atcormedical.com). SphygmoCor uses a *generalised* transfer function (GTF) and the validity of this in determining the central aortic waveform has been debated in the literature. Initially, it was shown to be reasonably accurate in determining central aortic waveforms at rest in patients with CAD (Karamanoglu *et al.*, 1993) and, in a study that measured radial waveforms with tonometry and aortic waveforms invasively, individualising the transfer function added little to the accuracy of determining central BP and aortic AIx, even after haemodynamic challenges such as the Valsalva manoeuvre and infusion of GTN (Chen *et al.*, 1997). However, Soderstrom *et al* (2002) found that the GTF tended to underestimate aortic systolic BP by 6-8 mmHg and overestimate aortic diastolic BP by about 4 mmHg, and Hope *et al* (2002) have suggested that gender-specific transfer functions may be more reliable than a GTF. In two studies the correlation coefficient of directly-measured and reconstructed aortic AIx was around 0.66 and, in both, reconstructed aortic AIx tended to underestimate the measured value (Segers *et al.*, 2000; Segers *et al.*, 2001). Furthermore, the correlation was weaker after GTN administration, and inter-individual variation in the relationship of

aortic AIx obtained directly and from the reconstructed waveform was highlighted (Segers *et al.*, 2001). More recently, the GTF has been shown to more accurately estimate central BP if the waveform is calibrated to radial artery BP rather than brachial artery BP, because of PP amplification between these 2 sites (Verbeke *et al.*, 2005). The error in constructing the central waveform from a peripheral one using a GTF is minimised if the peripheral waveform is calibrated to invasively, rather than non-invasively, measured BP (Hope *et al.*, 2004; Wilkinson *et al.*, 2004a). While this provides support for the validity of the GTF itself, it is only non-invasive BP that is available in clinical practice.

It may not even be necessary to determine the central aortic pressure waveform at all. Peripheral artery waveforms, such as the radial, are also characterised by an inflection point that relates to pressure wave reflection (see Figure 1.3). Hope *et al* (2004) have recently demonstrated that many characteristics of the radial artery waveform, including AIx, are similarly or more closely correlated with the equivalent characteristics of the aortic waveform than are values derived using a GTF. Millasseau *et al* (2003) also found a high degree of correlation between derived aortic AIx and radial AIx (RAIx) and also found that GTN and norepinephrine produced parallel changes in each of these. Thus, RAIx might provide as much information on cardiovascular prognosis or the haemodynamic effects of interventions as does aortic AIx derived using a GTF, with the advantage that simpler and less expensive equipment would be required.

Aortic AIx increases with age (Cameron *et al.*, 1998; Hayward *et al.*, 1997; Kelly *et al.*, 1989; McEniery *et al.*, 2005) and, compared to matched controls, is also higher in patients with type I diabetes (Wilkinson *et al.*, 2000b) and hypercholesterolaemia (Wilkinson *et al.*, 2002b), despite similar peripheral BP. Compared to PWV, there are relatively few data on the value of aortic AIx in predicting cardiovascular outcome. In a cross-sectional analysis non-invasively determined aortic AIx was an independent predictor of the presence of premature CAD at angiography (Weber *et al.*, 2004). In a prospective study of subjects with established CAD, invasively measured aortic AIx independently predicted a combined endpoint of acute coronary

events, stroke or death (Chirinos *et al.*, 2005). Non-invasively determined aortic AIx, corrected for HR, has also been demonstrated to predict cardiovascular events in subjects undergoing percutaneous coronary intervention (Weber *et al.*, 2005). AIx can also be directly measured, using applanation tonometry, relatively close to the central aorta, at the carotid artery. High carotid AIx is an independent predictor both of cardiac ischemic threshold during exercise in patients with CAD (Kingwell *et al.*, 2002) and of all-cause and cardiovascular mortality in patients with ESRF (London *et al.*, 2001). Of particular note from the latter study, carotid AIx predicted mortality even in patients considered to have a normal PWV (<11 m/s), highlighting the importance of assessing arterial wave reflection, rather than just arterial stiffness.

1.4.3 The effects of cardiovascular drugs on arterial stiffness

As arterial stiffness has become established as a cardiovascular risk factor in its own right it has also emerged as a potential target for intervention. Indeed, it is conceivable that reduction of arterial stiffness may become a major primary goal of treatment in particular patients at risk of cardiovascular disease. However, for this situation to arise increased arterial stiffness will not only have to be established as an important independent cardiovascular risk factor, but also reducing arterial stiffness will need to be shown to reduce risk, independent of other effects of treatment. Establishing whether currently used cardiovascular drugs exert their clinical benefit through improvements in arterial elasticity may lead to more appropriate targeting of these treatments. However, many of these agents also lower BP and this effect must be differentiated from any intrinsic effects, either structural or functional, on arterial wall stiffness.

The effects of organic nitrates, especially GTN, on the central aortic waveform have been well characterised. GTN can reduce aortic AIx and central systolic BP and PP at doses that have little or no effect on peripheral arterial resistance, peripheral BP, or aortic PWV (Fitchett *et al.*, 1988; Kelly *et al.*, 1990; Yaginuma *et al.*, 1986). This effect is probably the result of reduced peripheral wave reflection due to dilatation of muscular conduit arteries (Bank *et al.*, 1999; Jiang *et al.*, 2002).

β-blockers appear to reduce large artery stiffness, but their effects on arterial wave reflection and the central arterial waveform are less favourable. After six months treatment in hypertensives, atenolol was as effective as the ACE inhibitor cilazapril in increasing aortic elasticity (Savolainen *et al.*, 1996). However, atenolol was less effective than either fosinopril, after eight weeks treatment (Chen *et al.*, 1995), or perindopril, after one month of treatment (Pannier *et al.*, 2001), in lowering directly-measured carotid AIx. In a further study, treatment for 1 year with atenolol or perindopril/indapamide similarly reduced aortic PWV but only the ACE inhibitor/diuretic combination reduced carotid AIx (Asmar *et al.*, 2001). As a confounding variable, HR reduction with β-blockade largely accounts for these differences, which may, at least in part, explain why β-blockers appear to be less effective than the other major antihypertensive classes in the prevention of cardiovascular events in the treatment of hypertension (see section 1.3.1).

CCBs, ACE inhibitors and HMG-CoA reductase inhibitors (statins) also appear to have beneficial effects on arterial elasticity independent of effects on distending pressure, whereas there are conflicting data on the effects of diuretics (for full discussion see Oliver *et al.*, 2003).

1.4.4 Effect of PDE5 inhibition on arterial stiffness and wave reflection

PDE5 inhibitors might be expected to have organic nitrate-like properties on arterial wave reflection and aortic AIx, given that both increase vascular smooth muscle cGMP concentrations. In the study by Mahmud *et al* (2001) in which sildenafil 50 mg reduced BP in treated hypertensives (see section 1.3.2.2) there was also a reduction in aortic AIx. However, it is likely that the reduction in systemic BP that was observed fully accounted for the effects on aortic AIx. In hypertensive cardiac transplant patients in whom normal medicines were not withdrawn prior to study, sildenafil reduced aortic AIx by a mean maximum 7.5% (Schofield *et al.*, 2003). MAP was reduced by 9 mmHg, which, once again, is probably sufficient to account

for the effect on aortic AIx. There was no placebo comparator in this study. In men with CAD (mean age 69 years) who had been withdrawn from vasoactive drugs for 24 hours, in comparison to placebo sildenafil 50 mg acutely reduced aortic PWV by ~0.4 m/s and aortic AIx by 2-3% (Vlachopoulos *et al.*, 2003). The effect on PWV was statistically independent of the effects on systemic BP (reduction of ~10/9 mmHg compared to placebo) leading the authors to conclude that sildenafil reduced stiffness through an active effect on the aortic wall. The same authors also found that sildenafil 50 mg acutely reduced aortic PWV in patients with heart failure, again statistically independently of the reduction in systemic BP that was also observed (Hirata *et al.*, 2005). There was also a reduction in aortic AIx. The patients in this study were withdrawn from vasoactive medicines for only 12 hours. Therefore, the effects of sildenafil on arterial stiffness and wave reflection have only been investigated in single dose studies in which interactions with other drugs may have contributed to the observed effects. There have been no studies on the effects of tadalafil or vardenafil on arterial stiffness or wave reflection.

1.5 ENDOTHELIAL DYSFUNCTION

1.5.1 Measuring endothelial function

Impairment of the physiological function of the endothelium, commonly referred to as endothelial dysfunction, has become recognised as a key step in the development of atherosclerosis, the progression of atherosclerotic plaque and also in atherosclerotic complications (Bonetti *et al.*, 2003). A major characteristic of endothelial dysfunction is a reduction in the production and release of NO by the endothelium (Kuvin *et al.*, 2003; Vogel, 2003). This can be assessed *in vivo* by methodologies that indirectly quantify the amount of NO production as the degree of vasodilatation that occurs when endothelial NO is stimulated.

Local infusion of a substance that stimulates endothelial NO production and measurement of the vasodilatation that results is the gold standard in endothelium-dependent vasomotor function assessment. Suitable substances include ACh and

substance P and these can be infused either into the forearm or coronary circulations (Ludmer *et al.*, 1986; Newby *et al.*, 1997; Newby *et al.*, 2001). The degree of vasodilatation that occurs is quantified as the change in flow to the tissue, using venous occlusion plethysmography in the forearm (Wilkinson *et al.*, 2001c) and either quantitative angiography or Doppler ultrasound in the coronary circulation (Ludmer *et al.*, 1986; Newby *et al.*, 2001). An impaired response to the "endothelium-dependent" substance could be a result of either impaired production and release of NO or, alternatively, impaired sensitivity of the adjacent vascular smooth muscle to NO. Therefore, the response to a substance that relaxes vascular smooth muscle directly is also measured as an "endothelium-independent" control. NO-donor drugs, such as GTN or sodium nitroprusside, are commonly used for this purpose. The major limitation of these methodologies is that they are invasive, which restricts their use to the research setting and, even then, to relatively small studies. However, non-invasive methodologies exist for the assessment of endothelium-dependent vasomotor function and 2 of these, brachial artery flow-mediated dilatation (FMD) and the response of arterial wave reflection to $ß_2$-adrenoreceptor stimulation, are used in the studies described in this thesis.

1.5.1.1 FMD

FMD provides a measure of conduit artery endothelium-dependent vasomotor function (Corretti *et al.*, 2002; Deanfield *et al.*, 2005). It is most commonly performed at the brachial artery but can also be performed at the radial, femoral and posterior tibial arteries. The stimulus to NO release is increased shear stress at the wall of the artery under study and the degree of vasodilatation is measured by imaging the artery directly, usually with ultrasound. For the brachial artery, the arm is outstretched perpendicular to the body and a longitudinal image of the artery is obtained just above the elbow. The ultrasound probe is then held in place with a clamp for the remainder of the study. After recording the artery for a period at baseline, a BP cuff placed around the upper forearm, i.e. distal to the site of measurement, is inflated to above systolic BP to occlude blood flow into the forearm. The cuff remains inflated for 5 minutes and during this time the forearm vascular bed vasodilates considerably. As a result, when the cuff pressure is released there is a

high rate of blood flow into the forearm (reactive hyperaemia). The increased flow increases shear stress at the brachial artery wall which stimulates NOS to produce NO that, in turn, dilates the artery. Thus, the extent of dilatation in response to reactive hyperaemia provides a measure of endothelium-dependent vasomotor function. After a short rest period to allow the artery to recover, GTN can be given (sublingually) as an endothelium-independent control. The flow stimulus is quantified as the Doppler flow signal in the artery under study.

L-NMMA has no effect on the extent of reactive hyperaemia but abolishes FMD, indicating the NO-dependence of the response (Doshi *et al.*, 2001; Joannides *et al.*, 1995). A number of studies have found that brachial artery FMD correlates with responses to ACh in the coronary circulation (Anderson *et al.*, 1995; Teragawa *et al.*, 2005). However, in a large population study of elderly subjects there was no correlation between brachial artery FMD and responses to ACh in the forearm (Lind *et al.*, 2005). Depressed FMD has been demonstrated both in subjects with atherosclerosis and in subjects with cardiovascular risk factors (Brunner *et al.*, 2005; Celermajer *et al.*, 1996; Celermajer *et al.*, 1992; Celermajer *et al.*, 1994). In most of the studies demonstrating impaired FMD in particular populations GTN responses have generally been reported to be preserved. However, on closer inspection of many of these studies there is a tendency, albeit not statistically significant, for GTN responses to actually be slightly reduced. Indeed, in a large study (n=800) of asymptomatic subjects, reduced FMD was independently associated with reduced response to GTN, indicating a degree of vascular smooth muscle dysfunction as well as reduced endothelium-dependent vasomotor function (Adams *et al.*, 1998). Although non-invasive, FMD is technically challenging and operators need to be properly trained. For this reason, its use is likely to be confined to research.

1.5.1.2 β_2-adrenoreceptor agonist-induced reduction in arterial wave reflection

The β_2-adrenoreceptor agonist salbutamol is a vasodilator in the peripheral circulation and this response is dependent on endothelial NO synthesis (Dawes *et al.*, 1997). When given systemically via inhalation salbutamol reduces peripheral artery wave reflection and this response is also NO-dependent (Chowienczyk *et al.*, 1999;

Hayward *et al*., 2002b; Wilkinson *et al*., 2002a). Therefore, measurement of the change in arterial wave reflection with inhaled $ß_2$-adrenoreceptor agonists has potential as a simple, non-invasive methodology for assessing endothelium-dependent vasomotor function that could be used in large scale population studies and possibly even in clinical practice. This methodology was first described by Chowienczyk *et al* (1999) in a study in which salbutamol was shown to reduce the height of the inflection point on the digital volume pulse obtained by photoplethysmography. Subsequently, salbutamol was shown to reduce aortic AIx (Wilkinson *et al*., 2002a) and RAIx (Hayward *et al*., 2002b). As with FMD, GTN can be given sublingually as an endothelium-independent control.

Blunted responses to inhaled salbutamol have been demonstrated in subjects with type II diabetes (Chowienczyk *et al*., 1999), hypercholesterolaemia (Wilkinson *et al*., 2002a), and CAD (Hayward *et al*., 2002b). GTN responses were not significantly impaired in these subjects, although in CAD patients there was a trend to a blunted response of RAIx to GTN which was almost statistically significant ($P = 0.07$). This study compared 12 CAD patients with 10 controls and it is possible that if the sample had been larger a statistically significant difference in GTN responses would have been observed.

Several further studies, all of which measured the effects on aortic AIx, have been reported. Wykretowicz *et al* (2005) found that the response to salbutamol was blunted in CAD patients with newly diagnosed diabetes or impaired glucose tolerance compared to CAD patients with normal glucose tolerance. Salbutamol responses were also blunted in obese pre-menopausal women and improved after weight reduction (Park *et al*., 2005; Suh *et al*., 2005). Responses to both salbutamol and GTN were found to be blunted in chronic ambulatory peritoneal dialysis patients compared to haemodialysis patients (Covic *et al*., 2004), and responses to both agents also improved in haemodialysis patients following successful renal transplant (Covic *et al*., 2003).

1.5.2 Endothelium-dependent vasomotor function as a predictor of cardiovascular events

Over the past few years a number of studies, although not all, have reported that the assessment of endothelium-dependent vasomotor function can predict the occurrence of cardiovascular events independently of established risk factors (these studies are summarised in Table 1.4). Not only do these data suggest that assessment of endothelial function may be of use in the stratification of cardiovascular risk in clinical practice, but they also lend support to the contention that endothelial dysfunction is important in the pathogenesis of atherosclerotic cardiovascular disease.

Table 1.4. Summary of prospective studies on the value of endothelium-dependent vasomotor function in predicting cardiovascular events.

Study	Details
Coronary circulation	
Schachinger *et al* (2000)	In patients undergoing diagnostic coronary angiography or coronary angioplasty impaired response to both ACh and GTN independently predicted cardiovascular events
Suwaidi *et al* (2000)	In patients with mild CAD cardiac events only occurred in those with severely impaired ACh responses
Halcox *et al* (2002b)	Impaired epicardial and microvascular responses to ACh independently predicted acute cardiovascular events in patients with and without CAD
Targonski *et al* (2003)	Impaired response to ACh independently predicted cerebrovascular events in patients with CAD
von Mering *et al* (2004)	In women undergoing diagnostic angiography impaired response to ACh independently predicted cardiovascular events
Bugiardini *et al* (2004)	In women with chest pain and initially normal coronary angiograms impaired response to ACh predicted the development of angiographically evident CAD
Forearm circulation	
Perticone *et al* (2001)	Impaired response to ACh independently predicted cardiovascular events in patients with never-treated hypertension
Heitzer *et al* (2001)	Impaired response to ACh independently predicted cardiovascular events in patients with CAD
Fichtlscherer *et al* (2004)	Impaired response to ACh independently predicted cardiovascular events in patients with recent acute coronary syndrome; improvement in ACh response predicted further event-free survival

Continued on next page

Table 1.4
Continued from previous page.

Study	Details
Brachial artery FMD	
Neunteufl *et al* (2000)	Impaired FMD independently predicted cardiovascular events in patients with chest pain
Gokce *et al* (2002b)	Reduced FMD predicted cardiovascular events within 30 days of vascular surgery
Modena *et al* (2002)	In hypertensives cardiovascular events were much more frequent in those in whom there was no improvement in FMD with treatment compared to those in whom there was an improvement
Brevetti *et al* (2003)	In patients with peripheral artery disease, reduced FMD was an independent predictor of cardiovascular events and added to the prognostic value of ankle-brachial pressure index
Chan *et al* (2003)	In patients with significant carotid artery atherosclerotic plaque burden cardiovascular events were higher in those with impaired FMD
Gokce *et al* (2003)	Reduced FMD predicted cardiovascular events in patients with peripheral vascular disease
Fathi *et al* (2004)	In patients with or at increased risk of cardiovascular disease, reduced FMD correlated with but was **not** an independent predictor of cardiovascular events
Rossi *et al* (2004)	In apparently healthy postmenopausal women reduced FMD predicted future development of hypertension
Frick *et al* (2005)	FMD did **not** predict cardiovascular events in subjects undergoing coronary angiography
Katz *et al* (2005)	Reduced FMD independently predicted mortality in subjects with heart failure
Meyer *et al* (2005)	In patients with heart failure reduced FMD independently predicted worsening heart failure or death
Patti *et al* (2005)	Reduced FMD independently predicted in-stent restenosis in patients undergoing percutaneous coronary intervention

1.5.3 A brief note on the wider assessment of endothelial function

Although there have been countless clinical studies in which endothelium-dependent vasomotor function has been measured, it is important to remember that the endothelium has a range of functions in addition to the control of vasomotor tone and that measuring these may provide additional and novel insights into endothelial function and dysfunction. For example, one aspect of endothelial function in which there is growing clinical research interest is the stimulated release of tissue plasminogen activator (t-PA) from the endothelium (Oliver *et al.*, 2005b). Reports of preserved endothelium-dependent vasodilatation in smokers (Pretorius *et al.*, 2002) and in patients with hypertension (Hrafnkelsdóttir *et al.*, 2004; Hrafnkelsdóttir *et al.*, 1998) despite reduced acute t-PA release suggest that, in some circumstances, reduced t-PA release may be a more sensitive marker of endothelial dysfunction. On the other hand hypercholesterolaemia is associated with impaired endothelium-dependent vasodilatation, but no reduction in t-PA release (Newby *et al.*, 2002) while vascular inflammation impairs endothelium-dependent vasodilatation but augments t-PA release (Chia *et al.*, 2003). These findings highlight the complexity of vascular biology, and that it is perhaps naive to expect endothelial dysfunction to be expressed by a uniform phenotype irrespective of the insult. Indeed, the dominant use of term endothelial dysfunction to refer to endothelium- and NO-dependent vasodilatation seems unnecessarily restrictive and inaccurate.

1.5.4 The endothelium as a regulator of arterial stiffness

Endothelial dysfunction and increased arterial stiffness often co-exist in patients at increased risk of atherosclerotic disease, suggesting that the endothelium might regulate arterial stiffness. Indeed, studies showing that NO locally regulates the stiffness of the iliac arties of both sheep (Wilkinson *et al.*, 2002c) and humans (Schmitt *et al.*, 2005), strongly support this contention. The potential implication of this is that interventions that improve endothelial function, particularly the local bioavailability of NO, may, as a consequence, also reduce arterial stiffness.

1.5.5 Effects of PDE5 inhibition on endothelial function

Given that the methodologies used to assess endothelium-dependent vasomotor function are essentially surrogate measures of endothelial NO release, PDE5 inhibitors might be expected to improve these responses. Although this would not constitute an improvement in endothelial function as such, because release of NO and other elements of endothelial function would not be expected to change, it might nevertheless be of clinical benefit, given the general vasculoprotective actions of NO.

The effects of sildenafil on endothelium-dependent vasomotor function have been assessed by a number of investigators. Halcox *et al* (2002a) found that it augmented the response to ACh in the coronary circulation but had no effect on the response to verapamil, an endothelium-independent control vasodilator. In this study the effect on brachial artery FMD was also investigated. Sildenafil did not affect peak FMD but did prolong the post-reactive hyperaemia dilatation. Other studies have found an improvement in peripheral endothelial vasomotor function with sildenafil in heart failure (Guazzi *et al*., 2004a; Guazzi *et al*., 2004b; Hryniewicz *et al*., 2005) and in hypertensive cardiac transplant patients (Schofield *et al*., 2003), whereas in healthy subjects it does not appear to affect endothelial responses (Dishy *et al*., 2001; Guazzi *et al*., 2004a; Guazzi *et al*., 2004b; Halcox *et al*., 2002a). In patients with CAD, systemic sildenafil did not affect either vasodilatation in response to ACh or t-PA release in response to substance P (Robinson *et al*., 2006). There are conflicting data on the effect of sildenafil on endothelial vasomotor function in smokers, with an improvement shown in two studies (Kimura *et al*., 2003; Vlachopoulos *et al*., 2004) but no effect in a further study (Dishy *et al*., 2004). Chronic administration of tadalafil, a longer-acting PDE5 inhibitor, improved endothelial vasomotor function in men at increased cardiovascular risk (Rosano *et al*., 2005).

1.6 OTHER EFFECTS OF PDE5 INHIBITION ON THE CARDIOVASCULAR SYSTEM

In addition to the effects on BP, arterial stiffness and wave reflection and endothelium-dependent vasomotor function, the effects of PDE5 inhibition on other aspects of cardiovascular function have also been investigated in clinical studies.

Sildenafil is a vasodilator in the pulmonary vascular bed as well as in the systemic circulation. In subjects with normal pulmonary artery BP the effects are mild (Herrmann *et al.*, 2000; Jackson *et al.*, 2005) but in patients with pulmonary arterial hypertension (PAH) substantial reductions in pulmonary artery BP occur (Michelakis *et al.*, 2002). This physiological effect translates into improved functional capacity when sildenafil is taken regularly for PAH (Galiè *et al.*, 2005; Michelakis *et al.*, 2003) and it has recently been licensed in both Europe and the USA for this indication. PDE5 inhibitors may also be an effective treatment for Raynaud's phenomenon. A recent study found that regular sildenafil reduced the frequency and duration of acute attacks (Fries *et al.*, 2005).

Studies in animals have suggested that sildenafil can attenuate the myocardial damage associated with ischaemia-reperfusion, an effect that is dependent upon opening of mitochondrial ATP-sensitive K^+ channels (Bremer *et al.*, 2005). Sildenafil has also recently been shown to reduce ischaemia-reperfusion-induced endothelial dysfunction in man (Gori *et al.*, 2005). Although further work is required, minimisation of ischaemia-reperfusion injury could be a further use for these drugs, for example in coronary artery bypass surgery (Fung *et al.*, 2005). Sildenafil has also recently been shown to blunt the effects of ß-adrenoreceptor stimulation on the heart, which could translate into clinical benefit in conditions such as hypertension, left ventricular hypertrophy (LVH) and heart failure (Borlaug *et al.*, 2005). Sildenafil also reduces ADP-stimulated platelet activation (Berkels *et al.*, 2001; Halcox *et al.*, 2002a), although whether this translates into protection against arterial thrombosis clinically is not known.

Asymmetric dimethylarginine (ADMA) is a naturally occurring inhibitor of NO synthase. Circulating ADMA concentration is correlated with endothelial dysfunction and cardiovascular risk, although causal relationships have not yet been established (Vallance *et al.,* 2004). There has been some recent interest in the effect of PDE5 inhibition on ADMA. In a rat model of hypercholesterolaemia-induced PED, chronic administration of a novel PDE5 inhibitor reduced plasma ADMA concentration (Kang *et al.,* 2005). However, in men with PED there was no change in plasma ADMA concentration after 70 days of sildenafil therapy (Wierzbicki *et al*,, 2006).

1.7 SAFETY OF PDE5 INHIBITORS

In the late 1990s case reports of myocardial infarction (MI) associated with sildenafil use prompted concern over its cardiovascular safety (Arora *et al*., 1999; Feenstra *et al*., 1998). However, it was recognised that patients with PED often have increased cardiovascular risk (Grover *et al*., 2006) and also that sexual intercourse itself is associated with a small increased risk of MI (Muller *et al*., 1996). Demonstration that sildenafil had minimal or no effect on various cardiac haemodynamic parameters in patients with severe CAD provided some reassurance on the drug's safety (Herrmann *et al*., 2000). The concern that sildenafil might cause harm in the setting of the physical exertion of sexual activity was also somewhat allayed by a study in which sildenafil did not affect exercise duration or exercise-induced cardiac ischaemia in patients with stable CAD (Arruda-Olson *et al*., 2002). There are similar data for tadalafil (Patterson *et al*., 2005) and vardenafil (Thadani *et al*., 2002). Pooled data from clinical trials (Jackson *et al*., 2004; Mittleman *et al*., 2005) as well as prescription event monitoring data (Boshier *et al*., 2004; Shakir *et al*., 2001) have found no increased risk of acute MI with sildenafil or tadalafil use. Over recent years there have been a number of case reports of non-arteritic anterior ischaemic optic neuropathy, a cause of sudden onset, untreatable and irreversible visual loss, occurring after PDE5 inhibitor use (Pomeranz, 2006). It is not possible to determine if PDE5 inhibitors are a cause of this eye condition, but the Food and Drug Administration in the USA has advised patients to stop taking them immediately if

they experience sudden deterioration in vision and also to tell their doctors if they have ever had severe loss of vision (FDA, 2005).

1.8 AIMS

The overall aim of the work contained within this thesis was to further characterise the systemic vascular effects of PDE5 inhibition.

Initially, to further develop the methodologies used in the non-invasive assessment of endothelium-dependent vasomotor function, studies were performed to characterise, in healthy subjects (chapter 3):

1. The dose-response relationships of sublingual GTN to change in brachial artery diameter and change in arterial wave reflection, with a view to informing the most appropriate dose of sublingual GTN that should be used as a control in non-invasive endothelial function studies.
2. The effects of age and gender on the extent to which inhaled salbutamol reduces arterial wave reflection.
3. The relationship between brachial artery FMD and the effect of inhaled salbutamol on arterial wave reflection.

The following specific hypotheses were then investigated:

1. Endothelium-dependent vasomotor function measured as the reduction in arterial wave reflection with inhaled salbutamol is impaired by smoking (chapter 4).
2. In smokers sildenafil improves endothelium-dependent vasomotor function measured as the reduction in arterial wave reflection with inhaled salbutamol (chapter 4).
3. The interaction of the hypotensive effects of sildenafil (100 mg) and sublingual GTN (400 μg spray) is limited to within 8 hours of giving sildenafil in a group of typical male patients with stable angina (chapter 5).
4. Compared to placebo, regular administration of sildenafil reduces BP, arterial stiffness and arterial wave reflection, and improves endothelium-dependent vasomotor function in otherwise untreated hypertensive patients (chapter 6).
5. Combined sildenafil and ISMN reduces BP in patients with treatment-resistant hypertension when given in addition to their usual antihypertensives (chapter 7).

CHAPTER 2

METHODS

2.1 GENERAL REQUIREMENTS

2.1.1 Subjects

For all studies subjects were asked to refrain from alcohol for at least 24 hours and tea, coffee, other caffeinated drinks and food for at least 12 hours before each study visit. With the exception of the study investigating the effect of sildenafil on endothelial function in smokers (chapter 4), smoking was also not permitted for 12 hours before each study visit.

2.1.2 Study environment

Studies were conducted in a quiet room kept between 22°C and 24°C.

2.1.3 Research governance and ethics

Studies were approved either by a Local Research Ethics Committee or a Multi-centre Research Ethics Committee and were performed in accordance with the Declaration of Helsinki of the World Medical Association. Written informed consent was obtained from all subjects.

2.2 METHODOLOGIES

The methodologies used in each of the studies are described here. Specific protocols for each of the studies are described in the relevant chapters.

2.2.1 BP and HR

Systolic BP, diastolic BP and HR were recorded using a validated oscillometric sphygmomanometer, the Omron HEM-705CP (Omron Healthcare (UK) Ltd, Milton Keynes; O'Brien *et al.*, 1996), except in the study investigating the time course of the interaction on BP between sildenafil and sublingual GTN (chapter 5) in which a Dinamap Pro 100 BP monitor (GE Healthcare, Chalfont St Giles; Chang *et al.*, 2003) was used. Where standing BP and HR were measured they were recorded after 2 minutes standing. PP was calculated as the difference between systolic BP and

diastolic BP. True MAP was derived from integration of the radial artery pulse waveform obtained with PWA recordings (see section 2.2.3).

2.2.2 Ambulatory BP

Ambulatory BP was recorded at the brachial artery using a validated Spacelabs 90217 ambulatory BP monitor (Spacelabs Medical Inc, Issaquah, WA, USA; Baumgart *et al.*, 1998). Measurements were taken every 30 minutes for 24 hours. Subjects recorded the time that they went to sleep and the time that they awoke so that daytime average BP and night time average BP, as well as 24-hour average BP, could be calculated.

2.2.3 PWA

Peripheral pressure waveforms were recorded from the radial artery at the wrist by applanation tonometry using a high fidelity micromanometer (SPC-301; Millar Instruments, Texas, USA) and the SphygmoCor apparatus (AtCor Medical Pty Ltd West Ryde, Australia) running SphygmoCor software version 6.3. (Figure 2.1).

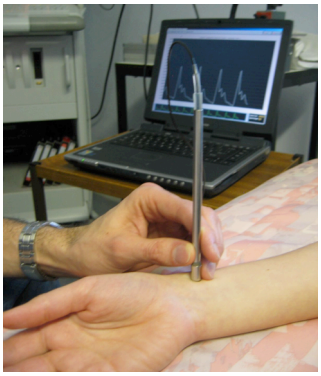


Figure 2.1. PWA measurement.
The tonometer is applied lightly over the radial artery at the wrist. The radial artery waveform can be seen on the computer's screen.

Averaged peripheral and corresponding central (ascending aortic) pressure waveforms were generated from the last 10 seconds of each radial artery recording. RAIx was calculated from the averaged peripheral waveform as:

$$RAIx = 100 \times \frac{(\text{sec}\,ond\ systolic\ peak - diastolic\ BP)}{(first\ systolic\ peak - diastolic\ BP)}$$

Central systolic BP, central diastolic BP, central PP, aortic AIx, and aortic AIx@75 were calculated from the averaged central waveform. Aortic AIx was calculated as explained in Figure 1.5 (page 24). Aortic AIx@75 is aortic AIx adjusted to a standard HR of 75 bpm. To derive aortic AIx the SphygmoCor software adjusts aortic AIx at an inverse rate of 4.8% for each 10 bpm increment in HR.

2.2.4 CF-PWV

The SphygmoCor apparatus was also used to measure CF-PWV. During continuous ECG monitoring pulse wave recordings were made first at the carotid artery and then at the femoral artery. The software identified the foot of the pulse wave, as the beginning of the sharp systolic upstroke, and the wave transit time was calculated using the R wave of the simultaneously recorded ECG as a reference frame. Surface distance between the two recording sites was measured and CF-PWV was calculated as:

$$CF-PWV = \frac{Distance\ travelled}{Wave\ transit\ time}.$$

2.2.5 Flow-mediated dilatation

The experimental set-up for FMD is shown in Figure 2.2. The right arm was extended approximately perpendicular to the body across a platform with the palm of the hand facing upwards. The brachial artery was continuously scanned longitudinally with B-mode ultrasound (Acuson XP 128, Siemens plc, Bracknell, UK) 5 cm above the elbow using a linear array transducer with an imaging frequency of 11 MHz. A stereotactic clamp was used to hold the probe in the same position throughout the study. Every 3 seconds end-diastolic (ECG R-wave triggered) frames were acquired on a computer equipped with a DT-3152 progressive scan frame grabber (Data Translation Ltd, Basingstoke, UK) and image acquisition software (CVI Acquisition version 1.5, Information Integrity Inc, USA). Baseline diameter was recorded for 1 minute, after which a paediatric-sized cuff placed just below the elbow was inflated to 220 mmHg (or 50 mmHg above systolic BP if systolic BP was

>170 mmHg) for 5 minutes. Following deflation of the cuff the artery was scanned for a further 5 minutes. For the assessment of the response to GTN, the brachial artery was scanned at baseline for 1 minute. Sublingual GTN was then administered and the artery was scanned for a further 5 minutes. Ultrasound recordings were stored on videotape. Brachial artery diameter was calculated off-line from the stored images using semi-automated wall-tracking software (Brachial Analyzer, Medical Imaging Applications, Iowa, USA).

Using pulsed wave Doppler at 70° to the vessel, blood flow was recorded continuously with the range gate (1.5 mm) positioned midway between near and far artery walls. The velocity time integral (VTI), the area under the velocity/time curve, was measured off-line at baseline and every 3 seconds for 18 seconds after cuff deflation. Blood flow was calculated as $VTI \times HR \times \pi \times (diameter/2)^2$. Reactive hyperaemia was quantified as the peak change in flow, expressed as percentage change from baseline.

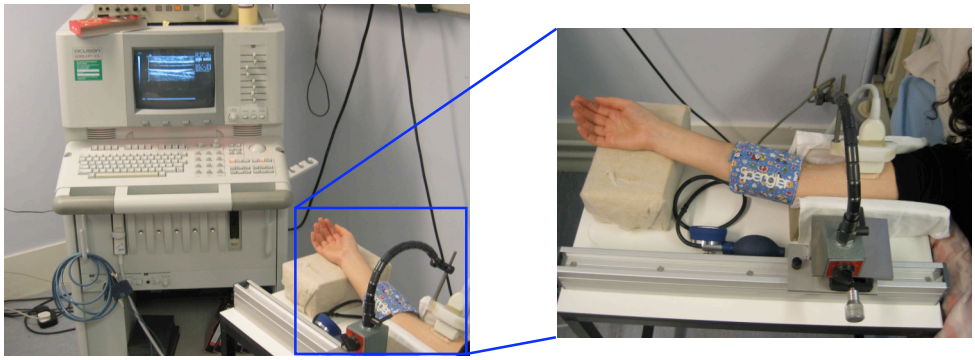


Figure 2.2. FMD set-up.
The ultrasound probe is fixed in place by a stereotactic clamp. An image of the brachial artery can be seen on the ultrasound machine's screen.

2.2.6 Assessment of LVH

ECG criteria for LVH were gender-specific (Alfakih *et al.*, 2004). For males the Cornell criteria were used (LVH present if the sum of the R-wave in lead aVL and

the S-wave in lead V3 is greater than 25 mm). For females the Sokolow-Lyon product was used (LVH present if the QRS duration multiplied by the sum of the S-wave in lead V1 and the R-wave in lead V5 or V6 is greater than 2970).

2.2.7 Screening blood samples

With the exception of the study investigating the dose-response relationship between GTN and brachial artery diameter/arterial wave reflection (section 3.2), blood samples were taken from subjects for full blood count, serum urea, creatinine, sodium, potassium and lipid profile [total, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol, and triglyceride], and plasma glucose. These samples were analysed by the Western General Hospital (WGH) Haematology and Biochemistry laboratories.

2.3 DRUGS

2.3.1 Salbutamol and salbutamol placebo

Salbutamol 400 μg or placebo training inhaler (Allen & Hanburys, Stockley Park, UK) were inhaled through a spacer device (Volumatic® Allen & Hanburys, Stockley Park, UK). Two puffs (200 μg of salbutamol) were initially placed into the spacer and were inhaled via a full inspiration. Subjects then held their breath for 10 seconds, exhaled, and once again fully inhaled through the spacer and held their breath for a further 10 seconds. This procedure was then repeated with a further 2 puffs. The casing for the salbutamol inhaler was grey and that for the training inhaler white. Where double blinding was required, this was achieved by salbutamol and placebo being administered by personnel independent of the study and subjects keeping their eyes closed during inhaler administration.

2.3.2 GTN

GTN was administered sublingually in a number of studies. The preparations used varied between these studies and, therefore, are described in the methods sections of the relevant chapters.

2.3.3 Sildenafil

Sildenafil 50 mg and 100 mg, and matched placebo tablets were obtained from Pfizer Ltd, Sandwich, UK.

2.3.4 ISMN

ISMN 10 mg was from APS Ltd, Eastbourne, UK. Placebo tablets for ISMN were manufactured by Tayside Pharmaceuticals, Dundee, UK, and were of different appearance to the ISMN tablets. Double blinding was achieved by ISMN and ISMN placebo being administered by personnel independent of the study and subjects keeping their eyes closed during administration.

2.4 DATA ANALYSIS

Methods used to analyse data differed between studies and, therefore, are described in the relevant chapters. Microsoft Excel 2004 for Macintosh and GraphPad Prism 4 for Macintosh were used for statistical analyses. A *P* value of <0.05 was considered significant. Where changes in variables, for example from baseline, are presented these are absolute rather than relative, unless stated otherwise.

2.4.1 Repeatability analysis

Ninety-five percent limits of agreement were used to assess repeatability of 2 measurements (Bland *et al.*, 2003). Using this method, only 5% of pairs of measurements on the same subject would fall outside of the 95% limit of agreement. Means and SDs of the differences between 2 measurements were calculated and the 95% limit of agreement was defined as the mean difference ±1.96 SDs. Standard errors of the limits were calculated as $\sqrt{3SD^2/n}$ and 95% confidence intervals (CIs) for the limits of agreement were calculated as ±1.96 standard errors. Repeatability data are presented graphically as plots of the difference between 2 measurements against the mean of the measurements (Bland-Altman plots).

CHAPTER 3

METHODOLOGY DEVELOPMENT

3.1 CHAPTER STRUCTURE

Two methodology development studies were performed and these are considered separately in the present chapter. The first investigated the relationships between GTN dose and change in both brachial artery diameter and arterial wave reflection. The second investigated the effects of age and gender on the extent to which inhaled salbutamol changes arterial wave reflection, and also compared these responses to brachial artery FMD.

3.2 RELATIONSHIPS OF DOSE OF SUBLINGUAL GTN TO CHANGE IN BRACHIAL ARTERY DIAMETER AND ARTERIAL WAVE REFLECTION

3.2.1 Background

When endothelial function is assessed *in vivo* the NO-dependent, endothelium-dependent response, for example increase in brachial artery diameter secondary to increased shear stress in FMD or reduction in arterial wave reflection with inhaled salbutamol, is compared to an NO-dependent but endothelium-independent response to control for vascular smooth dysfunction. Most commonly, GTN is used for this purpose.

The dose of GTN used in FMD studies varies among different investigators. Although a relatively high dose, such as 400 μg (Celermajer *et al.*, 1993; Doshi *et al.*, 2002; Gokce *et al.*, 2002a; Woodman *et al.*, 2004; Wu *et al.*, 2004; Zilkens *et al.*, 2003), has most commonly been used, including in children (Celermajer *et al.*, 1992; Pena *et al.*, 2004), some studies have used 50 μg (Ghiadoni *et al.*, 2000) or 25 μg (Bennett-Richards *et al.*, 2002; Cross *et al.*, 2003; Ghiadoni *et al.*, 2001; Ghiadoni *et al.*, 2003), stating that the degree of brachial artery vasodilatation that occurs with these doses more closely approximates the dilatation that occurs following reactive hyperaemia-induced increased shear stress. However, the dose-response relationship between sublingual GTN and brachial artery dilatation has not been published, nor is there currently any consensus on the most appropriate dose to use and why. The effect of salbutamol on arterial wave reflection has been compared to the effect of GTN given as a 500 μg tablet placed sublingually for 3 minutes (Wilkinson *et al.*, 2002a) and sublingual GTN 250 μg (Hayward *et al.*, 2002b). As with the effects on the brachial artery, the dose-response relationship of sublingual GTN to change in arterial wave reflection is not known.

3.2.2 Aims

In order to inform the most appropriate dose of sublingual GTN that should be used as a control in endothelial function studies, the aims of the present study were to characterise:

1. The dose-response relationship of sublingual GTN to change in brachial artery diameter.
2. The dose-response relationship of sublingual GTN to change in arterial wave reflection.

3.2.3 Methods

3.2.3.1 Subjects

Suitable subjects were identified from a database of subjects who had previously taken part in research at the University of Edinburgh's Clinical Research Centre (CRC) and from those who responded to advertisements placed around the city of Edinburgh.

3.2.3.1.1 Inclusion criteria

- Healthy
- Male or female
- Aged 18 to 50 years
- Weight between 60 and 95 Kg

3.2.3.1.2 Exclusion criteria

- History of major cardiac, respiratory, neurological or renal disease
- Asthma
- Current smoker
- Diabetes
- Taking any drugs that act on the cardiovascular system
- Previous intolerance to GTN
- Pregnant
- Current alcohol or drug abuse
- Previous serious drug allergy

3.2.3.2 Study design

Randomised, placebo-controlled, double blind, 9-way crossover.

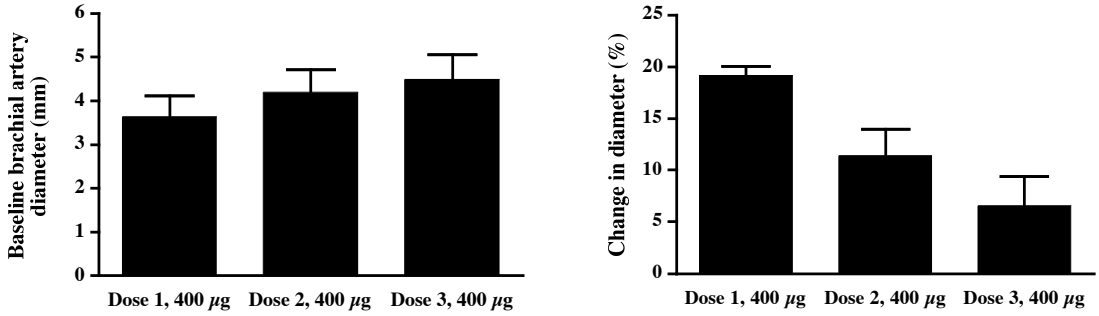
3.2.3.3 GTN and placebo

The doses of sublingual GTN investigated were 5, 10, 25, 50, 100, 200 and 400 μg. GTN 5 mg/mL for parenteral use (Faulding Pharmaceuticals plc, Leamington Spa, UK) was diluted to the appropriate concentration in sterile water. Administration was via a micropipette in a total volume of 80 μL. Placebo was 80 μL of sterile water.

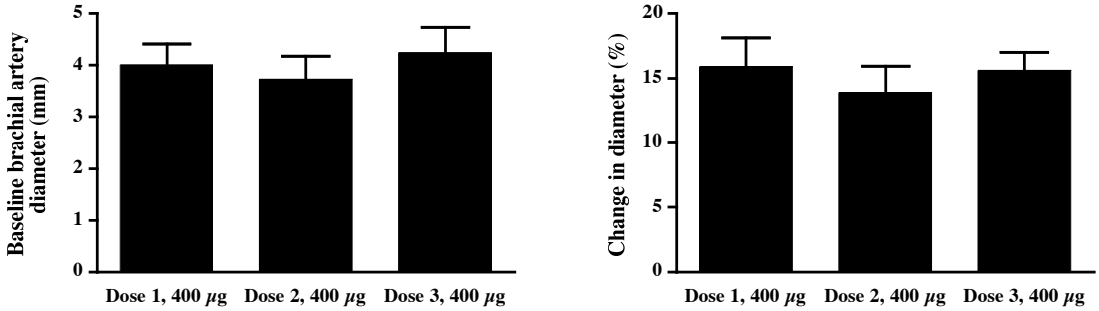
3.2.3.4 Protocol

Subjects attended the CRC on 3 separate days. At each visit measurements were made of FMD and of the responses of the brachial artery and arterial waveforms to 3 different doses of GTN or placebo. Because there were 7 different doses of GTN, placebo was administered on 2 occasions so that the format of each study day was identical. Two operators made recordings during each study. One, on each subject's right, performed brachial artery ultrasound and the other, on each subject's left, recorded HR, BP and radial artery tonometry. On each day subjects first rested semi-recumbent for 20 minutes, after which FMD was performed. After a further 15 minutes rest, baseline recordings were made of HR, BP and radial artery waveforms. Brachial artery ultrasound was then started and, after 1 minute, drug or placebo was administered sublingually. The brachial artery was scanned for a further 5 minutes. HR and BP were repeated at 4 minutes and tonometry at 5 minutes after drug administration. After a further 2 and 4 hours recordings were repeated in the same manner, but before and after different doses of GTN or placebo. Pilot studies, in which GTN was administered 3 times after differing time intervals, had shown that a period of 2 hours appeared to be sufficient for the effects of GTN on the brachial artery to return to baseline and also that there was no change in the sensitivity to GTN after this time (Figure 3.1).

A. GTN 400μg x 3, time interval 1 hour (n = 3)



B. GTN 400μg x 3, time interval 2 hours (n = 4)



C. GTN 50 μg, 400μg and 50 μg, time interval 2 hours (n = 4)

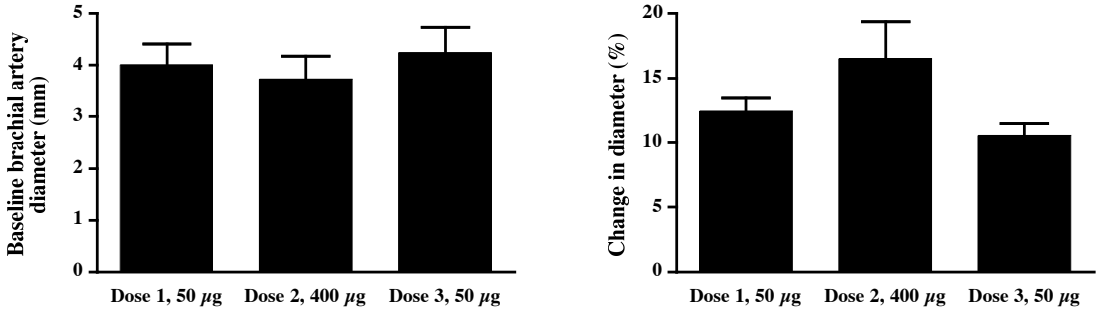


Figure 3.1. Results of pilot studies on the minimum time interval for repeated administration of GTN.
Baseline brachial artery diameter on the left and GTN-induced change in brachial artery diameter on the right (% change from baseline). With an interval of one hour there was an apparent progressive increase in baseline artery diameter and reduction in response to GTN (A). An interval of 2 hours appeared to be sufficient for the artery diameter to return to baseline and for the sensitivity of the artery to GTN to be maintained (B and C). Data are means and SEMs.

3.2.3.5 Randomisation process

The randomisation process was as follows:

1. For the first subject, a randomised sequence of the 9 different doses of GTN or placebo was created.
2. This sequence was used for all subjects, but the start point in the sequence was varied between subjects.
3. Subjects were randomised, in a balanced manner, to 1 of the 9 start points.

3.2.3.6 Analyses

Both FMD and the response to each dose of GTN or placebo on the brachial artery are expressed as percentage change from baseline. Aortic AIx is expressed as absolute change from baseline. RAIx is expressed primarily as percentage change from baseline, to facilitate comparison with the published effect of salbutamol on this parameter, but the absolute change from baseline is also presented. For each dose of GTN or placebo the baseline value of each parameter is that recorded immediately before drug administration. The effect of placebo was the mean of the 2 recordings made. For each subject mean baseline values for systolic and diastolic BP, resting brachial artery diameter, FMD, aortic AIx and RAIx were calculated from the first baseline recordings taken at each of the 3 visits.

3.2.4 Results

Seventeen subjects, all men, were recruited to the study. Their baseline characteristics are given in Table 3.1.

Age (years)	40 (9)
Systolic BP (mmHg)	124 (14)
Diastolic BP (mmHg)	77 (11)
FMD (%)	3.9 (2.4)
Aortic AIx (%)	14 (10)
RAIx (%)	64 (14)

Table 3.1. Mean (SD) subject characteristics.

The dose-response relationships are shown in Figure 3.2. With increasing GTN dose there was a progressive decrease in diastolic BP and increase in HR. In contrast, there was no clear dose-response relationship for GTN to systolic BP. With increasing dose of GTN there was also a progressive increase in brachial artery dilatation, and a progressively greater reduction in arterial wave reflection. The plateau of the dose-response relationship was not reached for any parameter.

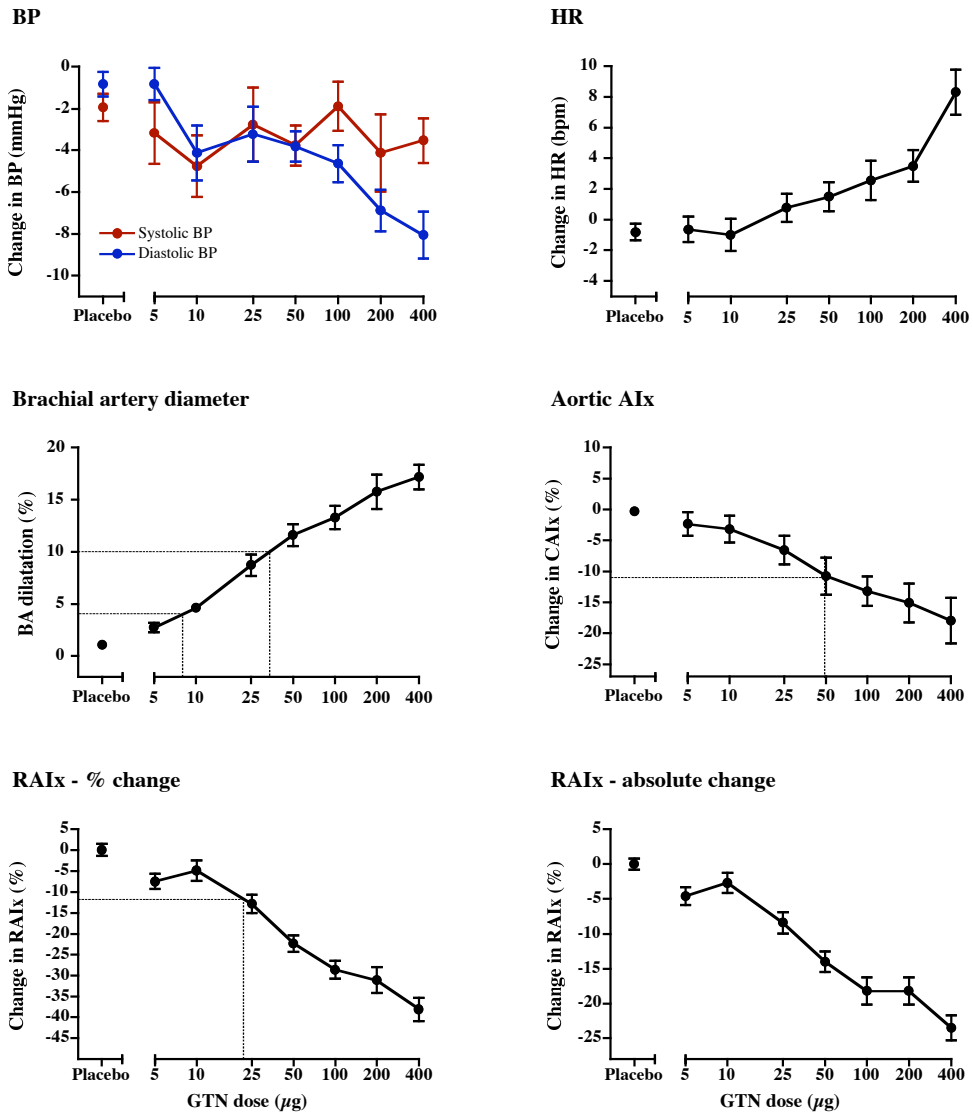


Figure 3.2. Log dose-response relationships of sublingual GTN to change in HR, BP, brachial artery diameter, aortic AIx and RAIx.
For brachial artery diameter the range of GTN doses that are equivalent to the normal FMD response (~4 to 10% dilatation) are indicated. For aortic AIx and RAIx (% change) the GTN doses that are equivalent to the normal responses to salbutamol are indicated. Values are means, error bars are SEMs. BA = brachial artery.

3.2.5 Discussion

3.2.5.1 Dose of GTN as a control in FMD studies

As has been suggested by other investigators (Bennett-Richards *et al.*, 2002; Cross *et al.*, 2003; Ghiadoni *et al.*, 2000; Ghiadoni *et al.*, 2001; Ghiadoni *et al.*, 2003), the present study confirms that lower doses of GTN dilate the brachial artery to a degree that is similar to the normal FMD response. It is not possible to define a single dose of GTN that equates to the normal FMD response because of variability in the degree of brachial artery dilatation following 5 minutes of ischaemia across published studies. Most studies quote FMD values in the range 6 to 10% for healthy controls (Celermajer *et al.*, 1996; Celermajer *et al.*, 1993; Celermajer *et al.*, 1994; Gokce *et al.*, 2002a; Raitakari *et al.*, 1999), although both higher (Celermajer *et al.*, 1992) and lower values (Betik *et al.*, 2004; Ghiadoni *et al.*, 2000) have been reported. In the present study FMD values were relatively low (mean 3.9%). For normal FMD values in the range 4 to 10% the equivalent dose range of GTN is ~8 to 35 μg. Most FMD studies that have used lower doses of GTN have used 25 μg (Bennett-Richards *et al.*, 2002; Cross *et al.*, 2003; Ghiadoni *et al.*, 2001; Ghiadoni *et al.*, 2003) and the data from this study suggest that this dose more closely approximates to the normal shear stress-induced response than does 50 μg (Ghiadoni *et al.*, 2000).

3.2.5.2 Dose of a GTN as a control in arterial wave reflection studies

In previous reports salbutamol 400 μg reduced aortic AIx (absolute change) by ~11% (Wilkinson *et al.*, 2002a) and RAIx (percentage change) by ~12% (Hayward *et al.*, 2002b) in healthy subjects. In the present study the dose of GTN that reduced aortic AIx by 11% was ~50 μg and the dose that reduced RAIx by 12% was ~25 μg. In the original study that investigated the effect of salbutamol on aortic AIx, GTN was given as a 500 μg tablet placed under the tongue for 3 minutes and reduced aortic AIx by ~12%. The present dose-response data suggest that 500 μg GTN would reduce aortic AIx by >18%. The disparity can largely be explained by the different formulations of GTN used in the 2 studies. Three minutes was not sufficient time for the GTN tablets to dissolve and, therefore, the dose of GTN delivered to the circulation could not easily be quantified, although it is likely that it was substantially less than 500 μg. In addition, it is probable that the variation between

subjects in the dose of GTN delivered to the circulation would be greater when delivery is via a sublingual tablet given for a period of time that does not allow for complete dissolution, than via a small volume of solution. For these reasons delivery of sublingual GTN in a small volume of solution may be preferable.

Although the SphygmoCor apparatus measures aortic AIx and RAIx simultaneously previous studies have not reported the effects on both. The dose-response data suggest that the doses of GTN that are equivalent to the effects of salbutamol 400 μg on aortic AIx and RAIx are different, presenting a difficulty if the effects of salbutamol on both aortic AIx and RAIx are measured in any given study. The site of action of GTN within the circulation is known to vary with dose (Jiang *et al.*, 2002), whereas the vascular site of action of salbutamol has not been precisely defined. Therefore, it is plausible that salbutamol and GTN would affect aortic AIx and RAIx differently. In addition, to date there are limited data on the effect of salbutamol on these 2 variables in healthy subjects. As has occurred with FMD, as further studies are published a clearer picture will emerge of the variability of these responses and the dose-response relationships presented here may be used as a future reference for any adjustment of the GTN dose that is necessary. It is critical that future studies should report the effects of salbutamol on both aortic AIx and RAIx. This will enable direct comparison of the effects on each variable and may elucidate whether one is of greater value than the other as a marker of endothelial function.

Following administration of GTN the brachial artery was continuously scanned for 5 minutes, which is sufficient time for peak vasodilatation to occur, at around 3 to 4 minutes (Corretti *et al.*, 2002). Aortic AIx and RAIx were both measured at 5 minutes after GTN. It is possible that, at least in some of the subjects, the effect of GTN on these parameters was not at peak at this time. However, the effect of sublingual GTN on haemodynamic parameters has previously shown to peak from 2 to 5 minutes and thereafter plateau until 6 to 9 minutes (Armstrong *et al.*, 1979; Bashir *et al.*, 1982; Nyberg *et al.*, 1981). Therefore, it is probable that the peak effect was indeed captured at the 5-minute time point.

3.2.5.3 The rationale for using particular doses of GTN as a control

When measuring endothelial function non-invasively one should be clear as to the rationale for using particular doses of GTN. Lower doses, in the range 8 to 50 μg, cause a change in brachial artery diameter, aortic AIx and RAIx to a degree that is similar to the endothelium-dependent stimulus, be it increased shear stress or salbutamol, in healthy subjects. It is for this reason that some researchers have recently used such doses of GTN in FMD studies. Thus, if a group of subjects has an impaired response to the endothelium-dependent stimulus but the response to low dose GTN is maintained one can conclude that the defect lies at the level of the endothelium. If the GTN response is also reduced then there is likely to be more generalised vascular dysfunction (for example, reduced vascular smooth muscle sensitivity to NO, impaired bioconversion of GTN to NO or increased inactivation of NO). When this is the case it is more difficult to determine the extent to which the endothelium itself is dysfunctional. In a recent FMD study, in which GTN 25 μg was given, Cross *et al* (2003) stated that higher doses of GTN may fail to allow discrimination between subtle differences of intrinsic smooth muscle reactivity. However, higher doses of GTN, on the plateau of the dose-response relationship, would provide a measure of the maximum capacity for NO-mediated dilatation, a different measure of vascular function and potential damage. Thus, it should be borne in mind that while lower doses are appropriate controls in the assessment of endothelial function, they may not be appropriate for assessing maximal responsiveness to NO. On the other hand, by 400 μg GTN there was no clear maximum effect plateau for any of the dose-response curves characterised in the present study. Whilst it is still possible that a maximal, or near-maximal, effect on each parameter is achieved with 400 μg GTN (i.e. this dose lies at the start of the plateau of the dose-response curves), it is likely that higher doses would be needed to assess maximum capacity for NO-mediated dilatation. However, systemic haemodynamic effects (increase in HR and reduction in BP) are clearly evident at higher doses and may act as important confounding factors. Certainly, aortic AIx would tend to fall with both BP reduction and increase in HR (Wilkinson *et al.*, 2000a). At lower doses of GTN, especially 25 μg, haemodynamic changes are unlikely to be of practical significance. That maximum effect plateaus were not

reached for brachial artery diameter, aortic AIx or RAIx until there were major systemic haemodynamic effects supports the argument for using lower doses of GTN, that produce equivalent effects to the endothelium-dependent stimuli.

As an alternative to using a single dose of GTN, in one recent study GTN 50 μg was administered repeatedly every 5 minutes until a cumulative dose of 200 μg had been given (Jarvisalo *et al.*, 2004), a principle first suggested by Corretti *et al* (2002) in a guideline paper for brachial artery FMD measurement. Brachial artery diameter was measured after each dose and dose-response curves constructed. In this study, children at increased risk for atherosclerosis, for example those with increased intima-media thickness, had impaired nitrate-mediated dilatation. The authors of this study stated that the subtle nature of the impairment in nitrate-mediated dilatation they reported would be unlikely to have had any significant effect on FMD. However, it is possible that a smaller dose of GTN would have been needed to directly address this. Indeed, it should be noted that the present study was performed in healthy adults. A further dose-response study should be performed in children. A further limitation to the cumulative dose approach is that the dose-response relationship cannot be determined accurately because GTN is rapidly metabolised, with a plasma half-life of 2 to 3 minutes. Nevertheless, the concept of cumulative doses of GTN is an interesting one that should be explored in future studies.

3.2.5.4 Summary

In summary, with increasing dose of GTN, from 5-400μg, there was a graded increase in brachial artery diameter, and decrease of aortic AIx and RAIx. The doses of GTN equivalent to endothelium-dependent stimuli were 8-35 μg for FMD, ~50 μg for the effect of salbutamol 400 μg on aortic AIx and ~25 μg for the effect of salbutamol 400 μg on RAIx.

3.3 CHANGE IN ARTERIAL WAVE REFLECTION AS A MEASURE OF ENDOTHELIAL VASOMOTOR FUNCTION: EFFECTS OF AGE AND GENDER, AND COMPARISON WITH FLOW-MEDIATED DILATATION

3.3.1 Background

The extent to which inhaled $ß_2$-adrenoreceptor agonists reduce arterial wave reflection holds promise as a non-invasive measure of endothelial vasomotor function that could be widely applied in population studies and even in clinical practice. However, to date there is relatively limited experience with this methodology, especially in comparison to other methodologies used to assess endothelial vasomotor function. For example, there are no published data on whether the arterial response to $ß_2$-adrenoreceptor agonist stimulation varies with age. At the outset of this study there were also no data on whether responses differ between men and women, or correlate with brachial artery FMD, an alternative non-invasive method for assessing endothelial vasomotor function. However, the population-based Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) Study, which recruited around 1000 subjects aged 70 years, either healthy, with, or at increased risk of cardiovascular disease, very recently reported no difference in the effect on arterial wave reflection of inhaled terbutaline (like salbutamol, a $ß_2$-adrenoreceptor agonist) between men and women and also no correlation between the effect of terbutaline and brachial artery FMD (Lind *et al.*, 2005).

3.3.2 Aims

The aims of this study were to investigate, in healthy subjects, the effects of age and gender on the extent to which inhaled salbutamol changes arterial wave reflection, and to also to compare these responses to brachial artery FMD.

3.3.3 Methods

3.3.3.1 Subjects

3.3.3.1.1 Identification of subjects

Suitable subjects were identified from a database of subjects who had previously taken part in research at the CRC and from those who responded to advertisements placed around the city of Edinburgh and on the internet.

3.3.3.1.2 Inclusion criteria

- Healthy
- Male or female
- Aged 20 to 79 years
- Weight between 60 and 100 Kg

3.3.3.2.1 Exclusion criteria

- History of major cardiac, respiratory, neurological or renal disease
- Asthma
- Current smoker
- Taking any drugs that act on the cardiovascular system
- Taking any drugs that might interact with salbutamol or GTN
- Pregnant
- Current alcohol or drug abuse
- Previous serious drug allergy

3.3.3.3 Study design

Randomised, placebo-controlled, double blind, 2-way crossover.

3.3.3.4 Protocol

Subjects attended 2 study visits on separate days and all studies were performed first thing in the morning. At the first visit height and weight were measured and a fasting blood sample was taken for full blood count and biochemistry. Otherwise, the protocol, summarised in Figure 3.3, was the same at both visits. After at least 30 minutes supine rest, brachial artery FMD was performed on the right arm. Following

this, baseline BP, HR and PWA were then measured 10 minutes and immediately before salbutamol 400 μg or placebo administration. These measures were repeated 5, 10, 15, 20 30, 45 and 60 minutes after drug administration. Sublingual GTN 50 μg (prepared from a solution for intravenous injection as in section 3.2.3.3, but given in a total volume of 25 μL) was then administered and BP, HR and PWA repeated after a further 5, 10, 15 and 20 minutes. BP, HR and PWA were measured in duplicate at each time point and the mean values entered into the dataset.

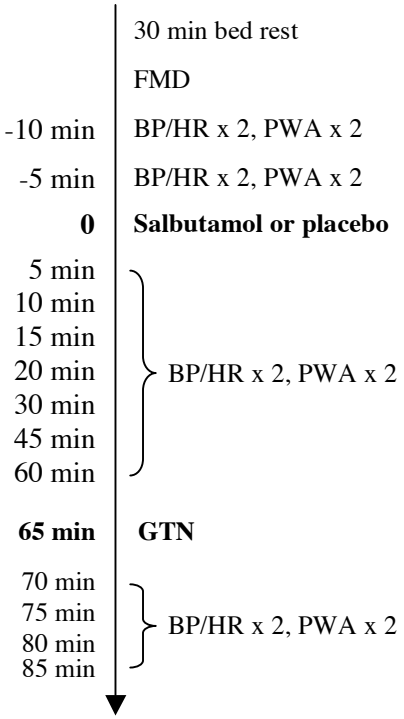


Figure 3.3. Protocol for each study visit.

3.3.3.5 Data analyses

Baseline brachial artery diameter and FMD for each subject were taken as the mean of the values from each visit. For BP, HR, aortic AIx, aortic AIx@75 and RAIx baseline values were those measured at -5 minutes. Changes from baseline were calculated at each time point after salbutamol or placebo inhalation. The -5 minute

measures, rather than those made at +60 minutes, were also used to calculate the changes from baseline following GTN.

Responses to salbutamol and placebo were quantified as both the absolute maximum change from baseline to 30 minutes after drug administration and as AUC of the absolute change from baseline to 60 minutes after drug administration. Responses to GTN were quantified as the absolute maximum change from baseline to 20 minutes after administration and as the AUC of the absolute change from baseline to 20 minutes after administration. Comparisons were made using Student's *t*-tests and correlations were analysed using the Pearson method. Data are given as means and SDs.

3.3.4 Results

3.3.4.1 Subjects

Forty-five subjects were initially recruited. Of these, 1 subject turned out to be hypertensive at his first visit and, therefore, did not complete the study. The data from the remaining 44 subjects were entered into the analyses. The baseline characteristics of the subjects are given in Table 3.2.

	All	Males	Females
Number	44	23	21
Age (years)	42 (14)	44 (14)	41 (13)
Weight (Kg)**	72 (12)	79 (10)	65 (9)
BMI (Kg/m^2)*	24 (3)	25 (3)	23 (3)
Systolic BP**	110 (13)	115 (9)	104 (14)
Diastolic BP	63 (7)	70 (8)	70 (5)
MAP*	82 (9)	85 (8)	79 (8)
Aortic AIx	14 (14)	12 (13)	17 (15)
Aortic AIx@75	5 (15)	4 (13)	7 (16)
RAIx	63 (16)	60 (14)	66 (18)
Plasma glucose (mmol/L)	4.6 (0.4)	4.7 (0.5)	4.6 (0.4)
Serum cholesterol:			
Total (mmol/L)	4.5 (0.8)	4.6 (0.8)	4.3 (0.8)
LDL (mmol/L)*	2.4 (0.7)	2.7 (0.6)	2.2 (0.8)
HDL(mmol/L)**	1.5 (0.4)	1.3 (0.4)	1.7 (0.5)
Total:HDL ratio**	3.2 (1.0)	3.6 (1.0)	2.7 (0.9)
Triglyceride (mmol/L)	1.2 (0.7)	1.3 (0.9)	1.0 (0.4)

Table 3.2. Baseline characteristics of the subjects.
Means (SDs) are given for continuous variables. BMI = body mass index. Values for haemodynamic variables are means of the baseline values from the 2 study visits. Comparisons between males and females by Student's *t*-tests (**P*<0.05, ***P*<0.01).

3.3.4.2 Flow-mediated dilatation

Seven of the visit 1 FMD studies and 6 of the visit 2 FMD studies were not suitable for analysis, due to image quality that was insufficient to reliably define the margins of the brachial artery or because there was significant movement of the position of the artery relative to the ultrasound probe. From 11 subjects there was only 1 suitable FMD study and in 1 subject neither FMD study was suitable.

Baseline brachial artery diameter increased with age (Figure 3.4) and was higher in males than in females [4.4 (0.5) mm vs 3.3 (0.4) mm, $P < 0.001$]. Both increasing age and increasing baseline brachial artery diameter were associated with reduced FMD (Figure 3.4). There was a trend to a lower FMD in males compared to females, but the difference did not achieve statistical significance [4.8 (2.2)% vs 6.4 (3.3)%, $P = 0.07$].

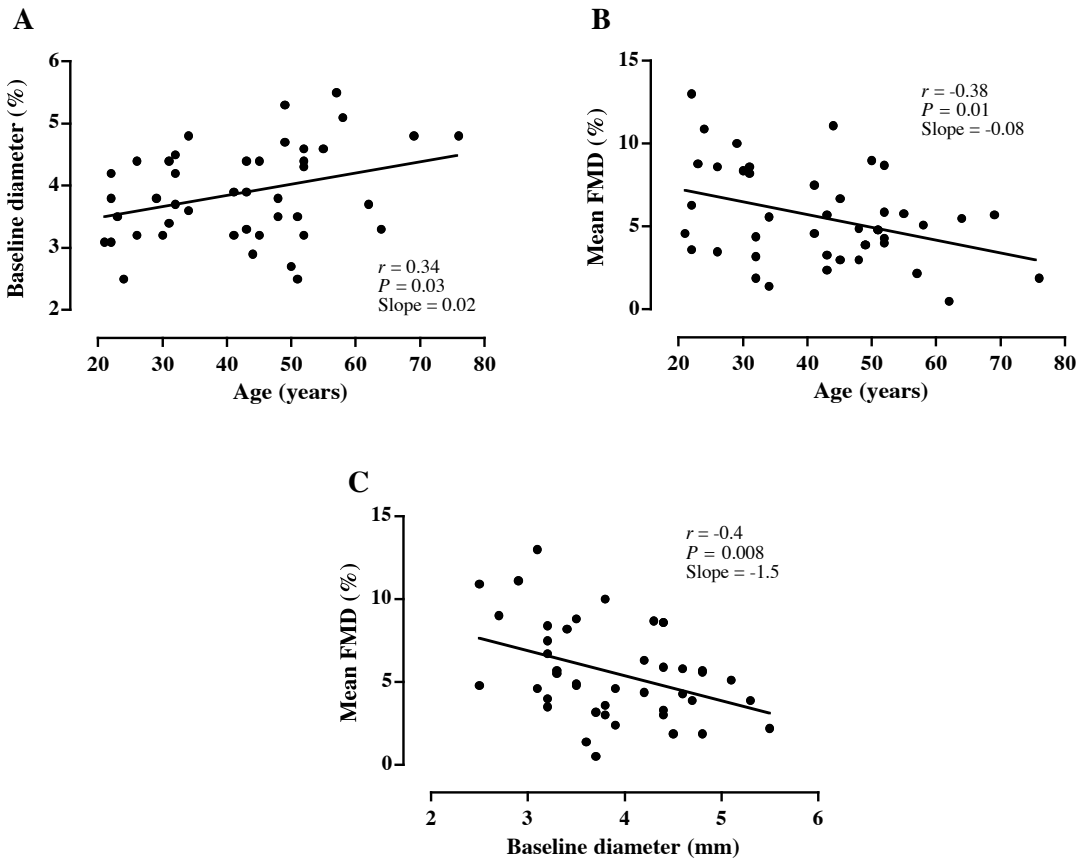


Figure 3.4. Relationships between age and baseline brachial artery diameter (A) and FMD (B), and between baseline brachial artery diameter and FMD (C).

3.3.4.3 Measures of arterial wave reflection at baseline

Mean baseline aortic AIx, aortic AIx@75 and RAIx were strongly related to age (Figure 3.5). Mean baseline MAP was not significantly related to age (Figure 3.5), aortic AIx (r = 0.2, P = 0.2) or RAIx (r = 0.14, P = 0.4) but was weakly correlated with aortic AIx@75 (r = 0.34, P = 0.02). There were no significant differences between males and females in aortic AIx [males 12 (12)% vs females 17 (15)%, P = 0.3], aortic AIx@75 [males 4 (13)% vs females 7 (16)%, P = 0.5], or RAIx [males 62 (15)% vs females 69 (18)%, P = 0.2].

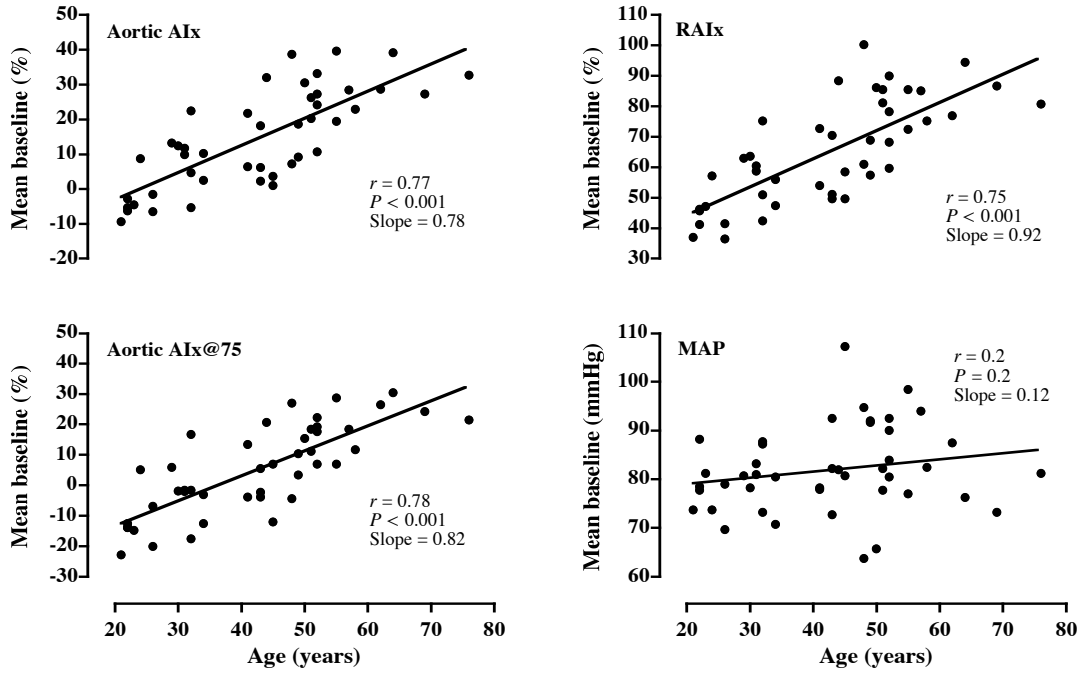
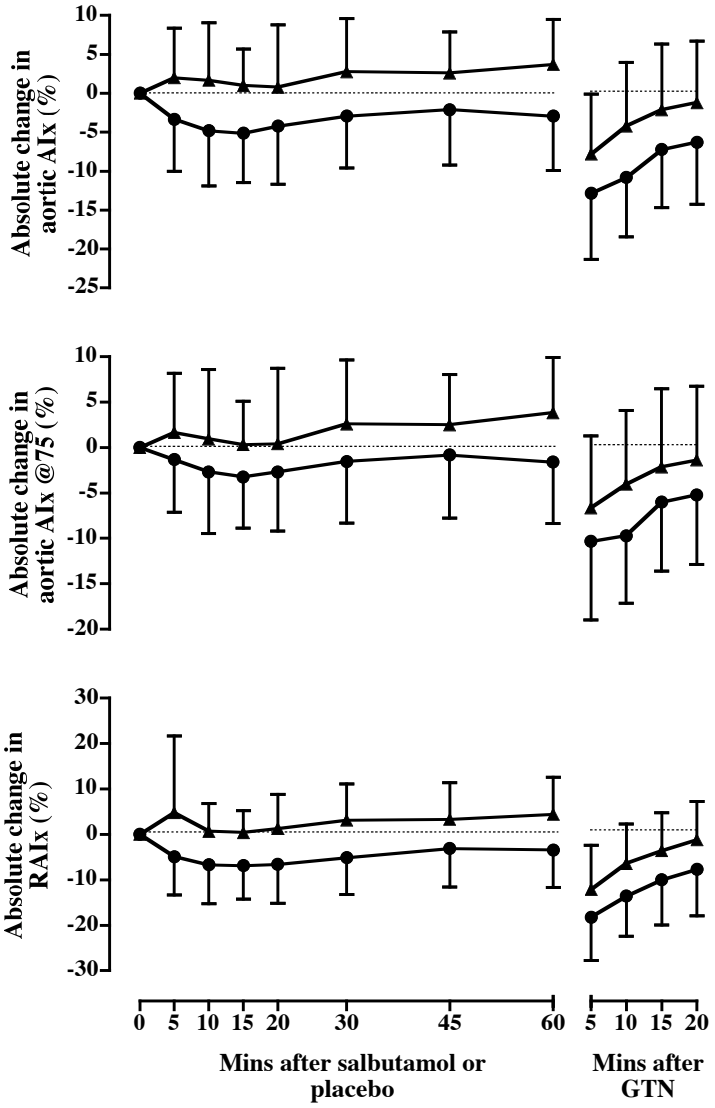


Figure 3.5. Relationships between age and aortic AIx, aortic AIx@75, RAIx and MAP.

3.3.4.4 Responses to salbutamol and glyceryl trinitrate

The effects of salbutamol and GTN, in all subjects, on aortic AIx, aortic AIx@75 and RAIx are shown in Figure 3.6 and on HR and MAP in Figure 3.7. Aortic AIx and RAIx were significantly lower 60 minutes after salbutamol than after placebo (aortic AIx: 13% vs 17%, P = 0.01; RAIx: 63% vs 68%, P = 0.003). Aortic AIx@75 also tended to be lower after salbutamol at this time (5% vs 8%, P = 0.051) and HR was

higher (59 bpm vs 56 bpm, $P = 0.008$), although there was little difference in MAP (84 mmHg vs 85 mmHg, $P = 0.3$).

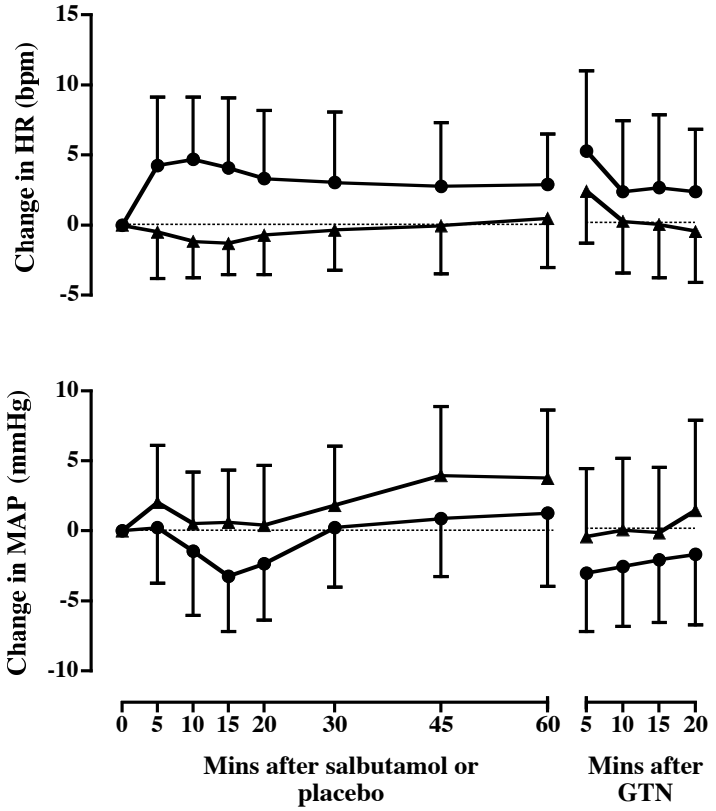


	Max Δ	AUC
Placebo	2(10)	130(287)
Salbutamol	-6(10)	-189(338)
***P* value**	**<0.001**	**<0.001**
GTN (plac)	-7(11)	-74(123)
GTN (salb)	-14(8)	-171(127)
***P* value**	**<0.001**	**<0.001**

	Max Δ	AUC
Placebo	2(11)	117(294)
Salbutamol	-4(10)	-100(313)
***P* value**	**0.01**	**0.001**
GTN (plac)	-5(12)	-67(127)
GTN (salb)	-11(10)	-145(125)
***P* value**	**0.004**	**0.003**

	Max Δ	AUC
Placebo	6(19)	165(333)
Salbutamol	-8(11)	-283(413)
***P* value**	**<0.001**	**<0.001**
GTN (plac)	-11(13)	-113(131)
GTN (salb)	-18(11)	-223(159)
***P* value**	**0.001**	**<0.001**

Figure 3.6. Effects on aortic AIx (top), aortic AIx@75 (middle) and RAIx (bottom) of placebo (▲) and salbutamol (●), and of GTN after placebo (▲) and salbutamol (●).
The tables give the mean maximum changes from baseline to 30 minutes (Max Δ) and the mean AUCs from baseline to 60 minutes. Comparisons are between salbutamol and placebo phases. GTN (plac) = GTN following placebo; GTN (salb) = GTN following salbutamol.



	Max Δ	AUC
Placebo	-1(5)	-21(129)
Salbutamol	7(6)	191(230)
***P* value**	**<0.001**	**<0.001**
GTN (plac)	2(5)	13(57)
GTN (salb)	6(7)	58(79)
***P* value**	**0.001**	**<0.001**

	Max Δ	AUC
Placebo	3(6)	130(185)
Salbutamol	-2(7)	-14(178)
***P* value**	**<0.003**	**<0.001**
GTN (plac)	1(8)	1(78)
GTN (salb)	-4(6)	-42(66)
***P* value**	**<0.001**	**<0.001**

Figure 3.7. Effects on HR (top) and MAP (bottom) of placebo (▲) and salbutamol (●), and of GTN after placebo (▲) and salbutamol (●).
The tables give the mean maximum changes from baseline to 30 minutes (Max Δ) and the mean AUCs from baseline to 60 minutes. Comparisons are between salbutamol and placebo phases. GTN (plac) = GTN following placebo; GTN (salb) = GTN following salbutamol.

Increasing age was associated with a greater maximum change in RAIx with salbutamol (r = -0.31, P = 0.04) but did not correlate with the change in RAIx quantified as the AUC of the change from baseline (r = -0.15, P = 0.3). Age was not significantly correlated with the effect of salbutamol on aortic AIx (r = -0.1, P = 0.5 for maximum change from baseline and r = -0.04, P = 0.8 for AUC of the change from baseline) or aortic AIx@75 (r = -0.2, P = 0.3 and r = -0.04, P = 0.8 respectively). There were no differences between males and females in the responses to salbutamol of aortic AIx, aortic AIx@75 or RAIx, HR or MAP (Table 3.3).

		Males	Females	*P* value
Aortic AIx	**Max Δ to 30 minutes (%)**	-6 (10)	-6 (9)	1
	AUC to 60 minutes (AU)	-193 (340)	-185 (344)	0.9
Aortic AIx@75	**Max Δ to 30 minutes (%)**	-5 (10)	-3 (10)	0.6
	AUC to 60 minutes (AU)	-127 (301)	-70 (332)	0.6
RAIx	**Max Δ to 30 minutes (%)**	-8 (11)	-9 (11)	0.8
	AUC to 60 minutes (AU)	-270 (402)	-298 (436)	0.8
HR	**Max Δ to 30 minutes (bpm)**	5 (5)	8 (7)	0.2
	AUC to 60 minutes (AU)	136 (188)	252 (259)	0.09
MAP	**Max Δ to 30 minutes (mmHg)**	-2 (7)	-1 (7)	0.4
	AUC to 60 minutes (AU)	-28 (175)	2 (185)	0.6

Table 3.3. Effects of salbutamol in males and females.

3.3.4.5 Relationships between FMD and responses to salbutamol

FMD was not significantly correlated with the responses of aortic AIx, aortic AIx@75 or RAIx to salbutamol (Table 3.4).

		Correlation with FMD	
		***r* value**	***P* value**
Aortic AIx	**Max Δ to 30 minutes**	-0.004	0.98
	AUC to 60 minutes	0.1	0.5
Aortic AIx@75	**Max Δ to 30 minutes**	0.15	0.3
	AUC to 60 minutes	0.14	0.4
RAIx	**Max Δ to 30 minutes**	0.13	0.4
	AUC to 60 minutes	0.16	0.3

Table 3.4. Relationships between FMD and responses to salbutamol.

3.3.5 Discussion

3.3.5.1 Main findings

The main findings from the present study are that in healthy subjects:

1. Age and gender do not influence the effect of inhaled salbutamol on arterial wave reflection.
2. There is no correlation between the effect of inhaled salbutamol on arterial wave reflection and brachial artery FMD.

3.3.5.2 Response to salbutamol as a measure of endothelial function

Consistent with previously published work, in this study FMD was inversely related to both baseline brachial artery diameter (Silber *et al.*, 2005) and age (Allen *et al.*, 2000; Celermajer *et al.*, 1994; Ryliskyte *et al.*, 2004) in healthy subjects. Baseline brachial artery diameter itself increased with age, but the age-related decline in FMD is known to occur independently of this (Celermajer *et al.*, 1994). So as not to influence the response to salbutamol, GTN was not given as a control to FMD in the present study (see section 3.3.5.4.1). However, it has previously been shown that dilatation of the brachial artery in response to GTN is not affected by age (Celermajer *et al.*, 1994), indicating that reduced FMD is the result of impaired endothelium-dependent vasomotion.

In contrast to the clear inverse relationship between age and FMD, there was no impairment of the systemic arterial response to salbutamol, whether measured as aortic AIx, aortic AIx@75 or RAIx, with increasing age. Indeed, there was a greater reduction in RAIx in older subjects. However, this was evident only when measured as the maximum change from baseline and not when measured as the AUC of the change from baseline. This was also only marginally statistically significant in the setting of multiple statistical testing and should, therefore, be interpreted with caution.

In addition to the established effect of aging on FMD, there is also a well documented progressive decline in muscarinic agonist, mainly ACh, stimulated vasodilatation in both the forearm (DeSouza *et al.*, 2000; Gerhard *et al.*, 1996;

Taddei *et al.*, 2001; Taddei *et al.*, 1997; Taddei *et al.*, 1995) and coronary (Egashira *et al.*, 1993; Yasue *et al.*, 1990) circulations that is evident from the 3rd or 4th decade of life. In these studies endothelium-independent vasodilatation, with either sodium nitroprusside, GTN or papaverine, was either unaffected or minimally affected, suggesting that ageing is associated with reduced endothelial NO release. Ageing is also associated with reduced vasoconstriction to the NO synthase inhibitor L-NMMA and to the cyclo-oxygenase inhibitor aspirin but not to the direct smooth muscle vasoconstrictor noradrenaline, suggesting impairment of basal endothelial NO and prostanoid pathways (Singh *et al.*, 2002).

Thus, there is a well characterised decline in both conduit vessel and resistance vessel endothelial vasomotor function with ageing, but systemic arterial responses to salbutamol are unaffected by age, even in a group of subjects in which there is a demonstrable age-related decline in FMD. Does this finding call into question the use of this novel methodology as a measure of endothelial vasomotor function, especially given the proven value of FMD in independently predicting cardiovascular events (Brevetti *et al.*, 2003; Fichtlscherer *et al.*, 2004; Patti *et al.*, 2005)? For example, it may be less sensitive than FMD in the detection endothelial dysfunction. However, there a number of alternative explanations for the lack of effect of ageing on salbutamol responses observed in the present study. For example, in contrast to muscarinic agonists, the degree of vasodilatation in the forearm with other endothelium-dependent agents, including bradykinin and substance P, is not affected by ageing (DeSouza *et al.*, 2002). This suggests that the age-related decline in endothelium-dependent vasodilatation may be agonist specific. It follows that salbutamol responses in the forearm might also be relatively preserved in older subjects, although this hypothesis has not been investigated directly. Even if vascular responsiveness to salbutamol is unaffected by ageing, it does not necessarily follow that there would also be no reduction in the response to salbutamol with other causes of endothelial dysfunction. For example, both smoking and hypercholesterolaemia are associated with impaired vasodilatation to substance P in the forearm (Newby *et al.*, 2002; Newby *et al.*, 1999). Indeed, hypercholesterolaemia is associated with

reduced systemic arterial effect of salbutamol, with individual salbutamol responses correlating with responses to ACh in the forearm (Wilkinson *et al*., 2002a).

Regular aerobic exercise prevents, and even reverses, the age-related decline in vasodilatation of the forearm vasculature to ACh (DeSouza *et al.*, 2000; Taddei *et al*., 2000). Thus, if a number of the subjects recruited to the study participated in regular exercise, a tendency to reduced responsiveness to salbutamol with age might have been concealed. A study specifically recruiting sedentary and exercise-trained subjects would address this issue. Just 4 subjects over 60 years of age were recruited to the study. Therefore, the possibility that the arterial response to salbutamol is relatively preserved until around 60 years and declines thereafter cannot be excluded.

In the present study there was a trend to a reduced FMD in males compared to females, which is consistent with the observation that the age-related decline in FMD is relatively delayed in females compared to males, with the deterioration in males evident from the early 40s and the deterioration in females evident from the early 50s (Celermajer *et al*., 1994). However, there were no differences, or even trends to differences, between males and females in the effects of salbutamol on arterial wave reflection. The recently reported PIVUS study found no differences between men and women in either FMD or in the effect of terbutaline on arterial wave reflection. The explanation for the lack of difference in FMD between the genders in PIVUS may be that all subjects were 70 years of age, which is old enough for the age-related decline in FMD to have affected both genders equally. One might speculate that the lack of even a trend to any difference between men and women in the response to salbutamol in the present study, which recruited a much younger population than did PIVUS, indicates that this methodology may be less sensitive than FMD in the detection of endothelial vasomotor dysfunction.

Vasodilatation in response to both increased shear stress and salbutamol are endothelium-dependent, largely through the acute release of endogenous endothelial NO (Dawes *et al*., 1997; Doshi *et al*., 2001). Despite this, there was no correlation between FMD and salbutamol responses. However, this finding is consistent with

data from PIVUS in which different methodologies for the assessment of endothelial vasomotor function were compared (Lind *et al.*, 2005). There was no correlation between FMD and the effect of terbutaline on arterial wave reflection. There was also no correlation between FMD and the degree of vasodilatation to ACh in the forearm. In contrast, responses to terbutaline were correlated, albeit rather weakly ($r = 0.12$, $P = 0.001$), with responses to ACh, which is consistent with the correlation shown between the responses to forearm ACh and systemic salbutamol in hypercholesterolaemia (Wilkinson *et al.*, 2002a). Endothelial vasomotor dysfunction as defined as impaired vasodilatation to ACh in the forearm is independently predictive of cardiovascular events, and so the correlation between local responses to ACh and systemic responses to $ß_2$-adrenoreceptor agonists provides some support for the hypothesis that the latter will also be of value in predicting cardiovascular outcome. Local responses to ACh and systemic responses to $ß_2$-adrenoreceptor agonists may be correlated because both stimulate NO release in resistance vessels. The lack of correlation of either of these techniques with FMD of the brachial artery may suggest that assessment of conduit artery endothelial vasomotor function provides different, perhaps even complementary, information on vascular health/risk. Interestingly, in the PIVUS study all 3 methodologies correlated with the Framingham risk score. Ultimately though, the relative value of each of these methodologies in cardiovascular risk assessment can only be assessed in longitudinal studies and, in this regard, the follow-up data on the PIVUS participants will be of particular interest.

3.3.5.3 Baseline arterial wave reflection

Although responses to salbutamol were not influenced by age, baseline measures of arterial wave reflection were strongly correlated with age. The observed relationship between age and aortic AIx is consistent with recently reported data from the Anglo-Cardiff Collaborative Trial (ACCT) a large population study of 4001 subjects, in which aortic AIx progressively increased with age, virtually linearly until around 60 years (McEniery *et al.*, 2005). It is not possible to comment on the relationship between age and aortic AIx in subjects older than 60 years from the present study because only 4 subjects of this age were recruited. However, in the ACCT there was

a progressive decline in the rate of rise of aortic AIx in older subjects. Aortic AIx@75 and RAIx were not reported by the ACCT, but the present study demonstrates that these are also strongly related to age.

3.3.5.4 Methodological considerations

3.3.5.4.1 GTN as a control

There is no consensus on whether GTN should be given before or after salbutamol in non-invasive endothelial function studies. Wilkinson *et al* (2002a) administered GTN (500 μg tablet kept sublingually for 5 minutes and then removed) 25 minutes *before* administering salbutamol, on the basis of pilot studies that had shown, in contrast to the pilot studies detailed in Figure 3.1 (page 53), that 20 minutes was sufficient for the haemodynamic effect of GTN to return to baseline, but that longer was required for salbutamol. In contrast, Hayward *et al* (2002b) administered sublingual GTN (250 μg, formulation not stated) 20 minutes *after* salbutamol. A recent placebo-controlled study in the CRC (not yet published) found that following sublingual GTN 50 μg aortic AIx fell acutely, remained lower than baseline for around 40 minutes and, from 60 minutes, tended to increase above baseline, suggesting compensatory neurohormonal activation, such as stimulation of the renin-angiotensin system. As a measure of endothelial vasomotor function, it is the change in arterial wave reflection in response to salbutamol that is the primary outcome of interest, with GTN being used as a control for vascular smooth muscle NO responsiveness. Therefore, given the possibility that the haemodynamic changes resulting from GTN might affect the response to $ß_2$-adrenoreceptor stimulation, salbutamol was administered first and GTN second.

The inclusion of a placebo arm in the present study facilitates analysis of the effect of prior salbutamol on GTN responses. Measures of arterial wave reflection were significantly lower and HR significantly higher 60 minutes after salbutamol compared to 60 minutes after placebo. Although this is consistent with the plasma half life of salbutamol of 4 to 6 hours, it means that GTN was administered at a time that salbutamol was still exerting an effect on the vasculature. The consequence of this was that the effect of GTN, when expressed as change from baseline, was greater

when administered after salbutamol (Figure 3.6 and Figure 3.7). Thus, a potential limitation of the methodology is that the response to GTN is affected by prior salbutamol and the response to salbutamol may be affected by prior GTN. Currently, the evidence that prior GTN affects the salbutamol response is indirect (haemodynamic changes suggesting neurohormonal activation) and a study comparing salbutamol responses after GTN to after GTN placebo would establish definitively whether or not this is an issue. If it is, then administering the drugs on separate days could be considered for future studies. Clearly, this would reduce the convenience of the methodology (one of its major strengths), although administering GTN could be restricted to a representative subset of subjects.

Based on the results of the previous study (section 3.2), the dose of GTN used, 50 μg, was chosen as that which would reduce aortic AIx to a similar degree as inhaled salbutamol 400 μg in healthy subjects. However, the effect of salbutamol on arterial wave reflection in healthy subjects was lower in the present study than in previous work, for example a mean maximum change in aortic AIx of 6% compared to around 10-11% in the study by Wilkinson *et al* (2002a). The consequence of this was that GTN had a greater effect on arterial wave reflection than did salbutamol. A lower dose, 25 μg, would most likely have more closely approximated the effect of the $ß_2$-adreoreceptor agonist (Oliver *et al*., 2005a). Another potential advantage of a lower dose is that it might result in less neurohormonal activation and influence any subsequent response to salbutamol to lesser degree.

3.3.5.5 Which measure of arterial wave reflection?

Previous studies have quantified the effect of salbutamol on arterial wave reflection either as the change in aortic AIx (Wilkinson *et al*., 2002a) or as the change in RAIx (Hayward *et al*., 2002b). The advantage of using RAIx is that it is directly measured. aortic AIx, on the other hand, is calculated from the aortic pressure waveform which, in turn, is derived from the peripheral pressure waveform using a GTF. This requires additional investment in equipment and one might also speculate that the potential for error in the calculation of aortic AIx may have the consequence that its response

to salbutamol will be less powerful than the response of RAIx in predicting future cardiovascular events.

Salbutamol increased HR by up to 7 bpm and this, in itself, would be expected to reduce aortic AIx (Wilkinson *et al.*, 2000a). Given that salbutamol has very little effect on BP, it is likely that the increase in HR is largely the result of a direct positive chronotropic effect through stimulation of cardiac ß-adrenoreceptors. The consequence of this is that some of the salbutamol-induced reduction in aortic AIx may occur independently of effects on the vascular endothelium. In this regard, the effect of salbutamol on aortic AIx@75, which is aortic AIx normalised to a HR of 75 bpm, might be a more appropriate measure of endothelial vasomotor function. As would be expected given its effect on HR, salbutamol significantly reduced aortic AIx@75 but not as much as it reduced aortic AIx. Currently, there is no consensus on whether the response to salbutamol is best quantified as the change in RAIx, in aortic AIx or in aortic AIx@75. The value of each in predicting cardiovascular events in prospective longitudinal studies will determine if one is superior to the others. In addition, in published studies, the responses of aortic AIx and RAIx to salbutamol and GTN have been expressed as maximum changes from baseline. An alternative method for quantifying the effects on arterial wave reflection of these drugs is as the AUC of the change from baseline. The relative value of these 2 approaches should also be evaluated in prospective studies.

3.3.5.6 Summary

In summary, the effect of inhaled salbutamol on arterial wave reflection is not influenced by age or gender and does not correlate with brachial artery FMD.

CHAPTER 4

ENDOTHELIAL FUNCTION IN HEALTHY SMOKERS AND THE EFFECT OF SILDENAFIL

4.1 INTRODUCTION

Smoking impairs endothelium-dependent vasodilatation and this has been demonstrated as reduced responses to endothelium-dependent agonists in both the coronary (Kugiyama *et al.*, 1996; Zeiher *et al.*, 1995) and forearm circulations (Heitzer *et al.*, 1996; Kimura *et al.*, 2003) and as reduced FMD of the brachial artery (Celermajer *et al.*, 1993). The effect of smoking on the systemic vascular responsiveness to salbutamol has not been investigated.

There are conflicting data on the effect of PDE5 inhibition on endothelial vasomotor function in smokers. Kimura *et al* (2003) found that oral sildenafil 100 mg augmented ACh-induced vasodilatation in the forearm and Vlachopoulos *et al* (2004) found that sildenafil 50 mg improved brachial artery FMD. In contrast, Dishy *et al* (2004) found that sildenafil 50 mg had no effect on brachial artery FMD. In all of these studies, other than being smokers, the subjects were healthy. The effect of PDE5 inhibition on the systemic vascular responsiveness to salbutamol in smokers has not been investigated.

4.1.1 Aims

The aims of this study were to investigate in healthy men:

1. The effect of smoking on endothelium-dependent vasomotor function measured as the change in peripheral arterial wave reflection with inhaled salbutamol.
2. The acute effect of sildenafil on endothelium-dependent vasomotor function in smokers using the same methodology.

4.2 METHODS

4.2.1 Subjects

4.2.1.1 Identification of subjects

Suitable subjects were identified from a database of subjects who had previously taken part in research at the CRC and from those who responded to advertisements placed around the city of Edinburgh.

4.2.1.2 Inclusion criteria

- Healthy male
- Aged 18 to 50 years
- Smokers: at least a 2 pack-year history of smoking
- Non-smokers: never smoked

4.2.1.4 Exclusion criteria

- History of major cardiac, respiratory, neurological or renal disease
- Known risk factors for cardiovascular disease
- Family history of premature cardiovascular disease
- Asthma
- Taking any regular medicine
- Current alcohol or drug abuse
- Previous serious drug allergy

4.2.2 Study design

Randomised, placebo-controlled, single-blind, 2-way crossover.

4.2.3 Protocol

Subjects attended on 2 occasions, each separated by at least 5 days. Smokers smoked a single cigarette 1 hour before resting supine. At the first visit height and weight were measured and a fasting blood sample was taken for full blood count and biochemistry. Otherwise, the protocol was the same at both visits. After at least 30 minutes rest, BP, HR and PWA were measured at -20, -10 and -5 minutes. Sildenafil

100 mg or matched placebo were administered at 0 minutes and the measurements were repeated after 30 and 55 minutes. Salbutamol 400 μg was administered at 60 minutes and measurements were again repeated every 5 minutes for a further 30 minutes. All measures were made in duplicate and the mean values were entered into the analyses.

4.2.4 Data analyses

Data are presented as means (SDs). Baseline was taken as the mean of the measures at -10 and -5 minutes. The effects of sildenafil at +55 minutes are presented as absolute changes from baseline. Response to salbutamol was defined, for each subject, as both the maximum absolute change from the +55 minute time point and as the AUC of the absolute change from the +55 minute time point until 30 minutes after salbutamol. Paired Students' *t*-tests were used for sildenafil vs placebo comparisons and unpaired Students' *t*-tests for smokers vs non-smokers comparisons.

4.3 RESULTS

4.3.1 Subjects

Twelve smokers and 11 non-smokers were recruited. There were no differences in the baseline characteristics between the smokers and non-smokers (Table 4.1). The mean number of pack years for the smoking group was 6.1 (7.8).

	Non-smokers	**Smokers**
Age (years)	25 (9)	24 (8)
Systolic BP (mmHg)	120 (11)	119 (7)
Diastolic BP (mmHg)	67 (9)	69 (6)
HR (bpm)	63 (11)	68 (7)
RAIx (%)	41 (11)	50 (12)
Aortic AIx (%)	0 (8)	4 (10)
Aortic AIx@75 (%)	-6 (10)	1 (11)
BMI (Kg/m^2)	25 (2)	24 (3)
Plasma glucose (mmol/L)	4.8 (0.3)	4.8 (0.4)
Serum cholesterol:		
Total (mmol/L)	4.5 (1.1)	4.5 (0.7)
LDL (mmol/L)	2.4 (0.9)	2.5 (0.8)
HDL (mmol/L)	1.3 (0.2)	1.4 (0.3)
Total:HDL ratio	3.4 (1.2)	3.4 (1.1)
Triglycerides (mmol/L)	1.4 (0.7)	1.3 (0.6)

Table 4.1. Baseline characteristics of the subjects.
For BP, HR, RAIx, aortic AIx and aortic AIx@75 values are the means of the baselines from each visit.

4.3.2 Baseline responses to salbutamol

Responses to salbutamol when administered 1 hour after placebo provided a measure of baseline endothelium-dependent vasomotion. When analysed as the AUC of the change in aortic AIx, smokers had a significantly reduced response to salbutamol compared to non-smokers. However, there were no significant differences between

smokers and non-smokers either in the response of aortic AIx when analysed as the maximum change, or in the response of any other parameter, however analysed.

	Maximum change*			AUC (AU)		
	Non-smokers	**Smokers**	***P***	**Non-smokers**	**Smokers**	***P***
SBP	3 (9)	-1 (10)	0.4	34 (158)	-34 (155)	0.3
DBP	-5 (6)	-1 (9)	0.2	-56 (84)	-23 (128)	0.5
HR	1 (13)	5 (11)	0.5	56 (212)	35 (183)	0.8
AAIx	-8 (8)	-4 (10)	0.3	-159 (124)	-29 (143)	**0.03**
AAIx@75	-5 (9)	-1 (12)	0.4	-104 (130)	5 (161)	0.09
RAIx	-5 (7)	-3 (11)	0.7	-79 (77)	-42 (174)	0.5

Table 4.2. Responses to salbutamol when administered after placebo.
SBP = systolic BP, DBP = diastolic BP. *For maximum changes units are mmHg for SBP and DBP, bpm for HR and % for aortic AIx (AAIx), aortic AIx@75 (AAIx@75) and RAIx.

4.3.3 Effect of sildenafil

The effects of sildenafil and placebo on each haemodynamic parameter is shown in Table 4.3. Compared to placebo, sildenafil reduced systolic BP in smokers but not in non-smokers. There were no significant effects of sildenafil or placebo on diastolic BP, HR or any measure of arterial wave reflection in either smokers or non-smokers, except for a significant reduction in aortic AIx@75 with placebo in smokers.

	Non-smokers			Smokers		
	Placebo	**Sildenafil**	***P***	**Placebo**	**Sildenafil**	***P***
Systolic BP (mmHg)	0 (4)	-1 (3)	0.9	-1 (7)	-9 (7)	**0.02**
Diastolic BP (mmHg)	0 (4)	-3 (6)	0.3	-3 (4)	-6 (5)	0.3
HR (bpm)	-2 (5)	-1 (9)	0.7	-5 (6)	-1 (7)	0.1
Aortic AIx	0 (9)	0 (6)	0.9	-1 (7)	2 (8)	0.1
Aortic AIx@75	0 (8)	0 (6)	0.7	-3 (8)	1 (8)	**0.02**
RAIx	2 (8)	1 (8)	0.9	0 (7)	-4 (15)	0.4

Table 4.3. Effects of sildenafil and placebo on haemodynamic parameters.
Data are changes from baseline to +55 minutes. Comparisons are between sildenafil and placebo.

There were no statistically significant effects of sildenafil on the responses to salbutamol, although there were trends to an improvement in smokers when analysed as AUCs of the changes from +55 minutes (Table 4.4).

	Non-smokers			Smokers		
	Placebo	**Sildenafil**	***P***	**Placebo**	**Sildenafil**	***P***
Max. change*						
SBP	3 (9)	-2 (10)	0.3	-1 (10)	2 (11)	0.4
DBP	-5 (6)	-2 (7)	0.3	-1 (9)	-3 (10)	0.5
HR	1 (13)	4 (11)	0.6	5 (11)	9 (7)	0.2
AAIx	-8 (8)	-10 (7)	0.7	-4 (10)	-3 (19)	0.8
AAIx@75	-5 (9)	-5 (10)	0.9	-1 (12)	1 (19)	0.8
RAIx	-5 (7)	-6 (9)	0.7	-3 (11)	-10 (11)	0.4
AUC (AU)						
SBP	34 (158)	-48 (151)	0.2	-34 (155)	68 (175)	0.08
DBP	-56 (84)	-49 (79)	0.8	-23 (128)	-47 (119)	0.5
HR	56 (212)	68 (211)	0.9	35 (183)	123 (133)	0.1
AAIx	-159 (124)	-140 (95)	0.8	-29 (143)	-96 (266)	0.2
AAIx@75	-104 (130)	-95 (113)	1	5 (161)	-31 (222)	0.5
RAIx	-79 (77)	-100 (108)	0.7	-42 (174)	-134 (110)	0.5

Table 4.4. Effects of salbutamol after sildenafil and after placebo.
Comparisons are between sildenafil and placebo. *For maximum changes units are mmHg for SBP and DBP, bpm for HR and % for aortic AIx (AAIx), aortic AIx@75 (AAIx@75) and RAIx.

4.4 DISCUSSION

4.4.1 Main findings

The main findings of the present study were:

1. The AUC of the change in aortic AIx with salbutamol was reduced in smokers compared to non-smokers.
2 Sildenafil reduced systolic BP in smokers but not in non-smokers.
3. There was a trend to an improvement in the response to salbutamol with sildenafil in smokers.

4.4.2 Baseline endothelium-dependent vasomotion

In the present study there was a reduced vascular response to salbutamol in smokers, indicating systemic endothelial vasomotor dysfunction. Although smoking is known to impair endothelial vasomotor function this has not previously been demonstrated using the salbutamol-based methodology. The data provide further evidence in support of the validity of the methodology in assessing endothelial vasomotor function, especially given its ability to detect endothelial vasomotor dysfunction in such a small, young population with a relatively short exposure to smoking.

This difference between smokers and non-smokers only achieved statistical significance with aortic AIx and, even then, only when analysed as the AUC of the change in this parameter following salbutamol. Nevertheless, consistent with this effect, there were trends to reduced responsiveness to the mean maximum change in aortic AIx, and also to the AUC of the changes and the mean maximum changes in aortic AIx@75 and RAIx. These data suggest that systemic endothelial vasomotor dysfunction might be most sensitively detected when the AUC of the change in aortic AIx is used and this should be specifically investigated in future studies that utilise the methodology. A potential explanation for the superiority of the AUC over the maximum change is that the noise related to the inherent variability in the measurement of aortic AIx (and the other wave reflection parameters) may be minimised when the AUC, rather than the maximum change, is calculated.

4.4.3 The effect of sildenafil on endothelium-dependent vasomotion

Although not statistically significant there was a trend to an improvement in salbutamol responsiveness in smokers with sildenafil, at least when analysed as AUCs of changes in measures of arterial wave reflection. Thus, sildenafil may improve systemic endothelial vasomotor function, but the study was probably underpowered to detect this effect of treatment. Indeed, sixty-two smokers would be required to detect a difference of 67 AUC units between sildenafil and placebo (the mean difference in the current study) in the effect of salbutamol on aortic AIx with 80% power at 5% significance.

The present study is the 4th to investigate the effect of sildenafil on endothelium-dependent vasomotor function in smokers. Of the 3 previous studies, 2 showed an improvement with sildenafil (Kimura *et al*., 2003; Vlachopoulos *et al*., 2004) and 1 showed no effect (Dishy *et al*., 2004). In terms of size and subject demographics these studies were similar. All were relatively small, (n = 9, 10 and 14) and all recruited relatively young, otherwise healthy men (mean ages 39, 33 and 28 years). In the study by Kimura *et al* the conditions may have been maximised to detect an effect of sildenafil. They used the highly sensitive gold standard methodology for the assessment of endothelium-dependent vasomotor function, forearm venous plethysmography, recruited subjects with a relatively extensive smoking history (≥20 cigarettes per day for ≥10 years) and gave sildenafil in a dose of 100 mg. If, as is thought, systemic salbutamol reduces arterial wave reflection by causing resistance artery vasodilatation (see sections 1.5.1.2 and 3.3.5.2), these data are consistent with the, admittedly statistically non-significant, data from the present study in suggesting that sildenafil improves endogenous NO-mediated responses at the resistance vessel level. The 2 other studies investigated the effect of sildenafil 50 mg on brachial artery FMD. As in the present study, but in contrast to that by Kimura *et al*, in both of these measurements were made following acute smoking. In addition, in both studies subjects with a relatively extensive history of smoking were recruited. Therefore, the discrepancy in the observed effects of sildenafil is difficult to explain. Overall, there remains some uncertainty on the effects of sildenafil on endothelium-

dependent vasomotor function in smokers, including whether effects differ between vascular sites.

4.4.4 Baseline arterial wave reflection

There was no statistically significant difference in baseline measures of arterial wave reflection between smokers and non-smokers, although there was a trend to an increase in smokers. In a much larger study in young subjects, aortic AIx was shown to be increased in smokers (Mahmud *et al.*, 2003) indicating that the present study was probably underpowered to detect a significant difference. The demonstration of impaired endothelial vasomotor function but less clear effect on arterial wave reflection may indicate that smoking affects endothelial function before it affects arterial wave reflection or arterial stiffness in smokers, in keeping with the hypothesis that endothelial dysfunction contributes causally to arterial stiffening (Oliver *et al.*, 2003; Wilkinson *et al.*, 2004b). However, appropriately designed longitudinal studies are required to properly address this question.

4.4.5 Effect of sildenafil on BP

Sildenafil reduced systolic BP only in smokers, even though baseline BP was no different to non-smokers. Although this contrasts with a previous study in which there was no effect of sildenafil 50 mg on BP in smokers (Dishy *et al.*, 2004), the possibility that smokers are more sensitive to the BP-lowering effect of PDE5 inhibition is worthy of further investigation.

4.4.6 Limitations

The effect on arterial wave reflection of an NO-dependent, endothelium-independent control such as sublingual GTN was not assessed. This is because of the potential interaction on BP between sildenafil and organic nitrates (see section 1.3.3). As a result, it is not possible to be absolutely confident that the impaired response of aortic AIx to salbutamol in smokers relates to dysfunction at the level of the endothelium. However, given that the salbutamol-induced changes in aortic AIx are endothelium-dependent (Wilkinson *et al.*, 2002a) and that smokers are known to

exhibit endothelial dysfunction, it is most likely that the mechanism is through reduced endothelial NO release.

Peak plasma concentrations of sildenafil occur between 0.5 and 2 hours after oral administration. The effect of sildenafil on endothelial function was assessed 1 hour after oral administration. Although this is the average time to peak plasma concentration, in some subjects the peak effect on endothelial function may not have been captured, limiting the ability to demonstrate a statistically significant effect. Unfortunately, a limitation of using systemic salbutamol is that it is not possible to make short-term serial measurements of endothelial function. The minimal interval between salbutamol challenges has not been determined, but given that the plasma half life of salbutamol is 4 to 6 hours further acute assessments of the effect of sildenafil are unlikely to be feasible.

4.4.7 Summary

Smokers exhibit impaired vascular responsiveness to inhaled salbutamol, indicating systemic endothelial dysfunction. Sildenafil may improve the vascular response to salbutamol in smokers.

CHAPTER 5

THE TIME COURSE OF THE INTERACTION BETWEEN ORAL SILDENAFIL AND SUBLINGUAL GLYCERYL TRINITRATE

5.1 INTRODUCTION

5.1.1 Background

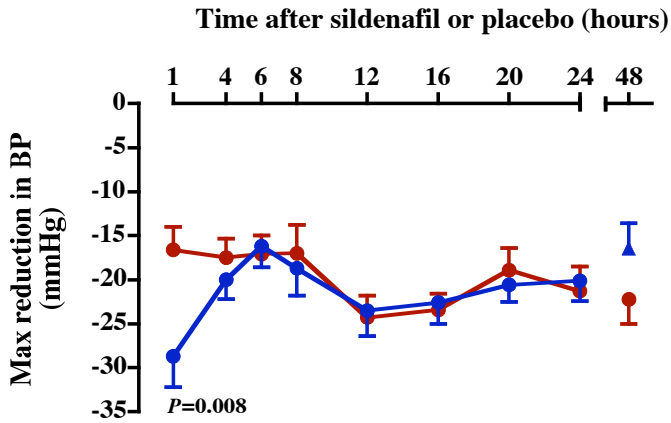
The simultaneous provision of exogenous NO from organic nitrates and inhibition of cGMP breakdown with PDE5 inhibition can result in substantial BP reduction and, as a result, the combined use of organic nitrates and PDE5 inhibitors is absolutely contraindicated. A recommendation has been made that organic nitrates should not be administered to patients who have taken sildenafil within 24 hours (Cheitlin *et al*., 1999). Organic nitrates, especially GTN, are highly effective and commonly used in the treatment of acute angina. Therefore, if a patient presents with acute angina treatment is more difficult if they have taken sildenafil in the previous 24 hours because GTN is not a therapeutic option.

Most studies on the interaction between sildenafil and organic nitrates have concentrated on the maximum interaction (Webb *et al*., 1999; Webb *et al*., 2000). However, in keeping with its plasma half-life of 3-5 hours, blood concentrations of sildenafil are very low 24 hours after a single dose. Therefore, it is possible that organic nitrates could safely be administered at a shorter interval after sildenafil than the 24 hours that is currently recommended. A previous study performed in the CRC found that the interaction of GTN 400 μg spray and oral sildenafil 100 mg lasted less than 4 hours after sildenafil administration (Figure 5.1). The main limitation of this study, which is not yet published, was that it was performed in healthy subjects and it may not be appropriate to extrapolate the findings to patients with angina, the clinically relevant population.

5.1.2 Aim

To characterise the time course of the interaction on BP of sildenafil 100 mg with sublingual GTN 400 μg in men with stable angina.

Systolic BP



Diastolic BP

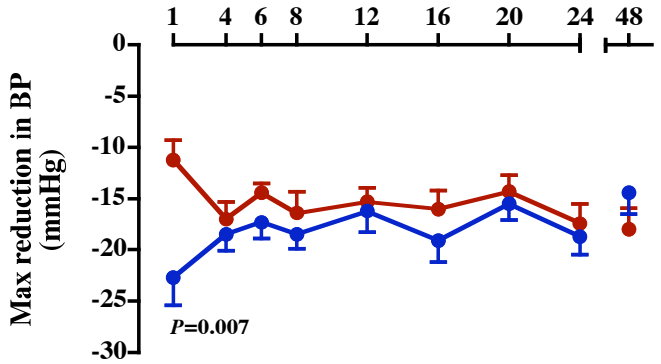


Figure 5.1. Effect of GTN on BP after sildenafil and placebo in healthy men. Sublingual GTN 400 μg spray was administered at different times (as indicated on the *x*-axis) after sildenafil (blue) and matched placebo (red). Sitting BP was measured at baseline (before sildenafil or placebo) and at regular intervals after each GTN challenge (every 3 minutes for the first 30 minutes and then every 15 minutes for a further 90 minutes). The maximum reduction in BP from baseline during each 2-hour monitoring period was recorded for each subject. The mean maximum changes, presented in the charts, were compared at each time point between sildenafil and placebo phases. Only at the 1-hour time point was the GTN-induced reduction in BP lower with sildenafil than with placebo.

5.2 METHODS

5.2.1 Subjects

5.2.1.1 Identification of subjects

Suitable subjects were identified from those attending the WGH for diagnostic coronary angiography and by searching the patient databases of 4 local General Practices.

5.2.1.2 Inclusion criteria

- Male
- Aged 30 to 80 years
- Weight between 60 and 100 Kg
- Stable angina with one of:
  - Classical history of exertional angina pectoris
  - Previous diagnostic exercise test
  - Angiographic evidence of CAD

5.2.1.3 Exclusion criteria

- Regular treatment with long-acting nitrates or nicorandil where these cannot be withdrawn 72 hours before the study
- MI, unstable angina, stroke or transient cerebral ischaemia within 3 months
- Systolic BP > 170 mmHg or diastolic BP > 100 mmHg
- Systolic BP < 100 mmHg or diastolic BP < 60 mmHg
- Orthostatic hypotension (> 20 mmHg fall in systolic BP on standing)
- Diabetes treated with oral hypoglycaemic agents or insulin
- Any clinically significant disease other than stable angina, excepting other cardiovascular disease risk factors, e.g. smoking, hypercholesterolaemia and diet-controlled diabetes
- Taking any drug that significantly interacts with sildenafil
- Evidence of drug abuse

5.2.2 Screening visit

Potentially suitable subjects who agreed to be considered for the study attended a screening visit at the CRC. At this visit the study was explained fully and written consent was obtained. A medical history was taken and a physical examination, including measurement of standing BP, and 12-lead ECG were performed. A non-fasting screening blood sample was also taken.

5.2.3 Study design

Randomised, placebo-controlled, double blind, 4-way crossover.

5.2.4 Drugs

GTN 400 μg (Nitrolingual Pumpspray®, Merck Pharmaceuticals, West Drayton, UK) was administered as a single spray sublingually. Sildenafil 100 mg and matched placebo were administered orally as single tablets.

5.2.5 Protocol

Subjects attended the research unit on 4 occasions, each separated by at least 5 days. On each study day, they took their regular morning medicines immediately after waking and had a light breakfast before attending the research unit at 07:00 hours. At visit 1 GTN was administered 4 and 8 hours after oral sildenafil or matched placebo and at visit 2 GTN was administered 4 and 8 hours after the alternative treatment (sildenafil or placebo). Similarly, at visits 3 and 4, GTN was administered 1 and 6 hours after sildenafil or placebo, with alternate treatments given at each visit. The order in which sildenafil and placebo were given was randomised between visits 1 and 2 and visits 3 and 4. Following at least 20 minutes rest, baseline sitting and standing BP and HR were recorded, in duplicate, 20 minutes and 5 minutes before sildenafil or placebo administration. Five minutes before and every 3 minutes for 33 minutes after each GTN dose further measurements of sitting BP and HR were made, duplicate measures before GTN and single measures after GTN. Standing BP and HR were repeated 36 minutes after each GTN dose. The protocol is summarised in Figure 5.2.

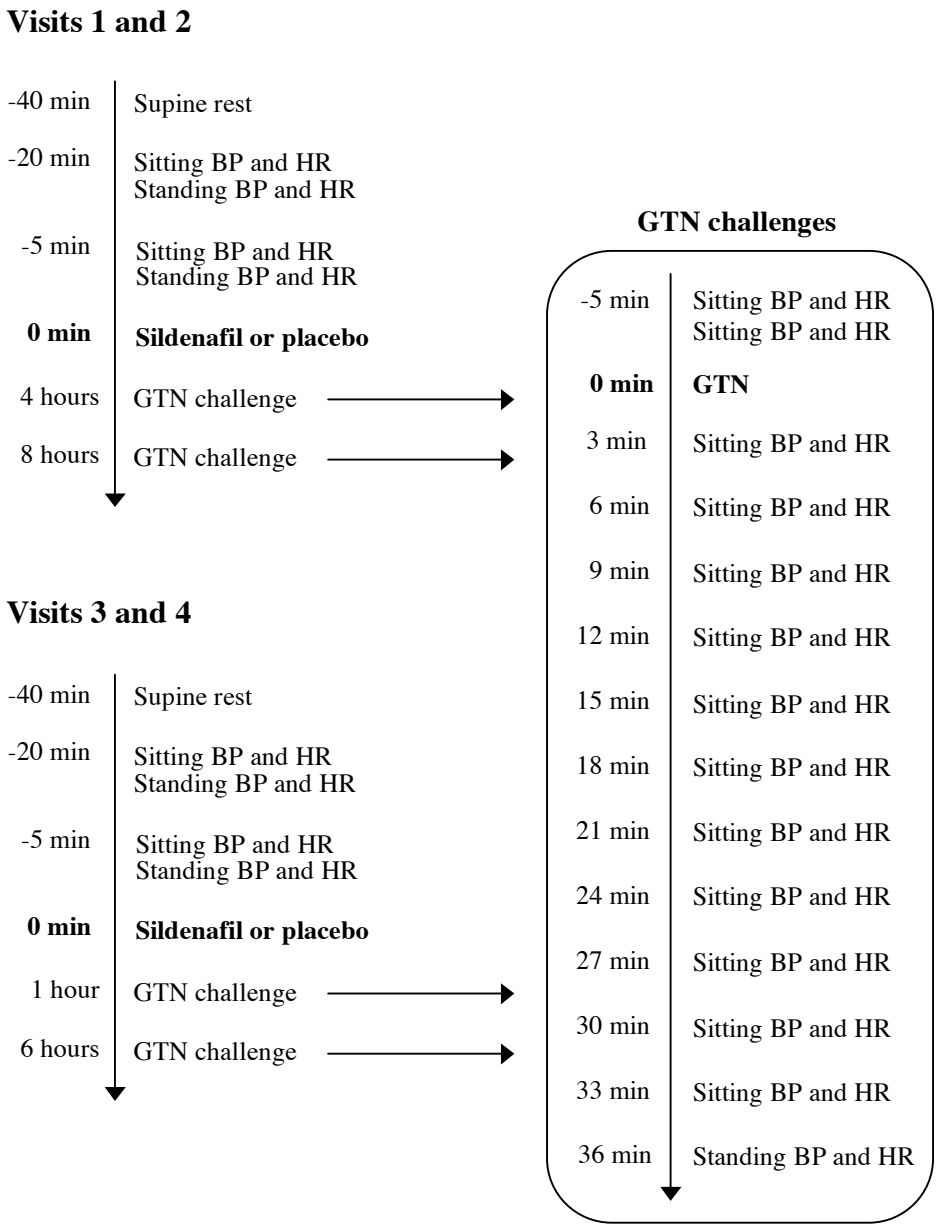


Figure 5.2. Study protocol.

5.2.6 Analyses

Baseline BP and HR were calculated as the mean of measurements taken 20 and 5 minutes before oral sildenafil or matched placebo administration. The pre-specified primary analysis was a comparison of the effects of the combination of sildenafil and GTN and the combination of placebo and GTN on the maximum reduction from

baseline in sitting BP and the maximum increase from baseline in HR that occurred in the 33 min following each GTN challenge.

Effects from pre-GTN values were also assessed for each time point. Pre-GTN BPs and HRs were calculated as the mean of the 2 measurements made before each GTN challenge. Maximum reductions in BP, maximum increases in HR and AUCs for changes in these parameters from pre-GTN values, during the 33 min following each GTN challenge, were compared between sildenafil and placebo phases. The effects of sildenafil and placebo on standing BP and HR 36 min after each GTN challenge were also compared.

Unless stated, data are presented as means (SEMs). All comparisons between sildenafil and placebo were made using 2-way ANOVA allowing for variation due to subject, treatment and treatment order. Differences in proportions between groups were analysed using the binomial test for comparison of proportions. Correlation coefficients were calculated using the Pearson method.

5.3 RESULTS

5.3.1 Subjects

Twenty-three subjects were formally assessed for suitability for the study and, of these, 2 were not suitable. Of the 21 recruited, 1 was withdrawn because his baseline BP was too low (90/58 mmHg) at his first visit. Analyses were performed using the data from the remaining 20 subjects. In 1 further subject, 4 hours after sildenafil administration on visit 2, BP had decreased from 120/70 mmHg to 86/46 mmHg and, as a result, GTN was not administered. These data were included in the comparison of the effects of sildenafil and placebo on pre-GTN BP and HR, but the comparison of sildenafil with GTN and placebo with GTN at 4 hours was analysed using the data from the remaining 19 subjects. GTN was administered to this subject at the 8 hour time point on the same day and at all time points at the other 3 visits. One subject was taking regular ISMN and this was withdrawn 72 hours prior each visit. None of the subjects had used sublingual GTN in the 24 hours preceding any of the visits. The baseline subject characteristics are given in Table 5.1.

	Number (%)		**Mean (SD)**
Current smokers	7 (35)	Age (years)	66 (8)
Ex-smokers	3 (15)	Plasma glucose (mmol/L)	5.5 (0.6)
Previous PCI	12 (60)	Serum cholesterol:	
Previous CABG	2 (10)	Total (mmol/L)	4.2 (0.5)
Hypertension	7 (35)	LDL (mmol/L)*	2.1 (0.5)
Current drugs:		HDL (mmol/L)	1.1 (0.2)
Aspirin	19 (95)	Total:HDL ratio	3.9 (0.9)
Clopidogrel	4 (20)	Triglyceride (mmol/L)	2.4 (1.6)
ß-blocker	19 (95)		
CCB	7(35)		
ISMN	1 (5)		
ACE inhibitor	9 (45)		
ARA	2 (10)		
Statin	19 (95)		
Thiazide diuretic	5 (25)		

Table 5.1. Subject baseline characteristics.
*Values from 18 subjects (in 2 subjects serum LDL concentration could not be calculated because serum triglyceride concentration was too high). PCI = percutaneous coronary intervention. CABG = coronary artery bypass graft.

5.3.2 Sitting BP and HR

5.3.2.1 Effect of sildenafil alone

Compared to placebo, sildenafil alone reduced sitting systolic BP and diastolic BP. This effect was evident from 1 hour, peaked at 4 hours [when placebo-corrected changes in systolic BP and diastolic BP were: -12 (6) mmHg (P = 0.01) and -8 (3) mmHg (P = 0.0008) respectively], and was maintained until at least 8 hours after administration (Figure 5.3). There was no effect of sildenafil alone on HR.

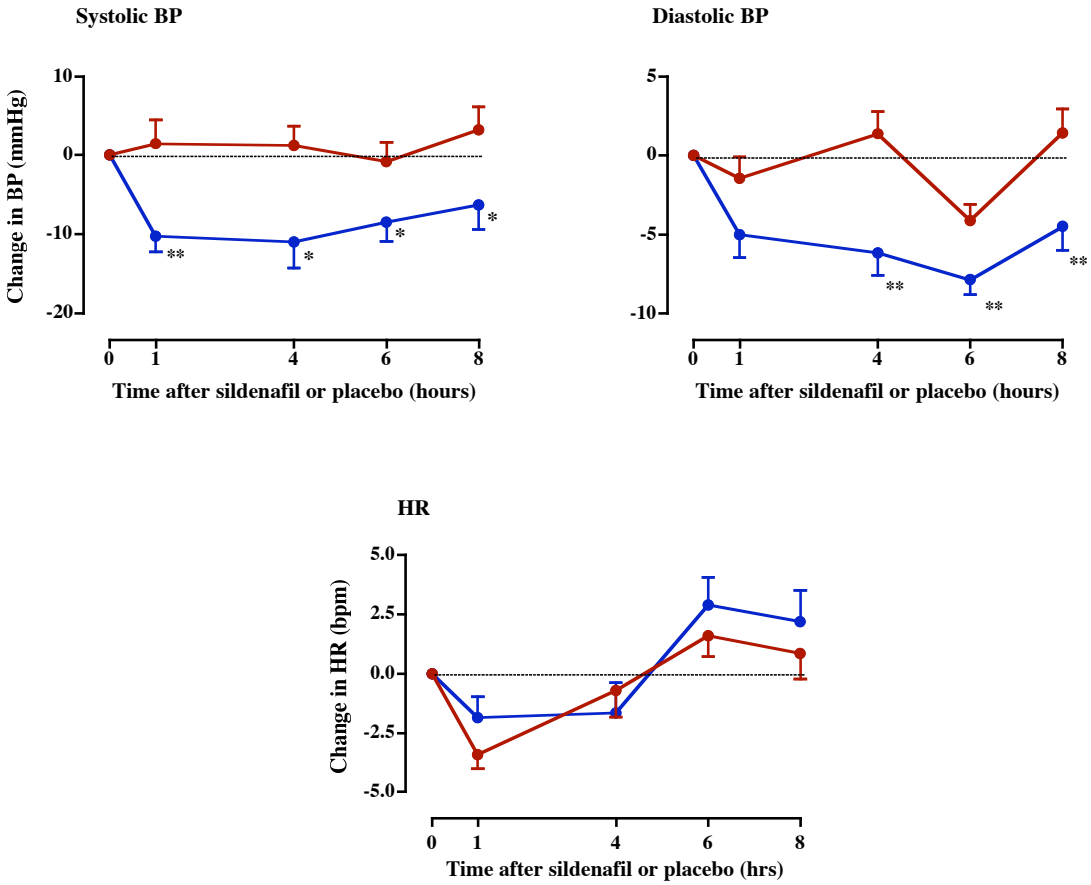


Figure 5.3. Effect of sildenafil alone on sitting BP and HR.
Sildenafil in blue and placebo in red. Measures are those taken before each GTN challenge. *P<0.05, **P<0.01.

5.3.2.2 Effect of the combination of sildenafil and GTN

The absolute effects on sitting BP and HR of GTN with sildenafil and GTN with placebo are shown in Figure 5.4.

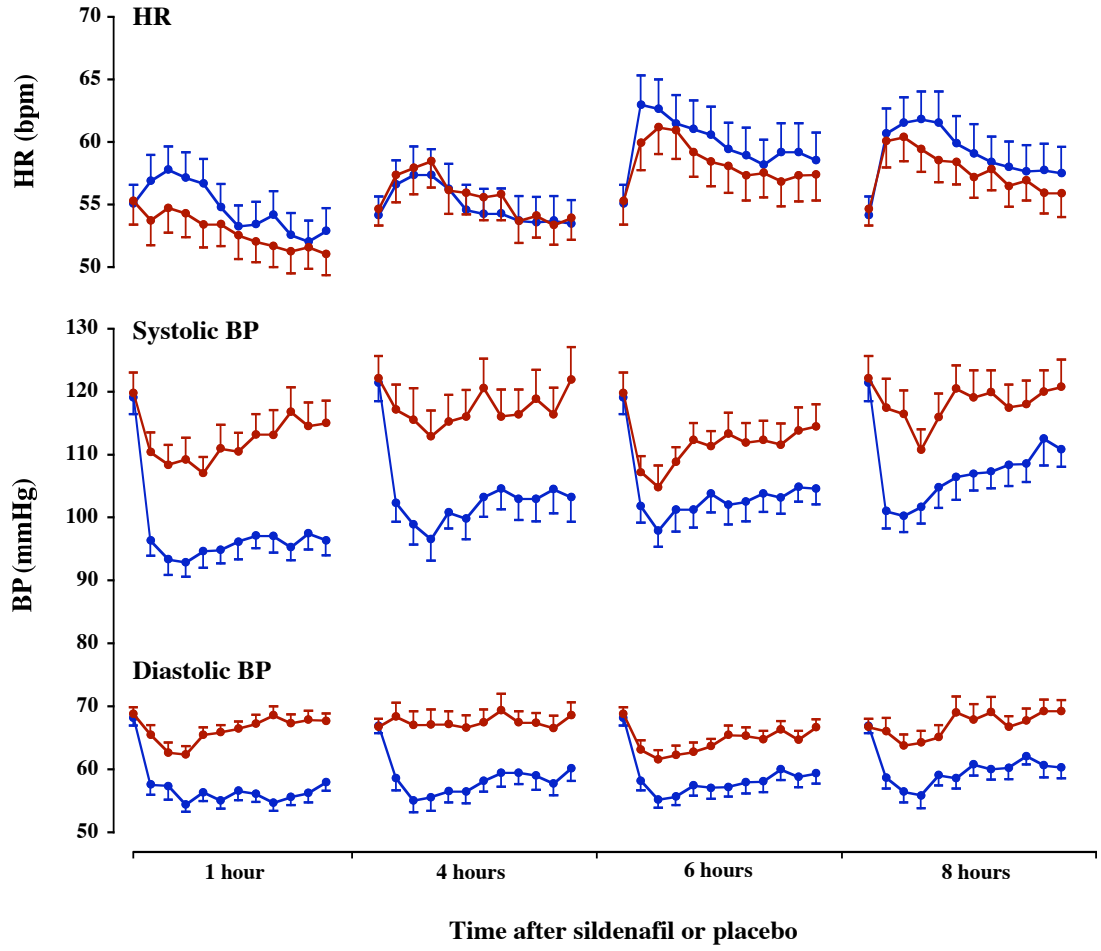


Figure 5.4. Mean effects on sitting BP and HR of GTN with sildenafil and with placebo.
Sildenafil in blue and placebo in red. Baseline values are those taken before sildenafil or placebo administration. For each time point, baseline values are followed by values measured every 3 minutes after GTN (pre-GTN values are not shown).

Compared to the combination of GTN and placebo, the combination of GTN and sildenafil resulted in greater mean maximum reductions in systolic BP at 1, 4 and 8 hours, and in diastolic BP at all time points (Figure 5.5). At 6 hours there was a trend

to a greater mean maximum reduction in systolic BP with sildenafil, but this did not achieve statistical significance. There was a greater effect on the maximum increase in HR with the combination of GTN and sildenafil than with the combination of GTN and placebo at 1 hour, but not at 4, 6 or 8 hours.

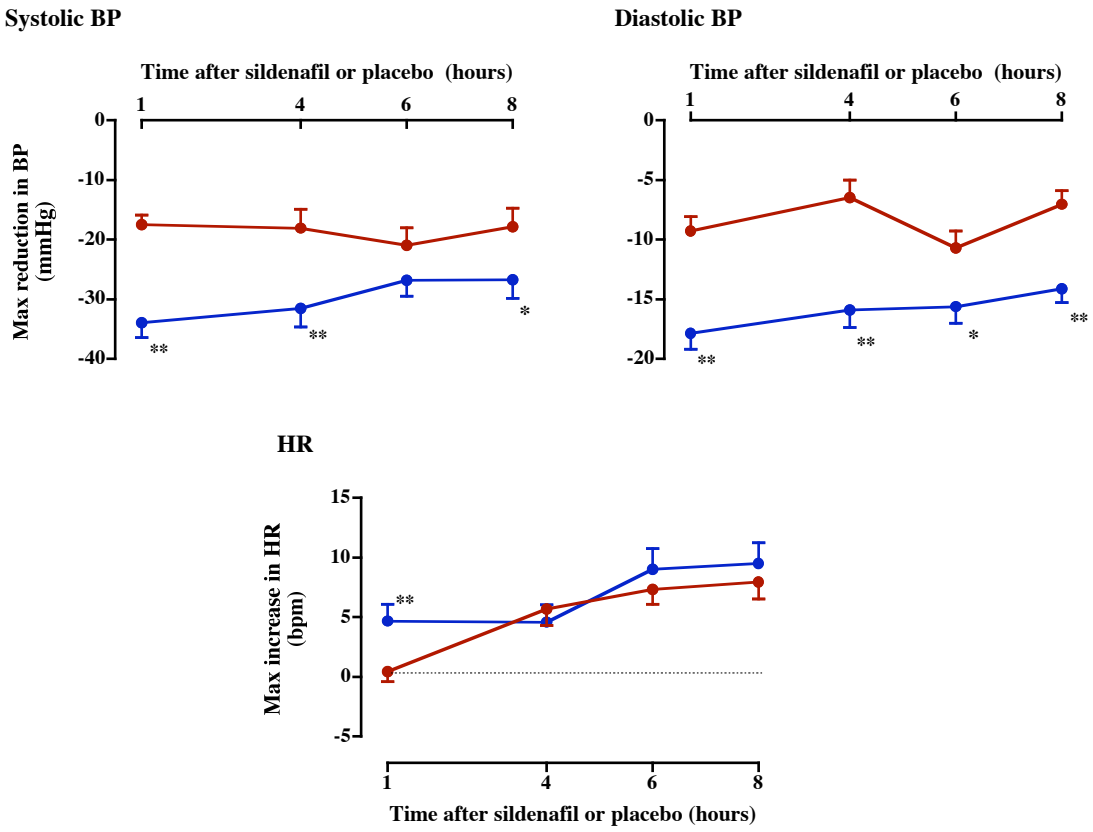


Figure 5.5. Mean maximum changes in sitting BP and HR with GTN given after sildenafil and after placebo.
Sildenafil in blue and placebo in red. Changes are from baseline recordings taken before sildenafil or placebo administration. *P<0.05, **P<0.01.

Table 5.2 shows how often systolic BP was reduced to less 100 mmHg, 90 mmHg and 80 mmHg and how often diastolic BP was reduced to less than 60 mmHg, 50 mmHg and 40 mmHg at some point during the monitoring period after each GTN challenge. Table 5.3 shows how often systolic BP was reduced by more than 20 mmHg, 30 mmHg and 40 mmHg and how often diastolic BP was reduced by more than 15 mmHg, 20 mmHg and 25 mmHg after each GTN challenge.

	Systolic BP <100 mmHg				Systolic BP <90 mmHg				Systolic BP < 80 mmHg			
	1 hr**	**4 hr***	**6 hr**	**8 hr**	**1 hr****	**4 hr****	**6 hr****	**8 hr**	**1 hr****	**4 hr**	**6 hr**	**8 hr**
Sildenafil	19 (95)	15 (79)	15 (75)	12 (60)	12 (60)	8 (42)	10 (50)	8 (40)	7 (35)	4 (21)	2 (10)	2 (10)
Placebo	11 (55)	9 (45)	12(60)	7 (35)	2 (10)	2 (10)	1 (5)	3 (15)	0 (0)	1 (5)	1 (5)	0 (0)

	Diastolic BP <60 mmHg				Diastolic BP <50 mmHg				Diastolic BP < 40 mmHg			
	1 hr**	**4 hr****	**6 hr***	**8 hr***	**1 hr****	**4 hr****	**6 hr**	**8 hr**	**1 hr**	**4 hr**	**6 hr**	**8 hr**
Sildenafil	19 (95)	18 (95)	17 (85)	17 (85)	11 (55)	9 (47)	6 (30)	6 (30)	0 (0)	0 (0)	0 (0)	0 (0)
Placebo	9 (45)	11 (55)	13 (65)	11 (55)	1 (5)	1 (5)	1 (5)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)

Table 5.2. Frequency with which BP was reduced below different thresholds at each GTN challenge.
Numbers (percentages) of subjects whose sitting BP fell to below different thresholds at some point in the 33-minute monitoring period after each GTN challenge. Comparisons between sildenafil and placebo were made of the proportions of subjects in whom BP fell below each of the thresholds (if significant indicated as *P<0.05 or **P<0.01).

	Systolic BP fall >20 mmHg				Systolic BP fall >30 mmHg				Systolic BP fall >40 mmHg			
	1 hr**	**4 hr***	**6 hr***	**8 hr**	**1 hr****	**4 hr***	**6 hr**	**8 hr**	**1 hr***	**4 hr***	**6 hr**	**8 hr**
Sildenafil	19 (95)	16 (84)	15 (75)	13 (65)	12 (60)	7 (37)	7 (35)	8 (40)	4 (20)	6 (32)	3 (15)	4 (20)
Placebo	6 (30)	9 (45)	8 (40)	8 (40)	1 (5)	2 (10)	4 (20)	4 (20)	0 (0)	1 (5)	2 (10)	1 (5)

	Diastolic BP fall >15 mmHg				Diastolic BP fall >20 mmHg				Diastolic BP fall >25 mmHg			
	1 hr**	**4 hr***	**6 hr**	**8 hr***	**1 hr****	**4 hr***	**6 hr**	**8 hr**	**1 hr**	**4 hr**	**6 hr**	**8 hr**
Sildenafil	12 (60)	7 (37)	9 (45)	8 (40)	8 (40)	4 (21)	4 (20)	3 (15)	1 (5)	3 (16)	2 (10)	0 (0)
Placebo	3 (15)	2 (10)	5 (25)	2 (10)	1 (5)	0 (0)	2 (10)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Table 5.3. Frequency with which BP was reduced by different thresholds with each GTN challenge.
Numbers (percentages) of subjects whose sitting BP fell by more than various thresholds at some point in the 33-minute monitoring period after each GTN challenge. Comparisons between sildenafil and placebo were made of the proportions of subjects in whom BP fell by more than each of the thresholds (if significant indicated as *P<0.05 or **P<0.01).

When the data from each of the GTN challenges following sildenafil administration were combined there was a significant correlation between baseline BP and the minimum BP recorded following GTN challenge (Figure 5.6). Correlations between baseline BP and minimum BP recorded following each GTN challenge are given in Table 5.4.

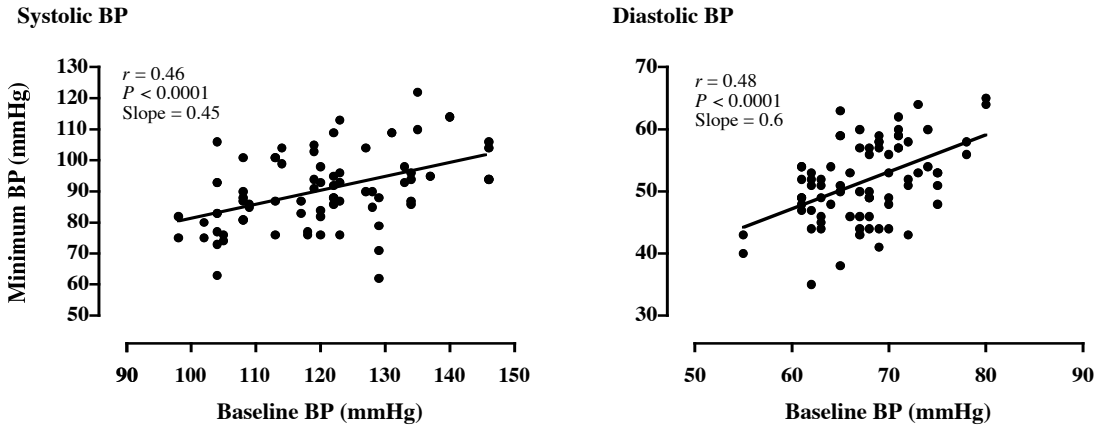


Figure 5.6. Relationships between baseline BP and minimum BP after GTN challenge following sildenafil administration.
Data from all 4 GTN challenges are included.

		Pearson *r*	*P* value
Systolic BP	**1 hour**	0.51	**0.02**
	4 hour	0.51	**0.03**
	6 hour	0.49	**0.02**
	8 hour	0.37	0.11
Diastolic BP	**1 hour**	0.33	0.16
	4 hour	0.62	**0.005**
	6 hour	0.41	0.07
	8 hour	0.6	**0.005**

Table 5.4. Correlations between baseline BP and minimum BP recorded after each GTN challenge following sildenafil administration.

The magnitude of the maximum reduction in BP with the combination of sildenafil and GTN was significantly correlated with baseline BP when all time points were combined (Figure 5.7), with baseline systolic BP at each time point and with baseline diastolic BP at 1 hour (Table 5.5).

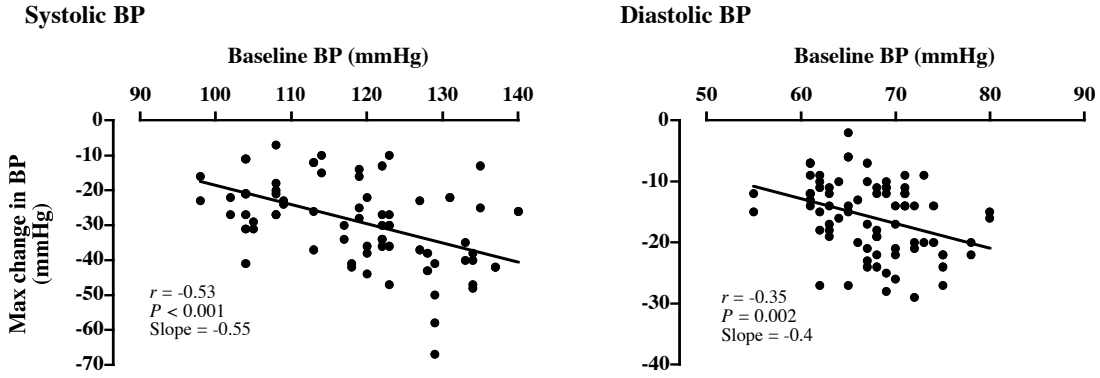


Figure 5.7. Relationships between baseline BP and maximum change in BP after GTN challenge following sildenafil administration.
Data from all 4 GTN challenges are included.

		Pearson *r*	*P* value
Systolic BP	**1 hour**	-0.62	**0.003**
	4 hour	-0.49	**0.03**
	6 hour	-0.48	**0.03**
	8 hour	-0.65	**0.003**
Diastolic BP	**1 hour**	-0.6	**0.006**
	4 hour	-0.06	0.8
	6 hour	-0.44	0.053
	8 hour	-0.28	0.23

Table 5.5. Correlations between baseline BP and maximum change in BP after each GTN challenge following sildenafil administration.

The absolute changes in sitting BP and HR from pre-GTN values are shown in Figure 5.8 and the maximum changes from pre-GTN values are shown in Figure 5.9.

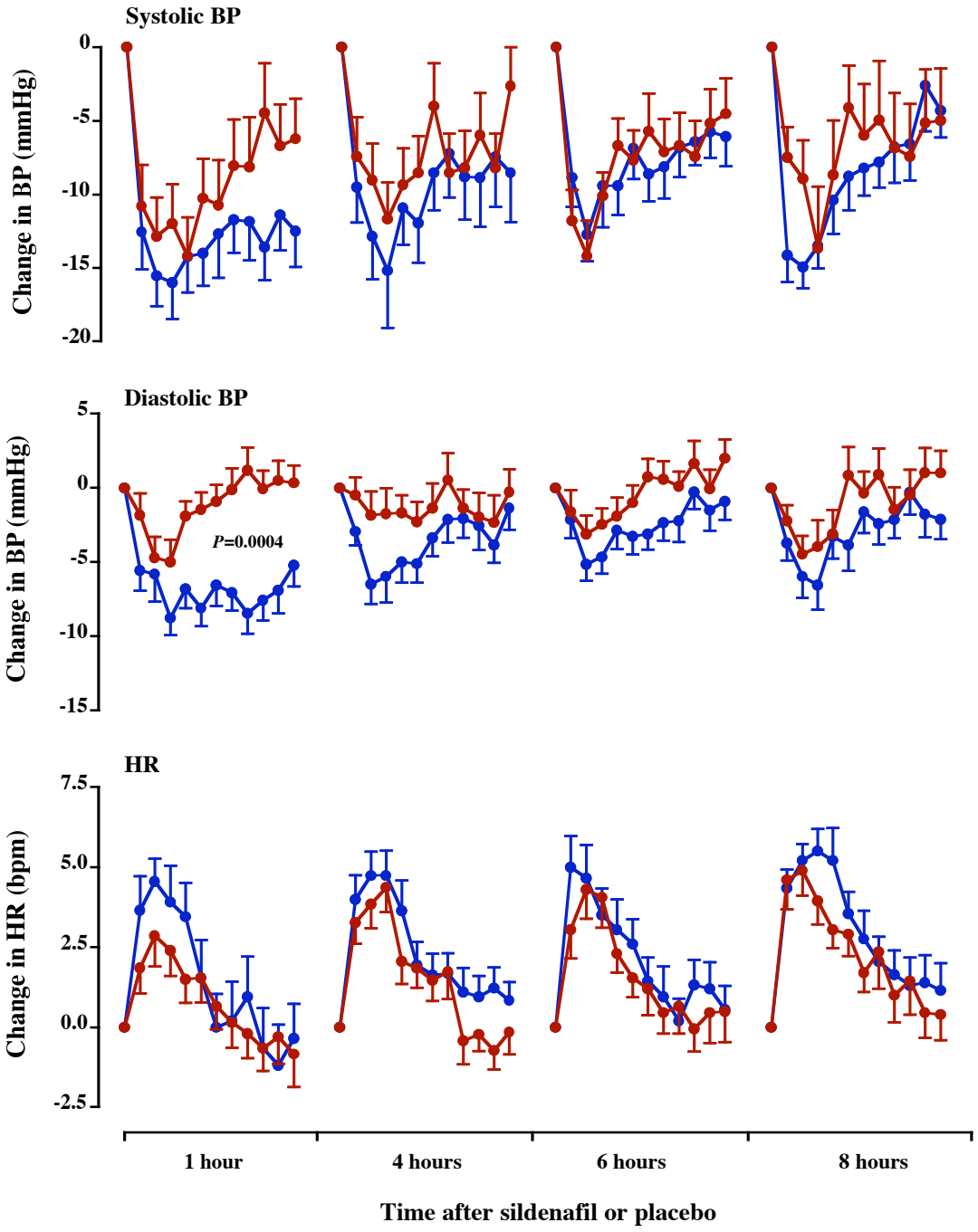


Figure 5.8. Mean changes in sitting BP and HR with GTN following sildenafil and placebo.
Sildenafil in blue and placebo in red. Changes are calculated from pre-GTN recordings. AUCs were compared statistically between sildenafil and placebo.

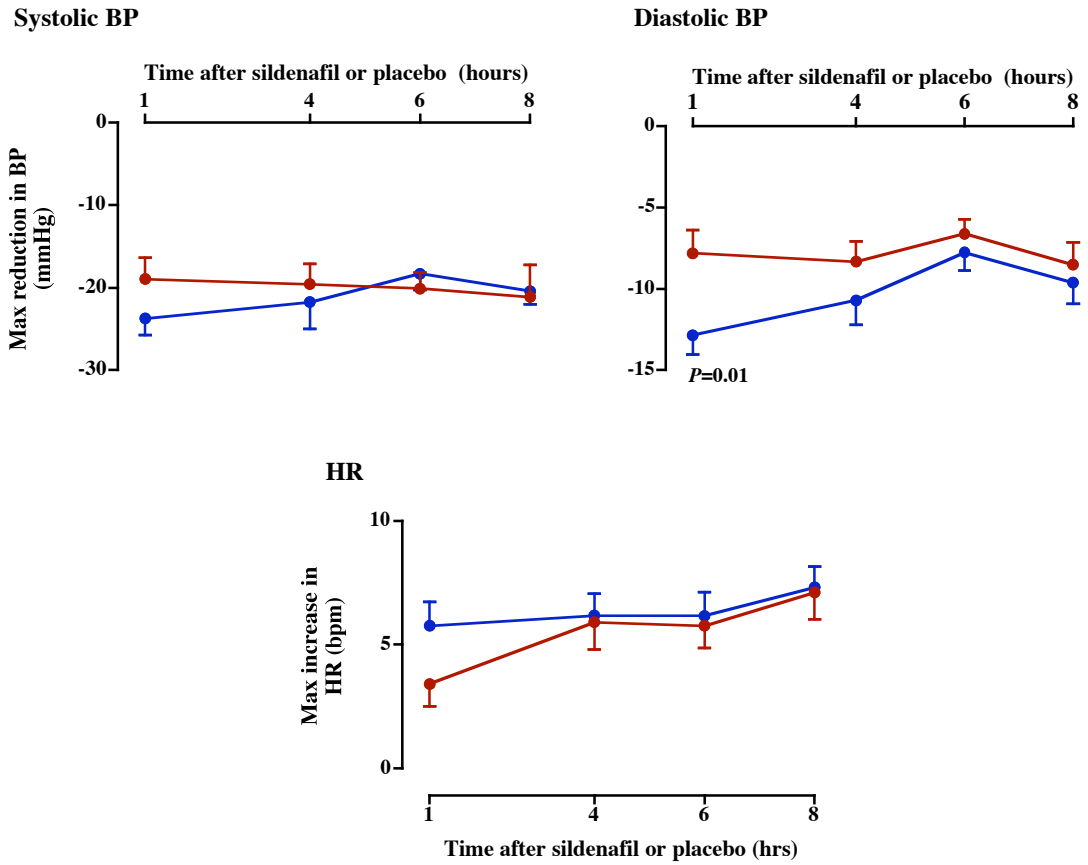


Figure 5.9. Mean maximum changes in sitting BP and HR with GTN given after sildenafil and after placebo.
Sildenafil in blue and placebo in red. Changes are from pre-GTN recordings.

At 1 hour, sildenafil appeared to prolong the duration of GTN-induced reduction in systolic BP. However, there were no statistically significant differences between sildenafil and placebo in the change in systolic BP from pre-GTN values at any time point, whether analysed as maximum reduction or as AUC. At 1 hour, the change in diastolic BP from the pre-GTN value was greater with sildenafil than with placebo, whether measured as the maximum reduction or as the AUC and, as with systolic BP, sildenafil appeared to prolong the duration of GTN action. At other time points there were no statistically significant differences between sildenafil and placebo in the change in diastolic BP from pre-GTN values. There were also no statistically significant differences between sildenafil and placebo in the change in HR from pre-

GTN values at any time point. Table 5.6 shows how often systolic BP was reduced by more than 20 mmHg, 30 mmHg and 40 mmHg and how often diastolic BP was reduced by more than 10 mmHg, 15 mmHg and 20 mmHg from pre-GTN values at some point during the monitoring period after each GTN challenge.

	Systolic BP fall >20 mmHg				Systolic BP fall >30 mmHg				Systolic BP fall >40 mmHg			
	1 hr*	**4 hr**	**6 hr**	**8 hr**	**1 hr**	**4 hr**	**6 hr**	**8 hr**	**1 hr**	**4 hr**	**6 hr**	**8 hr***
Sildenafil	15 (75)	8 (42)	8 (40)	9 (45)	3 (15)	5 (26)	1 (5)	3 (15)	1 (5)	3 (16)	1 (5)	0 (0)
Placebo	9 (45)	8 (40)	11 (55)	6 (30)	3 (15)	4 (20)	3 (15)	5 (25)	0 (0)	0 (0)	0 (0)	5 (25)

	Diastolic BP fall >10 mmHg				Diastolic BP fall >15 mmHg				Diastolic BP fall >20 mmHg			
	1 hr*	**4 hr**	**6 hr**	**8 hr***	**1 hr**	**4 hr***	**6 hr**	**8 hr**	**1 hr**	**4 hr**	**6 hr**	**8 hr**
Sildenafil	13 (65)	9 (47)	4 (20)	8 (40)	3 (15)	4 (21)	2 (10)	2 (10)	1 (5)	1 (5)	0 (0)	1 (5)
Placebo	7 (35)	5 (25)	2 (10)	7 (35)	3 (15)	2 (10)	0 (0)	2 (10)	1 (5)	1 (5)	0 (0)	1 (5)

Table 5.6. Frequency with which BP was reduced by different thresholds with each GTN challenge.
Numbers (percentages) of subjects whose sitting BP fell, from pre-GTN values, by more than various thresholds at some point in the 33-minute monitoring period after each GTN challenge. Comparisons between sildenafil and placebo were made of the proportions of subjects in whom BP fell by more than each of the thresholds (if significant indicated as *P<0.05 or **P<0.01).

When the data from each of the GTN challenges following sildenafil administration were combined the maximum reductions in both systolic BP and diastolic BP from pre-GTN values were significantly correlated with pre-GTN values (Figure 5.10). The correlations at each time point are shown in Table 5.7.

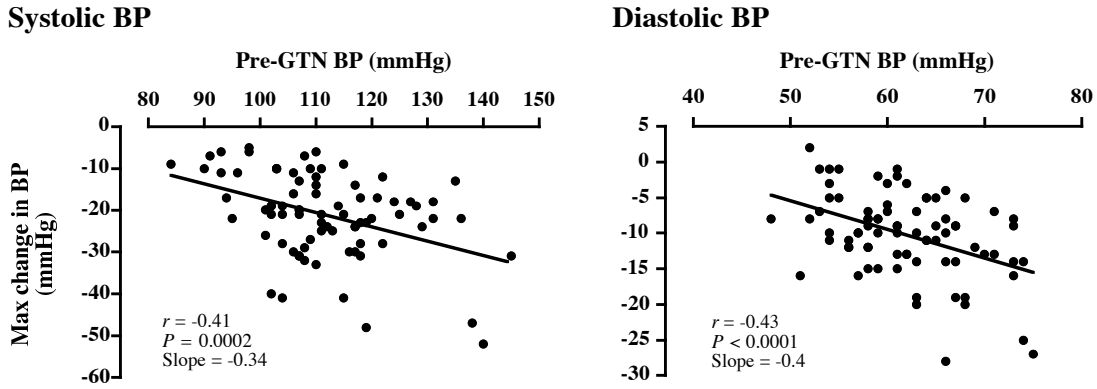


Figure 5.10. Relationships between pre-GTN BP and maximum change in BP from pre-GTN values after GTN challenge following sildenafil administration. Data from all 4 GTN challenges are included.

		Pearson *r*	*P* value
Systolic BP	**1 hr**	-0.4	0.08
	4 hr	-0.57	**0.01**
	6 hr	-0.25	0.29
	8 hr	-0.42	0.06
Diastolic BP	**1 hr**	-0.46	**0.04**
	4 hr	-0.26	0.3
	6 hr	-0.32	0.16
	8 hr	-0.6	**0.006**

Table 5.7. Correlations between pre-GTN BP and maximum change in BP from pre-GTN values after each GTN challenge following sildenafil administration.

5.3.3 Standing BP and HR

Symptomatic hypotension prevented standing measurements being made in 2 subjects 1 hour after sildenafil, but the data set was complete at all other time points. The effects on standing BP and HR of GTN with sildenafil and GTN with placebo are shown in Figure 5.11. Compared to the combination of GTN and placebo, the combination of GTN and sildenafil resulted in greater mean reductions in standing systolic BP and standing diastolic BP at all time points except systolic BP at 4 hours. There was also a greater increase in standing HR with the combination of GTN and sildenafil, although this achieved statistical significance only at 1 hour.

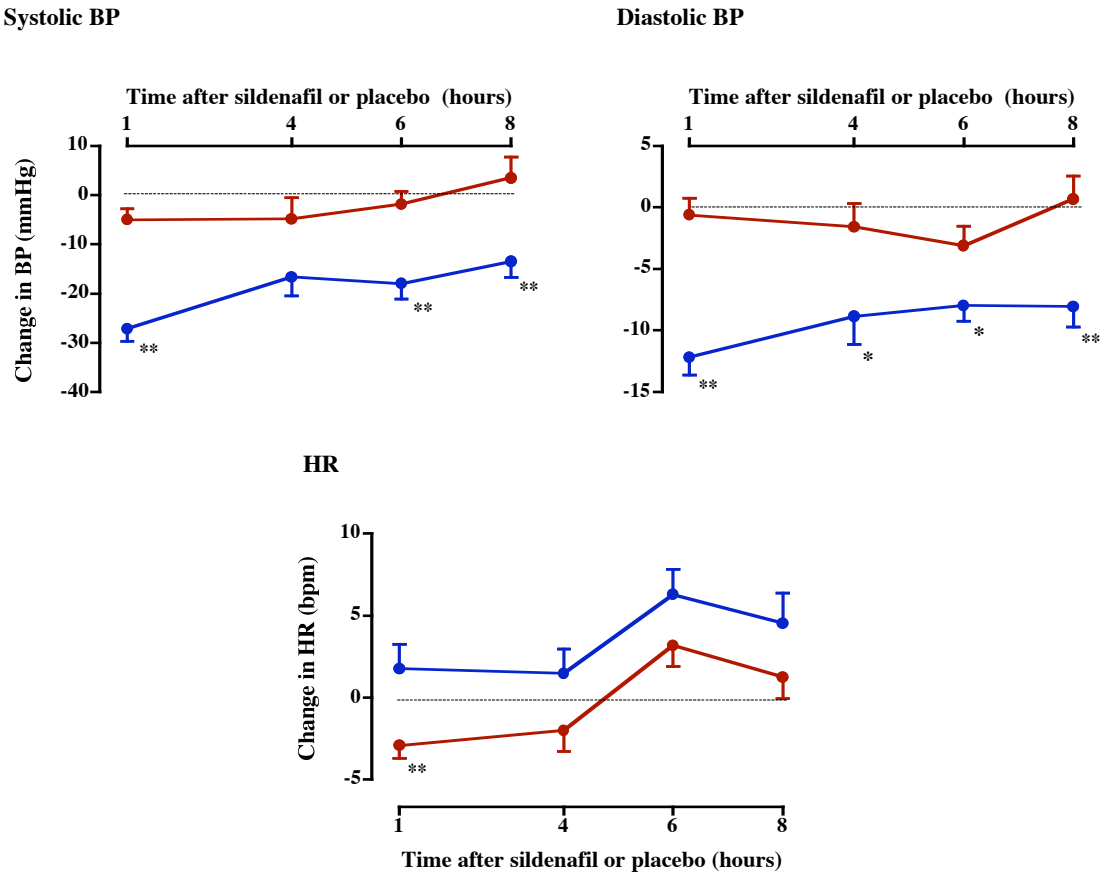


Figure 5.11. Mean changes in standing BP and HR with sildenafil and placebo. Sildenafil in blue and placebo in red. *P<0.05, **P<0.01.

There was no interaction, by ANOVA, between treatment and treatment order for any of the parameters, either sitting or standing, at any of the time points.

5.3.4 Side effects

Symptoms of hypotension (described as feeling dizzy or light headed, or having a "fuzzy" head) following GTN occurred in 8 subjects during the sildenafil phase but none of the subjects during the placebo phase. Of the 8 subjects who experienced hypotensive symptoms with sildenafil, 6 experienced them at the 1 hour GTN challenge (3 of whom needed to be lain supine with the foot of the bed elevated) and 3 experienced them at the 4 hour GTN challenge (2 of whom needed to be lain supine with the foot of the bed elevated). None of the subjects experienced hypotensive symptoms at the 6 and 8 hour GTN challenges. Hypotensive symptoms were not reported by any subject outwith the monitoring periods following each GTN administration. It can be seen from Figure 5.12 that those who had to be lain supine because of hypotensive symptoms tended to be those whose BPs fell to the lowest levels, especially at the 1 hour GTN challenge.

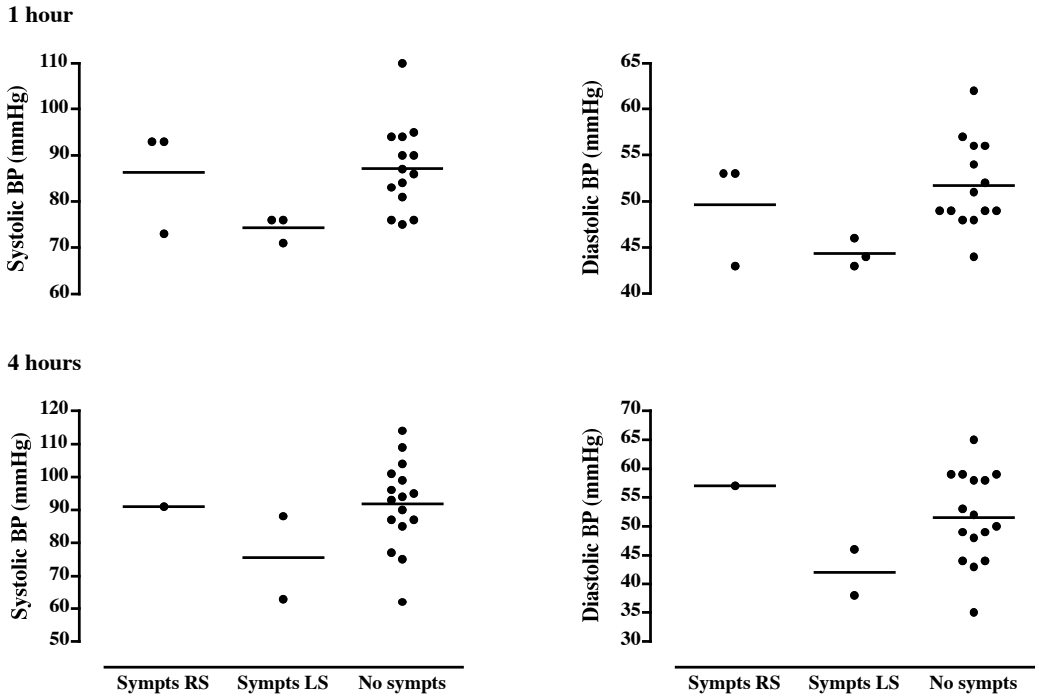


Figure 5.12. Minimum BP and hypotensive symptoms.
Scatter plot of minimum systolic (left) and diastolic (right) BPs after GTN challenges at 1 (top) and 4 hours (bottom) after sildenafil, stratified according to whether there were hypotensive symptoms but the subject remained sitting (Sympts RS), there were hypotensive symptoms requiring the subject to be lain supine (Sympts LS) or there were no hypotensive symptoms (No sympts). Horizontal lines are mean values.

A headache or “thick” head was experienced by 7 subjects after sildenafil (in 4 instances temporally related to GTN administration) and 3 subjects after placebo (in 1 instance temporally related to GTN administration and in a further instance a pre-existing headache was worsened with GTN). Indigestion/heartburn, stuffy nose and facial flushing were each experienced by 2 subjects following sildenafil but no subjects after placebo. One subject experienced a subconjunctival haemorrhage following sildenafil. This did not affect vision and resolved over several weeks, as would be expected. One subject experienced an episode of sickness, facial pain and headache that did not start until 1 hour following discharge (about 8 hours after sildenafil).

5.4 DISCUSSION

5.4.1 Main findings

In the present study there was a greater mean maximum reduction in BP with oral sildenafil 100 mg and GTN 400 μg than with matched placebo and GTN 400 μg for at least 8 hours after sildenafil administration, although the magnitude of this difference was greater at 1 and 4 hours than at 6 and 8 hours. In addition, sildenafil alone significantly reduced BP compared to placebo at all time points.

5.4.2 An additive or synergistic interaction?

That sildenafil alone significantly reduced BP, raises the question as to the degree to which the interaction on BP that was observed with the combination of sildenafil and GTN could be accounted for by additive effects of the two drugs. In this respect, analysis of the effects of GTN on BP from pre-GTN values provides some insight into nature of the interaction at each time point. At 6 and 8 hours the acute change in systolic and diastolic BPs from pre-GTN values was no different between sildenafil and placebo phases, suggesting that the overall greater reduction in BP observed with sildenafil and GTN compared to placebo and GTN was, at least on average, no more than additive. At 4 hours there was a trend, albeit statistically non-significant, to a greater reduction in diastolic BP with GTN following sildenafil than following placebo, perhaps suggesting that the interaction was more than simply additive (i.e. a possible degree of synergism). At 1 hour the reduction in BP, especially diastolic BP, with GTN was clearly greater following sildenafil than following placebo, suggesting a synergistic interaction between the two drugs. However, it should be noted that the study design does not allow for precise determination of the nature of the interaction in terms of additive or synergistic effects. Thus, the effects of sildenafil on BP were only measured at 1, 4, 6 and 8 hours (pre-GTN values). Although the peak plasma concentrations of sildenafil generally occur 1 hour after oral administration it is possible that its effect on BP continued to increase after 1 hour, potentially accounting for at least some of the difference observed between sildenafil and placebo phases in the change in diastolic BP following GTN at this time. In order to determine if this was the case, the effects of sildenafil with placebo

GTN and placebo sildenafil with placebo GTN would also have needed to be studied. This would have doubled the size of the study and meant that subjects would have had to attend eight long days, making it practically much more difficult to achieve. Moreover, these additional arms would not have altered the conclusion that the interaction between sildenafil and GTN on BP is, on average, no more than additive from 6 hours after sildenafil.

The discussion on whether the interaction is additive or synergistic at different time points has important clinical relevance. When treating a patient with acute angina who has recently taken sildenafil, the likely effect of GTN on BP will be the major factor in determining whether it might be safe to administer GTN. Although the BP at presentation will probably be lower than the patient's usual BP, due to the effect of sildenafil alone, if the expected further reduction in BP with GTN is likely to be no greater than would be expected if sildenafil had not been taken, then cautious use of GTN could be carefully considered. In contrast, if the interaction between sildenafil and GTN is synergistic then the potential for severe hypotension would mean that GTN should not be administered.

5.4.3 Symptoms of hypotension

Consistent with the effects on BP, symptoms suggestive of hypotension occurred at 1 hour and 4 hours, although more commonly at 1 hour, but not at 6 and 8 hours after sildenafil. It is notable that of the 3 subjects who experienced hypotensive symptoms at 4 hours, only 1 had also experienced them at 1 hour, suggesting variability between individuals in the time to peak interaction rather than some individuals being susceptible to the interaction for prolonged periods.

5.4.4 Comparison to effects in healthy men

In contrast to the current study, in the previous similar study in healthy men the interaction on maximum reduction in sitting BP between sildenafil and GTN only occurred at 1 hour after sildenafil (Figure 5.1). At subsequent time points (including 4, 6, and 8 hours) no interaction was observed. In this study sildenafil alone reduced diastolic BP at 1 hour but did not affect BP at other times (Figure 5.13).

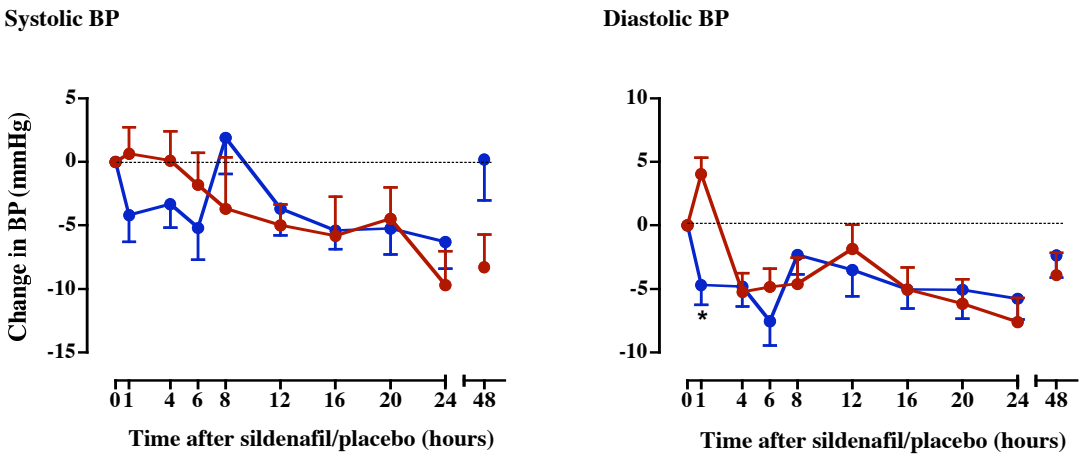


Figure 5.13. Effect of sildenafil and placebo alone on sitting BP and HR in healthy men.
Sildenafil in blue and placebo in red. Measures are those taken before each GTN challenge. *P<0.05.

Given that the interaction between sildenafil and GTN in angina patients was generally no more than additive at 6 and 8 hours, it is likely that the difference between the groups in the effect on BP of sildenafil alone largely explains the different effects on mean maximum reductions in BP between the studies. There are a number of differences between the angina patient group and the healthy volunteer group that may have contributed to their different haemodynamic responses to sildenafil. For example, in addition to the presence of CAD, in the present study the subjects were older (mean age 66 years vs 49 years) and were taking a variety of vasoactive and other medicines.

5.4.5 Standing BP

There was a significant interaction on standing BP, both systolic and diastolic, for up to 8 hours, although this was most marked at 1 hour. When given after placebo GTN had little, if any, effect on standing BP and it is likely that this is because standing BP was not assessed until 36 min after GTN administration. Standing BP was not assessed before each GTN challenge (so as not to disturb pre-GTN sitting measures) and it is, therefore, not possible to formally to assess whether the interaction between the 2 drugs was additive or synergistic. However, the greater magnitude of the

interaction at 1 hour might suggest a degree of synergism at this time point. It should be noted that although effects on standing BP may be of academic interest, they are not relevant to the clinical situation because patients with acute angina will be kept supine or semi-recumbent.

5.4.6 HR

Sildenafil alone did not significantly affect HR at any time point. The increase in HR with GTN was greater with sildenafil than with placebo, both sitting and standing, only at 1 hour. Although the magnitude of the difference was small and unlikely to be of clinical significance in itself, it is consistent with a compensatory response to the potentially synergistic hypotensive interaction between sildenafil and GTN that occurred at this time point. The mean maximum increase in HR with the combination of sildenafil and GTN was around 5 to 10 bpm. In the previous healthy volunteer study the mean maximum increase in HR was greater, around 16 to 25 bpm. All but one of the angina patients in the current study were taking a ß-blocker and this may explain the difference in HR responses between the studies.

5.4.7 Is it safe to administer sublingual GTN from 6 hours after sildenafil in clinical practice?

Although the data from the present study indicate that the interaction between sublingual GTN and sildenafil is on average no more than additive from 6 hours after sildenafil, it is important to note that the possibility of clinically significant hypotension occurring in some patients when GTN is administered from 6 hours after sildenafil cannot be excluded. At 6 hours systolic BP fell to less than 90 mmHg in 10 (50%) subjects after sildenafil but only 1 (5%) subject after placebo. At 6 and 8 hours systolic BP fell to less than 80 mmHg in 2 (10%) subjects after sildenafil whereas after placebo a systolic BP of less than 80 mmHg occurred in 1 (5%) subject at 6 hours and no subjects at 8 hours. At 6 and 8 hours diastolic BP fell to less than 50 mmHg in 6 (30%) subjects after sildenafil and 1 (5%) subject after placebo. Similarly, at 6 and 8 hours systolic BP fell by more than 30 mmHg or 40 mmHg and diastolic BP by more than 20 mmHg or 25 mmHg in more subjects after sildenafil than after placebo. The lack of statistical significance in the difference in the

frequencies with which systolic BP fell to less than 80 mmHg or by more than 30 or 40 mmHg and diastolic BP fell to less than 50 mmHg or by more than 20 or 25 mmHg at 6 and 8 hours should not be interpreted as there being no clinically significant difference in hypotensive effect at these times. The study was relatively small and was not powered to detect an increased frequency of relatively uncommon, let alone rare, severe hypotension. On the other hand, there was no consistent tendency at 6 and 8 hours for systolic BP to fall more than 20, 30 or 40 mmHg or for diastolic BP to fall more than 10, 15 or 20 mmHg from pre-GTN values with any greater frequency with sildenafil than with placebo, although, once again, a larger sample size would allow for greater confidence in this.

Overall, the hypotensive interaction between sildenafil and GTN is, on average, relatively diminished and not synergistic from 6 hours after sildenafil, but there is still a possibility of clinically significant hypotension in some patients given GTN up to 8 hours. Therefore, the study does not provide evidence that the recommended time interval between sildenafil and GTN should be reduced to less than 24 hours. However, these data are relatively reassuring and, when otherwise strongly indicated, cautious use of GTN could be considered in subjects with acute angina who have taken sildenafil at least 6 hours previously and who are haemodynamically stable. Bearing in mind that the subjects with lowest BPs at baseline were generally those in whom the lowest BPs occurred when sildenafil and GTN were co-administered, BP at presentation will be an important factor in the risk-benefit assessment under these clinical circumstances.

A number of other factors should be taken into consideration before administering GTN to a patient who has taken sildenafil. In this study, sublingual GTN was administered in a standard clinical dose of 400 μg. However, it is not uncommon for a dose of 800 μg to be used for acute angina and it should not be assumed that the time course of the interaction between sildenafil and GTN that we have characterised in this study would be similar for higher doses of GTN. It is possible that lower doses of either drug would be safer at earlier time points after sildenafil, although

this would need to be investigated specifically. Similarly, the data should not be extrapolated to the buccal or intravenous use of GTN.

Hepatic metabolism of sildenafil is reduced, and its plasma concentration increased, by a number of drugs, including cimetidine (Wilner *et al.*, 2002), macrolide antibiotics (Muirhead *et al.*, 2002a) and antifungal agents (Warrington *et al.*, 2002). Therefore, concomitant administration of these drugs could prolong the time after taking sildenafil that individuals would be susceptible to a significant interaction on BP with GTN.

The main outcome measure of the study was BP taken in the sitting position. When a patient presents to hospital with angina they are likely to be kept supine. Therefore, in this respect, our data may not be directly applicable to the clinical situation. However, we elected to measure sitting BP to maximise the observed effect on BP of sildenafil with GTN. If anything, lesser reductions in BP would have been expected in the supine position, so the conclusion that the interaction between sildenafil and GTN is, on average, no more than additive from 6 hours after sildenafil remains valid to the clinical situation.

Potential limitations

Subjects received two doses of GTN at each visit, either at 4 and 8 hours or at 1 and 6 hours after sildenafil or placebo. During the placebo phase of the study the effects of GTN on BP were no less at 8 and 6 hours than at 4 hours and 1 hour (Figure 5.5, page 98), indicating that no significant nitrate tolerance occurred following the first dose of GTN on each day.

When subjects experienced symptoms of hypotension it was often necessary to lie them supine and elevate the foot of the bed. Although this is clearly clinically appropriate, it has the potential to minimise the observed effect on BP – if subjects had remained sitting it is likely that their BP would have remained lower than in the supine position and may even have fallen further than the minimum BP that was actually recorded. However, this would not materially affect the main conclusion of

the study, as the differences between sildenafil and placebo would only be greater at 1 and 4 hours, whereas there would have been no effect on the data at 6 and 8 hours because it was not necessary to lie any subjects supine at these time points.

Subjects took their usual medicines, many of which were vasoactive, as normal during the study. These other medicines may have influenced the changes in BP that we observed with sildenafil and GTN, both alone and in combination. Indeed, as discussed above, concomitant use of other drugs may explain the difference in the effect on BP of sildenafil alone in the angina patients from the present study and the healthy volunteers from the previous study. However, withdrawing regular vasoactive medicines might have placed the participants at increased risk of symptomatic angina and, moreover, the study design was intended to be as applicable to the clinical situation as possible.

The 4 visits of the study were not completely randomised. Rather, GTN was administered at 4 and 8 hours after sildenafil or placebo during the first 2 visits and at 1 and 6 hours after sildenafil or placebo during visits 3 and 4 (the order in which sildenafil and placebo were given being randomised between visits 1 and 2 and visits 3 and 4). This design meant that there would have been an opportunity to prevent any subject who experienced severe hypotension at 4 hours from proceeding to visits 3 and 4 when they would have received GTN at 1 hour, in light of the data from the previous study the likely time of maximum interaction.

5.4.8 Summary

In men with stable angina there is an interaction on BP reduction between sildenafil 100 mg and sublingual GTN 400 μg for at least 8 hours after sildenafil administration, but this interaction is, on average, no more than additive from 6 hours after sildenafil administration.

CHAPTER 6

THE EFFECTS OF REGULAR SILDENAFIL ON BLOOD PRESSURE, ARTERIAL STIFFNESS, ARTERIAL WAVE REFLECTION AND ENDOTHELIAL VASOMOTOR FUNCTION IN UNTREATED HYPERTENSIVES

6.1 INTRODUCTION

6.1.1 Background

The major treatments for hypertension are diuretics, inhibitors of the renin-angiotensin system and non-NO-mediated vasodilators. The NO-cGMP pathway is not commonly targeted, even though NO is a potent vasodilator that tonically reduces systemic BP. The organic nitrates are NO-donor drugs but their use in hypertension is limited by the development of tolerance to their haemodynamic effects when given continuously (Munzel *et al*., 2005). Enhancement of the effects of endogenous NO by inhibiting PDE5 is a potential alternative approach to reducing BP in hypertension. Most studies have shown that PDE5 inhibitors reduce BP, both in healthy subjects and patients with vascular disease (see section 1.3.2). However, these have generally been single dose studies and in many, in particular those that recruited patients with or at risk of cardiovascular disease, sildenafil was administered in addition to other cardiovascular drugs. The effects on BP of PDE5 inhibition when given regularly as monotherapy for hypertension have not been investigated previously. Similarly, the effects of PDE5 inhibition on endothelium-dependent vasomotor function, arterial stiffness and arterial wave reflection have not been investigated under these conditions.

6.1.2 Aims

To investigate the effects of 16 days of treatment with sildenafil given 3 times daily on BP, endothelium-dependent vasomotor function, arterial stiffness and arterial wave reflection in otherwise untreated hypertensives.

6.2 METHODS

6.2.1 Subjects

6.2.1.1 Identification of subjects

Potentially suitable subjects were identified from patients attending nurse-led General Practice hypertension clinics, a database of subjects previously involved in research at the CRC, and from the WGH Cardiovascular Risk Clinic.

6.2.1.2 Inclusion criteria

- Male or female.
- At least 3 separate office measurements of systolic BP ≥160 mmHg or diastolic BP ≥100 mmHg, not taking antihypertensive drugs.
- Hypertension confirmed on ambulatory monitoring (average daytime systolic BP ≥145 mmHg or diastolic BP ≥95 mmHg), within 3 months of the screening visit.
- Subjects with 'borderline' hypertension were eligible if their calculated 10-year risk of cardiovascular disease was >20% or they had evidence of target organ damage. Borderline hypertension was defined according to ambulatory BP criteria alone as an average awake systolic BP ≥135 and <145 mmHg or diastolic BP ≥85 and <95 mmHg.
- Established hypertensives were eligible if their BP was controlled (systolic BP <160 mmHg and diastolic BP <100 mmHg) on a single antihypertensive agent (see section 6.2.1.4).

6.2.1.3 Exclusion criteria

- History of other major cardiac, respiratory, neurological or renal disease.
- Systolic BP consistently >210 mmHg or diastolic BP consistently >120 mmHg.
- Systolic BP consistently >180 mmHg or diastolic BP consistently >110 mmHg in those withdrawn from existing therapy .
- Current alcohol abuse.
- Diabetes.
- Taking vasoactive drugs.
- Previous serious drug allergy.

- Pregnant.
- Participation, within 6 months, in other research studies.

6.2.1.4 Procedure for recruiting patients taking treatment for hypertension

For any subjects who were identified as potentially suitable for the study who were already taking a regular antihypertensive, ABPM would have been performed 4 weeks after stopping treatment. They would have been eligible if, on ABPM, their average daytime BP was ≥145/95 mmHg. Office BP would have been recorded 2 and 4 weeks after stopping treatment, as well as during the study, and if systolic BP was consistently >180 mmHg or diastolic BP was consistently >110 mmHg subjects would have either not progressed or would have been withdrawn from the study and restarted on their usual therapy with immediate effect. After the study, subjects would have continued with their usual medicine as before (unless a clinical reason to change this was identified).

6.2.2 Screening visit

Potentially suitable subjects who agreed to be considered for the study attended a screening visit at the CRC. At this visit the study was explained fully and written consent was obtained. A medical history was taken and a physical examination and 12-lead ECG were performed. A non-fasting blood sample was also taken. A 24-hour ambulatory BP monitor was fitted to those subjects who had not had ambulatory BP monitoring performed in the previous 3 months.

6.2.3 Study design

Randomised, placebo-controlled, double blind, 2-way crossover.

6.2.4 Protocol

Subjects underwent the protocol outlined in Figure 6.1. Duplicate measures of BP, HR, PWA, and CF-PWV were made at each time point. The screening ambulatory BP was used as the baseline for both phases of the study.

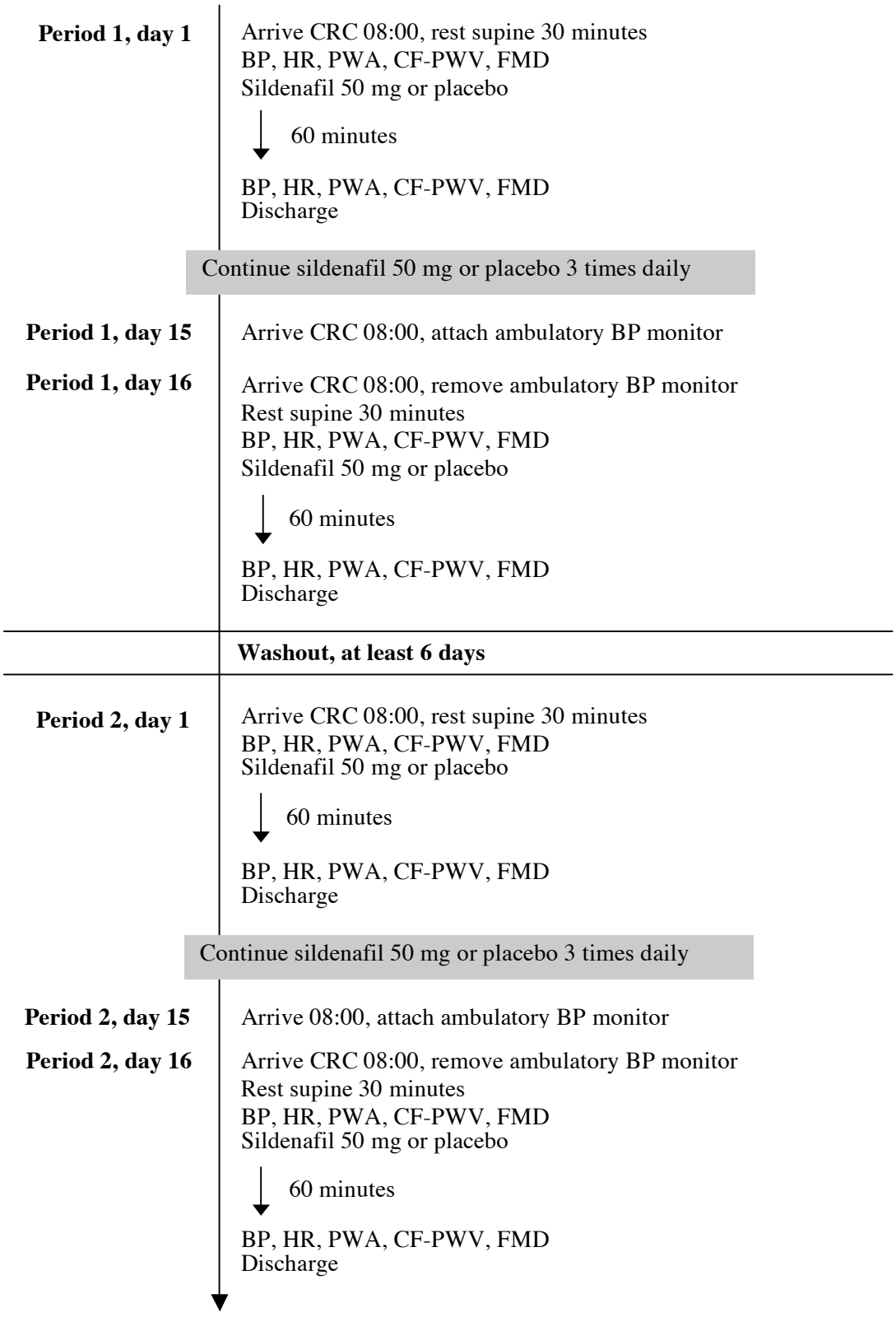


Figure 6.1. Study protocol.
BP, HR, PWA, CF-PWV and FMD were measured in the order shown.

6.2.5 Drugs

Tablets were packed by the WGH pharmacy. Subjects were instructed to take 1 tablet 3 times each day, at around 08:00, 14:00 and 22:00 hrs. They were given a diary card to record the time at which they took each tablet. During each phase of the study subjects were provided with a total of 46 tablets, 2 more than were actually required, and were asked to return all unused tablets.

6.2.6 Adverse effects

Subjects were issued with cards on which they were asked to write down any symptoms that they experienced during each period of the study and rate them as mild, moderate or severe. Details of symptoms experienced were clarified when subjects attended the CRC.

6.2.7 Analyses

Data are given as means and SDs. Means were compared by paired Student's *t*-tests. Correlation coefficients were calculated using the Pearson method.

The number of tablets returned was used to determine the 'maximum adherence', calculated as $\frac{100 \times (number\ returned - 2)}{44}$ and expressed as a percentage. Thus, a subject who returned 2 tablets may have taken all 44 tablets and would have a maximum adherence of 100%. A subject who returned 6 tablets can only have taken up to 40 of the 44 tablets and would have a maximum adherence of 91%.

6.3 RESULTS

6.3.1 Subjects

Thirty-six subjects, none of whom were taking regular antihypertensive treatment, underwent screening to assess eligibility for the study. Thirty subjects were identified from General Practice hypertension clinics, 4 from the CRC database and 2 from the WGH Cardiovascular Risk Clinic. Of the 36 screened, 10 did not meet the inclusion and exclusion criteria and 1 subject withdrew before starting the study. Of the 25 subjects who started the study, 3 were withdrawn due to side effects. Analyses were performed on the data from the remaining 22 subjects. The baseline characteristics of the subjects are given in Table 6.1.

Table 6.1. Baseline characteristics of the subjects.
Means (SDs) are given for continuous variables. Central BP, CF-PWV and wave reflection variables are a mean of the values obtained at baseline at each of the 2 study visits. Other values are those obtained at the screening visit.

	Subjects who completed (n=22)	**Subjects withdrawn (n=3)**
Male/female	18/4	1/2
Age (years)	60 (12)	60 (17)
24-hour BP (mmHg)	144 (7) / 84 (7)	149 (4) / 87 (8)
Daytime BP (mmHg)	153 (8) / 92 (6)	157 (8) / 94 (11)
Night time BP (mmHg)	124 (9) / 71 (7)	137 (8) / 75 (6)
Office BP (mmHg)	165 (16) / 96 (9)	179 (25) / 102 (15)
Central BP (mmHg)	144 (16) / 89 (7)	154 (20) / (98 (5)
Aortic AIx (%)	32 (9)	33 (11)
Aortic AIx@75 (%)	25 (8)	29 (8)
Radial AIx (%)	89 (13)	89 (16)
CF-PWV (m/s)	10.3 (2.6)	9.0 (0.4)
Weight (Kg)	89 (12)	87 (13)
BMI (Kg/m^2)	29 (3)	30 (5)

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Table 6.1
Continued from the previous page.

	Subjects who completed (n=22)	**Subjects withdrawn (n=3)**
LVH	1	0
Plasma glucose (mmol/L)	5.3 (0.6)	5.9 (1.2)
Serum cholesterol:		
Total (mmol/L)	5.4 (0.7)	5.9 (0.2)
LDL (mmol/L)	3.2 (0.7)	3.9 (0.3)
HDL (mmol/L)	1.3 (0.3)	1.3 (0.4)
Total:HDL ratio	4.5 (1.3)	5.0 (1.4)
Serum triglyceride (mmol/L)	2.0 (1.2)	1.6 (0.2)

6.3.2 Repeatability

There was good repeatability between the 2 baseline measures (placebo phase and sildenafil phase) for the various clinical parameters recorded during the study. Figure 6.2 shows Bland-Altman plots for peripheral clinic BP and central BP and Figure 6.3 shows Bland-Altman plots for arterial wave reflection parameters, CF-PWV and FMD.

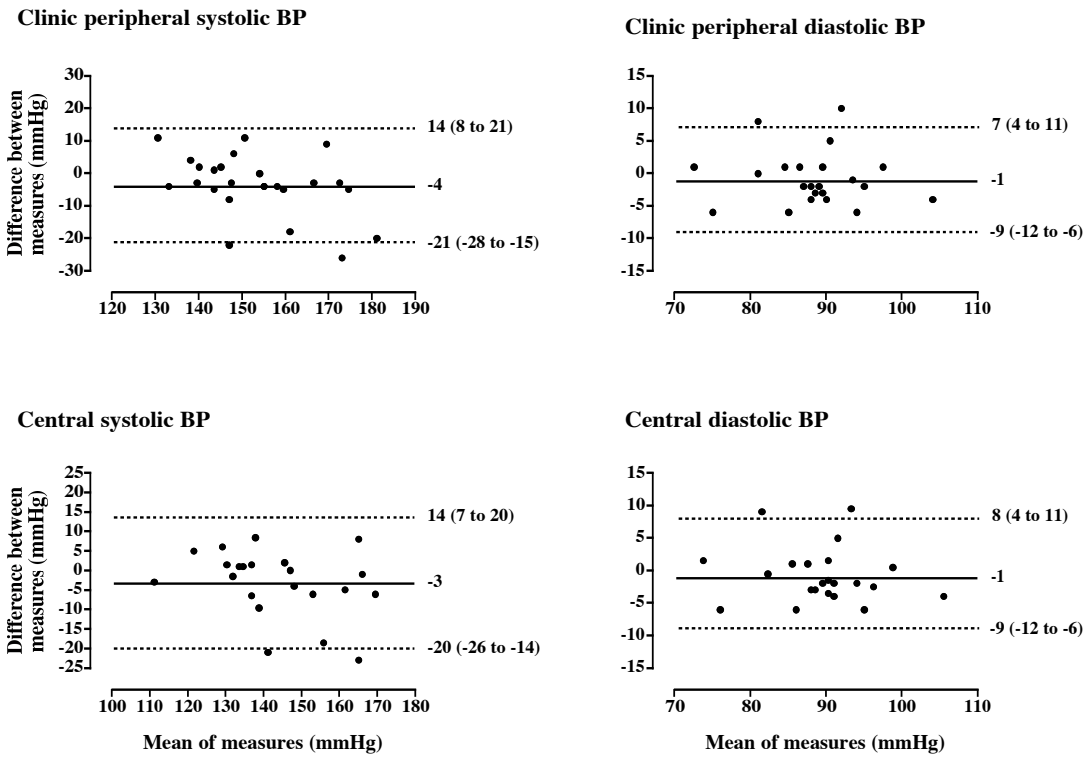

Figure 6.2. Bland-Altman plots of baseline measures of peripheral clinic BP and central BP.
Solid lines are mean differences and dotted lines are upper and lower 95% limits of agreement (for which 95% CIs are given in brackets).

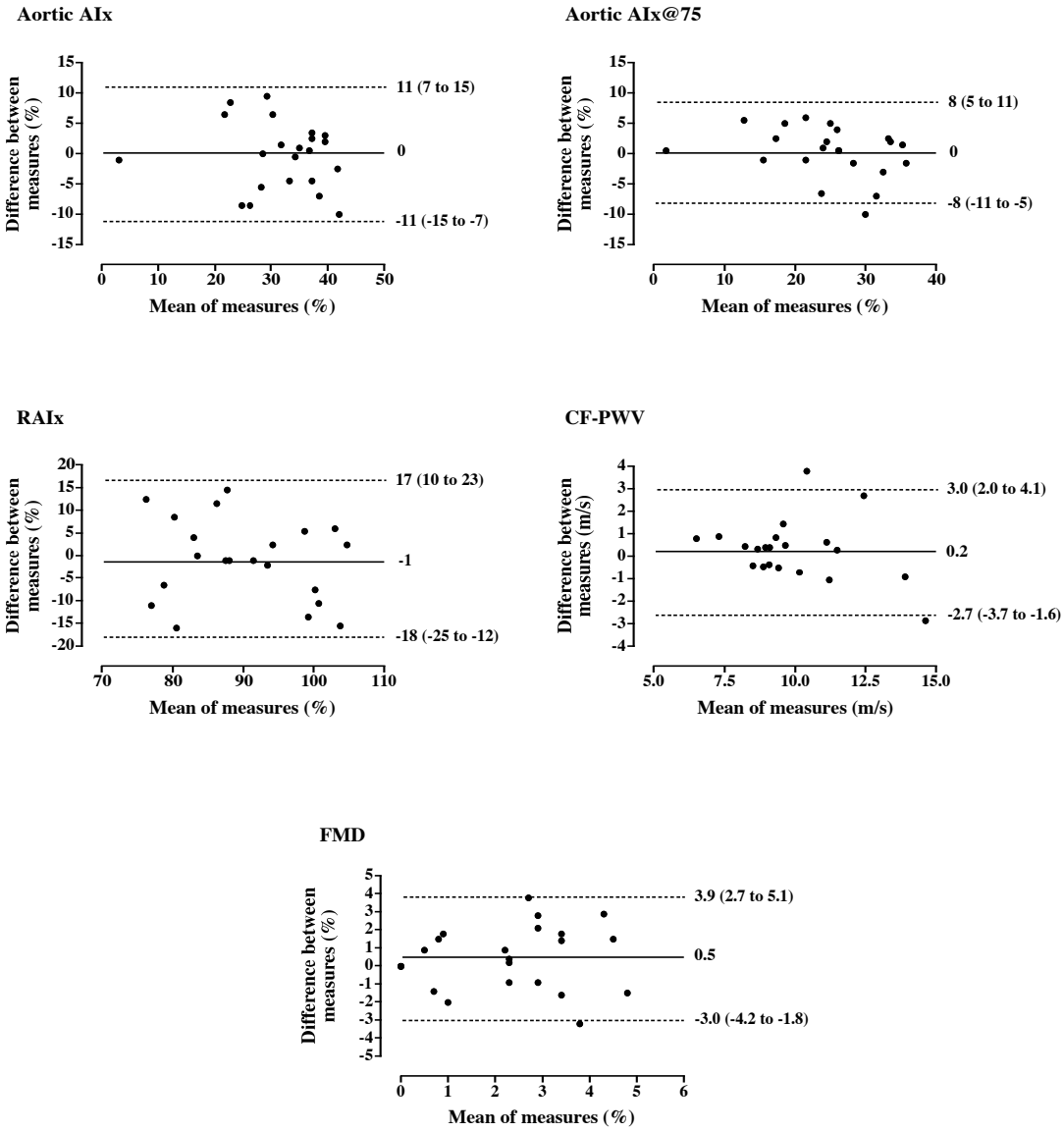

Figure 6.3. Bland-Altman plots of baseline measures of aortic AIx, aortic AIx@75, RAIx, CF-PWV and FMD.
Solid lines are mean differences and dotted lines are upper and lower 95% limits of agreement (for which 95% CIs are given in brackets).

6.3.3 Office BP and HR

Baseline office systolic BP, diastolic BP, MAP, PP and HR were not significantly different between placebo and sildenafil phases of the study.

The effects of sildenafil and placebo on office BP and HR are summarised in Table 1.1 (absolute values) and Table 1.2 (changes). Sildenafil reduced office systolic BP, diastolic BP and MAP acutely (1 hour after administration), but by day 16 of regular treatment the magnitude of this reduction in BP was reduced compared to the acute effect. Office diastolic BP and MAP were significantly lower than at baseline when measured prior to sildenafil administration on day 16 (trough), but systolic BP was not. Office systolic BP, diastolic BP and MAP were all significantly lower than baseline when measured 1 hour after sildenafil administration on day 16 (peak). Sildenafil did not affect office PP or HR, either acutely or after 16 days of regular treatment.

		Placebo			Sildenafil		
		Mean (SD)	*P* value, vs:		Mean (SD)	*P* value, vs:	
			baseline	trough, d 16		baseline	trough, d 16
Systolic BP (mmHg)	**Baseline**	154 (15)			150 (12)		
	1 hour	155 (15)	0.5		141 (13)	**<0.001**	
	Trough, d 16	152 (15)	0.3		146 (13)	0.1	
	Peak, d 16	158 (18)	0.07	**0.004**	145 (13)	**0.04**	0.7
Diastolic BP (mmHg)	**Baseline**	89 (7)			88 (7)		
	1 hour	90 (8)	0.2		82 (9)	**<0.001**	
	Trough, d 16	88 (8)	0.8		85 (7)	**0.003**	
	Peak, d 16	90 (9)	0.3	0.06	83 (8)	**<0.001**	0.1
MAP (mmHg)	**Baseline**	113 (10)			111 (8)		
	1 hour	113 (10)	0.7		102 (11)	**<0.001**	
	Trough, d 16	112 (10)	0.4		107 (9)	**0.006**	
	Peak, d 16	115 (12)	0.3	**0.03**	105 (9)	**0.001**	0.4

Continued on next page

Table 6.2. Effects of sildenafil and placebo on office BP and HR, absolute values.
Statistically significant comparisons are highlighted in bold; d = day.

		Placebo			Sildenafil		
		Mean (SD)	*P* value, vs:		Mean (SD)	*P* value, vs:	
			baseline	trough, d 16		baseline	trough, d 16
PP (mmHg)	**Baseline**	65 (13)			62 (12)		
	1 hour	66 (15)	0.7		59 (10)	0.07	
	Trough, d 16	63 (13)	0.2		62 (12)	0.7	
	Peak, d 16	68 (15)	0.2	**0.005**	62 (11)	0.98	0.6
HR (bpm)	**Baseline**	61 (8)			62 (9)		
	1 hour	58 (7)	**0.001**		63 (8)	0.5	
	Trough, d 16	61 (7)	0.8		62 (7)	0.8	
	Peak, d 16	60 (7)	0.3	0.2	61 (7)	0.3	0.1

Table 6.2
Continued from previous page.

		Placebo	Sildenafil	*P* value
Systolic BP (mmHg)	**Baseline to 1 hour**	1 (9)	-10 (10)	**<0.001**
	Baseline to trough, d 16	-2 (8)	-4 (11)	0.4
	Baseline to peak, d 16	4 (10)	-5 (11)	**0.003**
	Trough, d 16 to peak, d 16	6 (9)	-1 (10)	**0.01**
Diastolic BP (mmHg)	**Baseline to 1 hour**	2 (6)	-6 (6)	**<0.001**
	Baseline to trough, d 16	0 (5)	-3 (5)	0.06
	Baseline to peak, d 16	2 (7)	-5 (5)	**0.002**
	Trough, d 16 to peak, d 16	2 (4)	-2 (6)	**0.04**
MAP (mmHg)	**Baseline to 1 hour**	1 (7)	-8 (7)	**<0.001**
	Baseline to trough, d 16	-1 (6)	-4 (6)	0.1
	Baseline to peak, d 16	2 (8)	-5 (7)	**0.003**
	Trough, d 16 to peak, d 16	3 (6)	-1 (7)	**0.03**

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Table 6.3. Changes in office BP and HR with sildenafil and placebo.
Statistically significant comparisons are highlighted in bold; d = day.

		Placebo	Sildenafil	*P* value
PP (mmHg)	**Baseline to 1 hour**	1 (9)	-3 (8)	0.1
	Baseline to trough, d 16	-2 (7)	-1 (10)	0.7
	Baseline to peak, d 16	2 (8)	0 (8)	0.3
	Trough, d 16 to peak, d 16	4 (6)	1 (7)	0.1
HR (bpm)	**Baseline to 1 hour**	-4 (5)	0 (3)	**<0.001**
	Baseline to trough, d 16	0 (5)	0 (5)	0.7
	Baseline to peak, d 16	-1 (6)	-1 (6)	0.9
	Trough, d 16 to peak, d 16	-1 (4)	-1 (5)	0.7

Table 6.3.
Continued from previous page.

6.3.4 Ambulatory BP

The effects of 16 days of treatment with sildenafil and placebo on 24-hour, daytime and night time ambulatory BPs are shown in Table 6.4 (absolute values) and Table 6.5 (changes). Sildenafil significantly reduced both systolic and diastolic ambulatory 24-hour, daytime and night time BPs compared both to baseline and to placebo.

Baseline ambulatory systolic BP, whether 24-hour, daytime or night time, was significantly correlated with the change in ambulatory systolic BP that occurred after 16 days of sildenafil, with increasing baseline BP being associated with a greater reduction with treatment. In contrast, baseline ambulatory diastolic BP was not correlated with the change in ambulatory diastolic BP that occurred with sildenafil. These data are shown in Figure 6.4.

		Baseline (B)	Placebo (P)	Sildenafil (S)	*P* value, B vs P	*P* value, B vs S
24-hour	**Systolic BP**	144 (7)	147 (7)	138 (8)	0.2	**0.003**
	Diastolic BP	85 (7)	87 (10)	80 (9)	0.3	**<0.001**
Daytime	**Systolic BP**	153 (8)	155 (7)	145 (8)	0.4	**0.001**
	Diastolic BP	92 (6)	92 (10)	86 (8)	0.9	**<0.001**
Night time	**Systolic BP**	124 (9)	129 (10)	119 (9)	0.05	**0.02**
	Diastolic BP	71 (7)	74 (11)	68 (9)	0.08	**0.01**

Table 6.4. Effects of sildenafil and placebo on ambulatory BP (mmHg), absolute values.

	Systolic BP			Diastolic BP		
	Placebo	**Sildenafil**	***P* value**	**Placebo**	**Sildenafil**	***P* value**
24-hour	3 (10)	-7 (9)	**<0.001**	1 (6)	-5 (5)	**<0.001**
Daytime	2 (9)	-8 (9)	**<0.001**	0 (6)	-6 (5)	**<0.001**
Night time	5 (12)	-5 (9)	**0.02**	3 (8)	-4 (6)	**0.01**

Table 6.5. Effects of sildenafil and placebo on ambulatory BP (mmHg), changes from baseline.

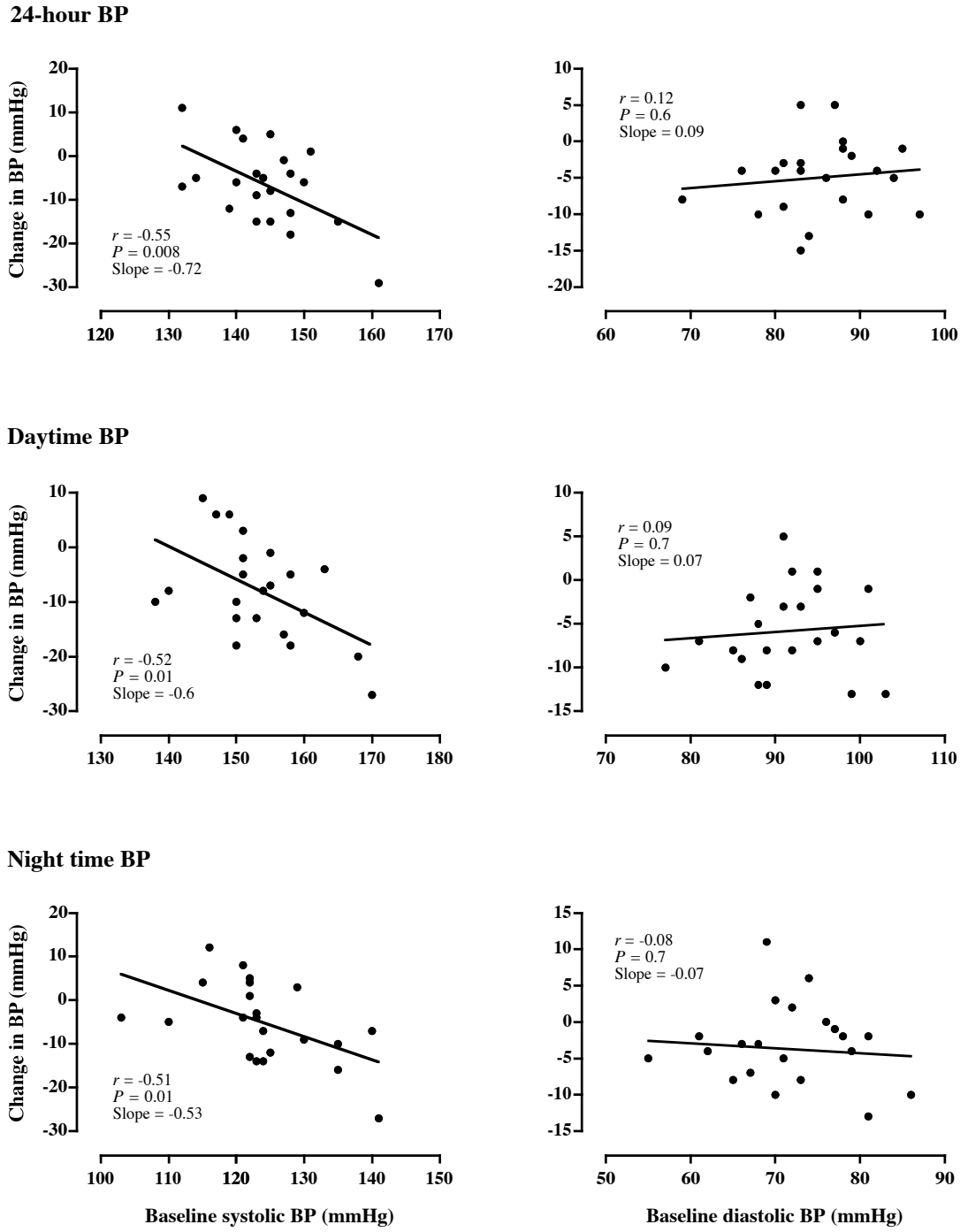

Figure 6.4. Correlations between baseline ambulatory BP and change with sildenafil.
Systolic BP on the left and diastolic BP on the right.

6.3.5 Pulse wave analysis

Baseline aortic AIx, aortic AIx@75, RAIx, central systolic BP, central diastolic BP and central PP were not significantly different between placebo and sildenafil phases of the study. The effects of placebo and sildenafil on each of these are summarised in Table 6.6 (absolute values) and Table 6.7 (changes). Sildenafil reduced aortic AIx, aortic AIx@75 and RAIx acutely (1 hour after administration), but as with the effects on office BP, by day 16 the magnitude of these effects was reduced compared to the acute effects. These parameters were not significantly different to baseline when measured prior to sildenafil administration on day 16 (trough) but were significantly lower than baseline when measured 1 hour after sildenafil administration on day 16 (peak). The changes from baseline in aortic AIx, aortic AIx@75 and RAIx with sildenafil were significantly different to placebo acutely but were not significantly different to placebo either before or after sildenafil administration on day 16.

Sildenafil reduced central systolic BP and central diastolic BP from baseline values acutely and both before and after sildenafil administration on day 16, although the changes in each of these parameters were not significantly different to the changes observed with placebo when measured before drug administration on day 16. Sildenafil reduced central PP acutely but not following chronic treatment.

		Placebo			Sildenafil		
		Mean (SD)	*P* value, vs:		Mean (SD)	*P* value, vs:	
			baseline	trough, d 16		baseline	trough, d 16
Aortic AIx (%)	**Baseline**	32 (10)			32 (9)		
	1 hour	33 (9)	0.4		28 (10)	**<0.001**	
	Trough, d 16	31 (11)	0.4		30 (9)	0.1	
	Peak, d 16	31 (11)	0.5	0.6	30 (10)	**0.03**	0.7
Aortic AIx@75 (%)	**Baseline**	25 (9)			25 (8)		
	1 hour	24 (9)	0.3		22 (9)	**<0.001**	
	Trough, d 16	24 (11)	0.5		23 (9)	0.1	
	Peak, d 16	23 (9)	**0.03**	**0.04**	23 (8)	**0.004**	0.3
RAIx (%)	**Baseline**	89 (14)			88 (13)		
	1 hour	90 (13)	0.3		82 (14)	**<0.001**	
	Trough, d 16	87 (15)	0.4		85 (13)	0.1	
	Peak, d 16	88 (14)	0.6	0.6	84 (13)	**0.002**	0.2

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Table 6.6. Effects of sildenafil and placebo on PWA-derived parameters, absolute values.
Statistically significant comparisons are highlighted in bold; d = day.

		Placebo			Sildenafil		
		Mean (SD)	*P* value, vs:		Mean (SD)	*P* value, vs:	
			baseline	trough, d 16		baseline	trough, d 16
Central systolic BP (mmHg)	**Baseline**	145 (17)			142 (15)		
	1 hour	148 (17)	0.2		130 (15)	**<0.001**	
	Trough, d 16	144 (17)	0.4		137 (15)	**0.03**	
	Peak, d 16	149 (20)	0.09	**0.005**	136 (16)	**0.01**	0.8
Central diastolic BP (mmHg)	**Baseline**	90 (8)			89 (7)		
	1 hour	90 (10)	0.9		82 (9)	**<0.001**	
	Trough, d 16	89 (8)	0.8		86 (7)	**0.002**	
	Peak, d 16	91 (9)	0.3	0.07	84 (8)	**<0.001**	0.2
Central PP (mmHg)	**Baseline**	55 (14)			53 (13)		
	1 hour	57 (16)	0.3		48 (11)	**0.005**	
	Trough, d 16	54 (14)	0.3		51 (12)	0.3	
	Peak, d 16	58 (16)	0.1	**0.004**	53 (13)	0.7	0.4

Table 6.6.
Continued from previous page.

		Placebo	Sildenafil	*P* value
Aortic AIx	**Baseline to 1 hour**	1 (4)	-4 (4)	**0.003**
	Baseline to trough, d 16	-1 (5)	-2 (5)	0.7
	Baseline to peak, d 16	-1 (5)	-2 (4)	0.3
	Trough, d 16 to peak, d 16	0 (3)	0 (3)	0.4
Aortic AIX@75	**Baseline to 1 hour**	-1 (3)	-3 (4)	**0.02**
	Baseline to trough, d 16	-1 (4)	-2 (4)	0.5
	Baseline to peak, d 16	-2 (4)	-1 (3)	0.7
	Trough, d 16 to peak, d 16	-1 (3)	-1 (3)	0.6
RAIx	**Baseline to 1 hour**	1 (7)	-6 (6)	**<0.001**
	Baseline to trough, d 16	-2 (8)	-3 (8)	0.6
	Baseline to peak, d 16	-1 (9)	-4 (6)	0.1
	Trough, d 16 to peak, d 16	1 (4)	-2 (6)	0.1

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Table 6.7. Changes in PWA-derived parameters with sildenafil and placebo.
Statistically significant comparisons are highlighted in bold; d = day.

		Placebo	Sildenafil	*P* value
Central systolic BP (mmHg)	**Baseline to 1 hour**	2 (9)	-12 (10)	**<0.001**
	Baseline to trough, d 16	-2 (9)	-5 (10)	0.1
	Baseline to peak, d 16	4 (11)	-6 (10)	**0.001**
	Trough, d 16 to peak, d 16	6 (9)	-1 (10)	**.02**
Central diastolic BP (mmHg)	**Baseline to 1 hour**	0 (8)	-6 (6)	**<0.001**
	Baseline to trough, d 16	0 (5)	-3 (4)	0.06
	Baseline to peak, d 16	1 (7)	-5 (5)	**0.002**
	Trough, d 16 to peak, d 16	2 (4)	-2 (6)	**0.04**
Central PP (mmHg)	**Baseline to 1 hour**	2 (8)	-5 (8)	**0.003**
	Baseline to trough, d 16	-1 (6)	-2 (8)	0.8
	Baseline to peak, d 16	3 (8)	-1 (7)	0.1
	Trough, d 16 to peak, d 16	4 (6)	1 (6)	0.1

Table 6.7.
Continued from previous page

6.3.6 CF-PWV

Baseline CF-PWV was not significantly different between placebo and sildenafil phases of the study. The effects of placebo and sildenafil on CF-PWV are summarised in Table 6.8 (absolute values) and Table 6.9 (changes). CF-PWV was not significantly different to baseline following sildenafil, either acutely or after 16 days of regular treatment.

	Placebo		**Sildenafil**	
	Mean (SD)	***P***	**Mean (SD)**	***P***
Baseline	10.2 (3.0)		10.4 (2.3)	
1 hour	10.4 (2.6)	0.3	10.2 (2.3)	0.3
Trough, day 16	10.7 (2.9)	**0.03**	9.9 (2.6)	0.1
Peak, day 16	10.3 (2.5)	0.4	9.6 (2.6)	0.053

Table 6.8. Effects of sildenafil and placebo on CF-PWV, absolute values.
Units are m/s. *P* values are vs baseline. Statistically significant comparisons are highlighted in bold.

	Placebo	**Sildenafil**	***P***
Baseline to 1 hour	0.2 (0.8)	-0.2 (0.9)	0.1
Baseline to trough, day 16	0.5 (0.9)	-0.5 (1.3)	**0.02**
Baseline to peak, day 16	0.1 (0.9)	-0.8 (1.8)	0.06

Table 6.9. Changes in CF-PWV with sildenafil and placebo.
Units are m/s. Statistically significant comparisons are highlighted in bold.

6.3.7 Baseline BP and baseline arterial stiffness and arterial wave reflection

For each subject, means of the 2 baseline measures of MAP, aortic AIx, RAIx and CF-PWV were calculated. The relationships between baseline values of MAP and each of aortic AIx, RAIx and CF-PWV are shown in Figure 6.5.

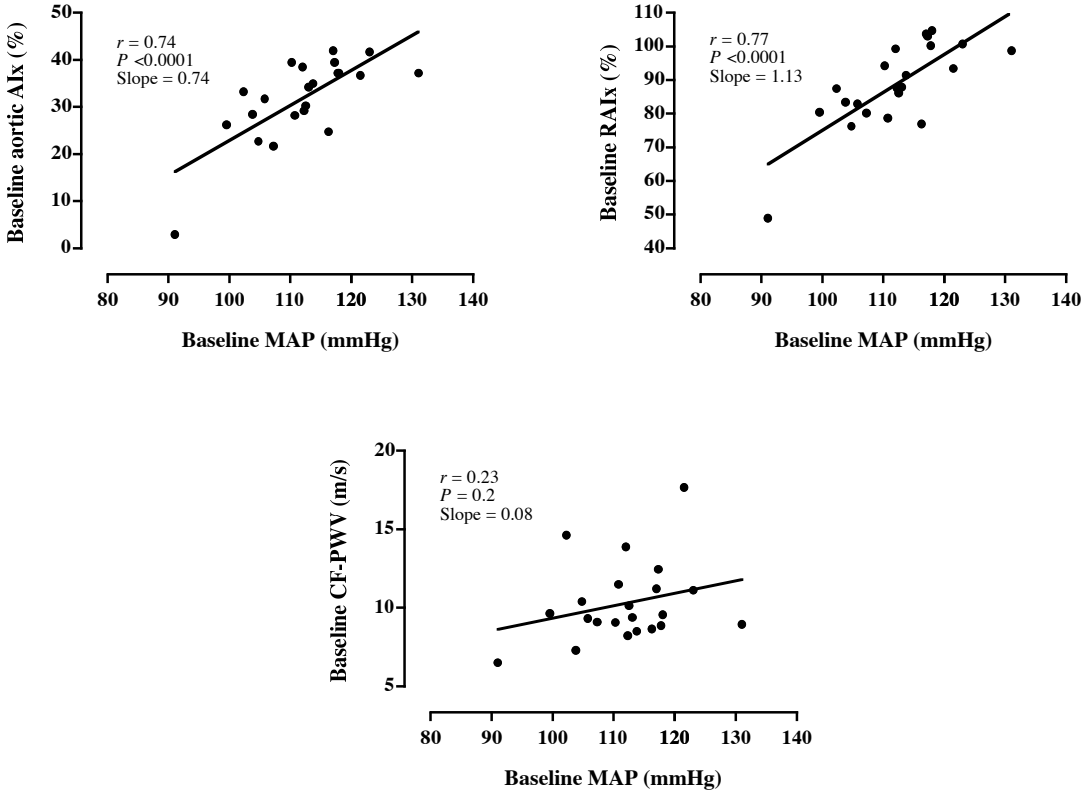

Figure 6.5. Relationships between baseline MAP and baseline aortic AIx, RAIx and CF-PWV.

6.3.8 Flow-mediated dilatation

Baseline FMD was not significantly different between placebo and sildenafil phases of the study. Sildenafil did not affect FMD either acutely or after 16 days of regular treatment. FMD responses during the sildenafil phase of the study are illustrated in Figure 6.6 and these responses, as well as those with placebo, are quantified as peak percentage dilatation and AUC of the change in brachial artery diameter over time in Table 6.10 (absolute values) and Table 6.11 (changes). Sildenafil also did not affect baseline brachial artery diameter or the degree of reactive hyperaemia at any point.

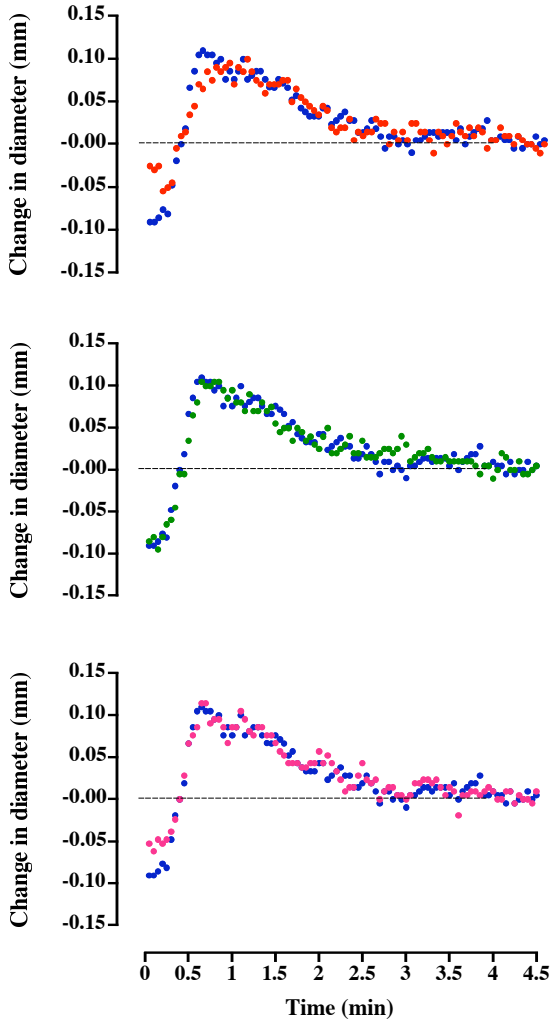

Figure 6.6. FMD responses during the sildenafil phase of the study.
Values are mean changes from baseline. Response at baseline (pre-sildenafil) is shown in blue. In red is the response at 1 hour (top), in green the response at trough on day 16 (middle) and in pink the response at peak on day 16 (bottom).

	Placebo		Sildenafil	
	Mean (SD)	***P***	**Mean (SD)**	***P***
Peak dilatation (%)				
Baseline	2.1 (1.8)		2.6 (1.7)	
1 hour	2.8 (2.1)	0.07	2.1 (1.9)	0.2
Trough, day 16	2.2 (1.6)	0.7	2.7 (1.7)	0.8
Peak, day 16	3.2 (2.1)	**0.02**	2.6 (2.9)	1
AUC of change from baseline (AU)				
Baseline	5.6 (8.7)		7.1 (8.8)	
1 hour	4 (14)	0.9	7.4 (10.5)	0.9
Trough, day 16	6.9 (8.1)	0.9	7.3 (9.9)	0.9
Peak, day 16	6.4 (10.2)	0.9	7.9 (11.6)	0.8

Table 6.10. Effects of placebo and sildenafil on FMD, absolute values.
P values are vs baseline.

	Placebo	Sildenafil	***P***
Peak dilatation (absolute %)			
Baseline to 1 hour	0.7 (1.7)	-0.4 (1.3)	0.05
Baseline to trough, day 16	0.1 (1.8)	0.1 (1.5)	0.8
Baseline to peak, day 16	1.1 (2.1)	0.0 (2.8)	0.1
AUC of change from baseline (AU)			
Baseline to 1 hour	-0.3 (12.9)	0.3 (13.0)	0.9
Baseline to trough, day 16	0.5 (11.9)	0.3 (12.8)	0.7
Baseline to peak, day 16	-0.3 (11.2)	0.8 (15.6)	0.9

Table 6.11. Absolute changes in FMD with placebo and sildenafil.

6.3.9 Subject withdrawals and adverse effects

Two of the 3 subjects who were withdrawn from the study were withdrawn while taking sildenafil. One of these subjects was withdrawn because of severe headache and the other because of back pain and feeling generally unwell. The subject withdrawn while taking placebo was withdrawn because of joint pains, nausea and headache.

A summary of the symptoms experienced during each phase of the study is given in Table 6.12. The most common adverse effect was indigestion/heartburn, which lasted up to 5 days. One subject was prescribed omeprazole and 7 other subjects took over-the-counter acid suppression treatments, all with good effect. Headache was the next most common adverse effect experienced. Headaches were generally mild and transient, even though severe headache was the reason that 1 subject was withdrawn. Sildenafil was also associated with low back/buttock/leg pain, which was generally described as a muscular aching sensation. This was usually responsive to paracetamol, although a few subjects also took ibuprofen, and tended to settle within a few days, with no further analgesic requirement. Plasma creatine kinase concentrations were measured in 4 of the subjects who experienced these symptoms and all were within the laboratory reference range.

	Sildenafil				Placebo			
	Total	Mild	Mod	Severe	**Total**	Mild	Mod	Severe
Indigestion/heartburn	**10 (40)**	5 (20)	4 (16)	1 (4)	**1 (4)**	1 (4)		
Headache	**8 (32)**	6 (24)	1 (4)	1 (4)	**1 (4)**			1 (4)
Back/buttock/leg ache	**7 (28)**	1 (4)	5 (20)	1 (4)	**1 (4)**	1 (4)		
Abdominal discomfort	**2 (8)**		2 (8)		**0**			
Fatigue	**2 (8)**		1 (4)	1 (4)	**0**			
Facial flushing	**2 (8)**	2 (8)			**0**			
Cramp	**1 (4)**	1 (4)			**1 (4)**		1 (4)	
Dizziness	**1 (4)**	1 (4)			**1 (4)**		1 (4)	
Insomnia	**1 (4)**		1 (4)		**0**			
Joint pain	**1 (4)**			1 (4)	**1 (4)**			1 (4)
Loin pain	**1 (4)**		1 (4)		**0**			
Nausea	**1 (4)**		1 (4)		**2 (8)**			2 (8)
Neck pain	**1 (4)**	1 (4)			**1 (4)**		1 (4)	
Urinary frequency	**1 (4)**	1 (4)			**0**			
Diarrhoea	**0**				**1 (4)**			1 (4)
Stress	**0**				**1 (4)**		1 (4)	
Vomiting	**0**				**1 (4)**		1 (4)	

Table 6.12. Symptoms experienced with placebo and sildenafil in all subjects recruited (n=25).
Values are numbers (percentages) of subjects. Mod = moderate.

6.3.10 Drug adherence

Mean maximum adherence was 96% (range 80 to 100%) during the placebo phase and 96% (range 87 to 100%) during the sildenafil phase. There was no indication that the level of maximum adherence had any influence on the measured effect of sildenafil on BP (Figure 6.7).

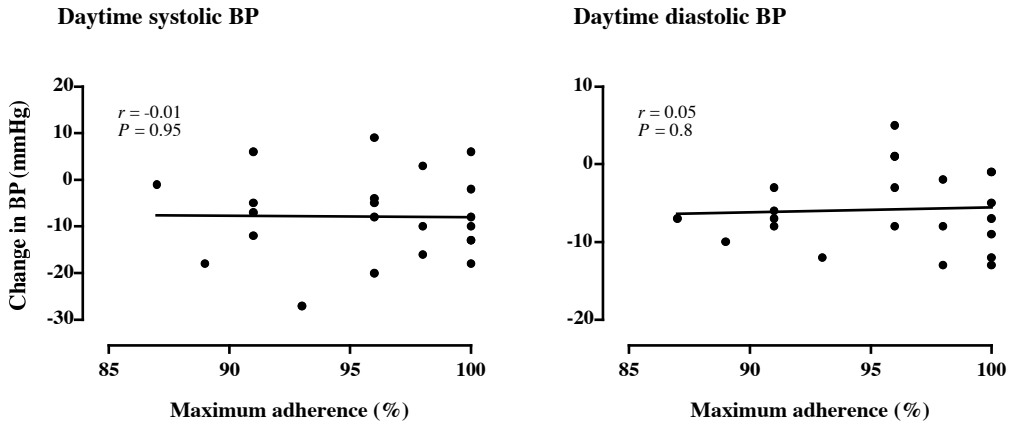

Figure 6.7. Relationship between maximum adherence and change in daytime ambulatory BP.

6.4 DISCUSSION

6.4.1 Main findings

The major findings from this study are that in untreated hypertensives regular oral sildenafil reduced BP and arterial wave reflection but had no effect on CF-PWV or brachial artery FMD.

6.4.2 Measurement repeatability

As can be seen from the Bland-Altman-plots, there was generally good repeatability of baseline measurements, with no tendency for repeatability to vary with mean values of each of the parameters. The repeatability of baseline aortic AIx and of baseline CF-PWV compare well with previously published data (Wilkinson *et al.*, 1998), particularly given that the 2 baseline recordings were made on different days, which is in contrast to the published data.

6.4.3 Effects on peripheral BP

Sildenafil reduced BP both acutely and after 16 days of regular administration. The reduction in average daytime BP was 8/6 mmHg compared to baseline and around 10/6 mmHg compared to placebo. A number of studies have compared the effects on BP of the major antihypertensive drugs when given as monotherapy for hypertension. In a large multi-centre, double-blind study that recruited 1292 hypertensive men (mean age 59 years and mean sitting BP 152/99 mmHg), subjects were randomised to placebo or 1 of 6 antihypertensives (Materson *et al.*, 1993). Subjects initially entered a dose titration phase lasting 4 to 8 weeks during which the assigned drug was started at the lowest dose and titrated until either a diastolic BP of less than 90 mmHg was achieved or the maximum dose was reached. At the end of the dose titration phase placebo-corrected reductions in BP were 11/5 mmHg with hydrochlorothiazide, 8/7 mmHg with atenolol, 6/5 mmHg with captopril and 10/9 mmHg with diltiazem.

In an open label, 4-way crossover study, 36 untreated hypertensives (age 22 to 51 years, mean supine BP 161/98 mmHg) were rotated through treatment with lisinopril

20 mg, bisoprolol 5 mg, hydrochlorothiazide/triamterene 25/50 mg and nifedipine (slow-release) 30 mg (Dickerson *et al.*, 1999). Each treatment was given for 1 month and there was a washout period of 1 month between treatments. At the end of each month of treatment the reductions in BP were ~10/12 mmHg with lisinopril, ~11/13 mmHg with bisoprolol, ~6/6 mmHg with hydrochlorothiazide/triamterene and ~7/7 mmHg with nifedipine. The same research group subsequently performed a similar crossover study in which 34 untreated hypertensives (age 28 to 55 years, mean supine BP 160/101 mmHg) each received double-blind treatment with 5 antihypertensives and placebo (Deary *et al.*, 2002). The placebo-corrected reductions in BP after 6 weeks of each treatment were ~13/9 mmHg with amlodipine 5 mg, ~10/8 mmHg with doxazosin 4 mg, ~17/11 mmHg with lisinopril 10 mg, ~10/12 mmHg with bisoprolol 5 mg and ~4/2 mmHg with bendroflumethiazide 2.5 mg.

It can be seen from these previous studies that the degree of BP reduction observed with sildenafil is similar to the degree of BP reduction that occurs with other, commonly used, antihypertensive drugs when they are given as monotherapy. The subjects recruited to the current study had relatively mild hypertension (average daytime BP 153/92 mmHg) and, given that higher baseline BP was associated with greater BP reduction, at least for systolic BP, it is likely that the absolute effect of sildenafil on BP would have been greater if the population had been more hypertensive.

Examination of the office BP data shows that there was some attenuation of the acute effect of sildenafil with chronic treatment. Sildenafil reduces BP directly through peripheral arterial vasodilatation secondary to preservation of vascular smooth muscle cGMP (Jackson *et al.*, 1999). Following the relatively rapid onset of this pharmacodynamic effect it is likely that neurohormonal counter regulatory mechanisms would be stimulated, including release of catecholamines and stimulation of the renin-angiotensin system. These responses would tend to counteract the sildenafil-induced BP reduction by promoting vasoconstriction and intravascular volume expansion.

An alternative mechanism that might explain the observed attenuation of the acute effect of sildenafil is that tolerance develops to its action as a vasodilator. Tolerance is a well described phenomenon with organic nitrates and is associated, within a few days of continuous administration, with loss of hypotensive as well as anti-anginal efficacy (Elkayam, 1991). *In vivo*, some of the attenuation of the acute effects of organic nitrates with continuous treatment can be explain by neurohormonal counter regulatory mechanisms with consequent volume expansion and vasoconstriction (Munzel *et al*., 1996; Stewart *et al*., 1986), so-called 'pseudo tolerance'. However, vascular nitrate tolerance also occurs, in which there is a loss of nitrovasodilator-responsiveness. Vascular nitrate tolerance appears to be the result of increased vascular superoxide production and, at least for GTN, impaired bioactivation of the organic nitrate to produce NO (Munzel *et al*., 2005). Whether or not chronic sildenafil administration is associated with a reduction in the responsiveness of the vasculature to PDE5 inhibition cannot be addressed directly from the *in vivo* data presented here. However, the persistent hypotensive effect of sildenafil with chronic treatment, even if slightly diminished compared to the acute effect, contrasts with the complete loss of hypotensive effect that is associated with chronic organic nitrate therapy, suggesting that little or no vascular tolerance occurs. Furthermore, in rats chronic sildenafil dosing, for up to 8 weeks, is associated with up-regulation of PDE5 expression but no attenuation of the ability of sildenafil to induce penile erection (Behr-Roussel *et al*., 2005; Musicki *et al*., 2005), providing further evidence that the effects of sildenafil on the vasculature do not undergo tolerance.

On day 16 of sildenafil treatment office BP was higher before the administration of sildenafil than at 1 hour afterwards. Indeed, the changes from baseline in both systolic BP and diastolic BP were not significantly different to placebo when measured before sildenafil administration, but were significantly lower than placebo when measured 1 hour after sildenafil administration. Although sildenafil effectively reduced average night time BP, these data suggest that the overnight dose interval of between 10 and 11 hours was sufficient for the antihypertensive effect of sildenafil to begin to wane. This may have important clinical implications. There is a recognised diurnal variation in BP with night time BP generally being 10-20% lower than day

time BP. Coincident with arousal and arising from overnight sleep there is an abrupt increase in BP. The incidence of cardiovascular events, both coronary (Muller *et al.*, 1985) and cerebrovascular (Wroe *et al.*, 1992), also follows a diurnal pattern, with the hour of awakening rather than the hour in the day being most predictive (Willich *et al.*, 1992). The early morning BP surge has been suggested as a trigger for cardiovascular events (Giles, 2005; Kario *et al.*, 2003b) and recent evidence has confirmed that patients with a higher morning BP surge are at greater risk of stroke (Kario *et al.*, 2003a). Therefore, despite reducing BP overall, the 3 times daily dosing regime used in the current study may provide suboptimal early morning protection, whereas alternative dosing strategies, for example using a modified-release formulation, or a longer acting PDE5 inhibitor, such as tadalafil, might afford better protection.

Sildenafil reduced both systolic BP and diastolic BP. As a result, it also reduced MAP but did not reduce PP. This suggests that the primary mode of action is through peripheral vasodilatation rather than through reduction in large artery stiffness. The lack of effect on PP might also reflect the population of hypertensives studied. Thus, subjects with raised systolic BP, diastolic BP or both were recruited. If the study had been confined to subjects with isolated systolic hypertension there might have been a preferential effect on systolic BP with a consequent reduction in PP.

6.4.4 Effects on arterial wave reflection, arterial stiffness and central BP

In keeping with the established relationships between BP and measures of arterial stiffness (Oliver *et al.*, 2003), baseline MAP was significantly correlated with aortic AIx and RAIx. Baseline CF-PWV also tended to increase with MAP, although these measures were less closely correlated. Sildenafil reduced aortic AIx, and did so in a manner that was similar to the effect on office BP, with a greater reduction acutely than was evident after 16 days. The effect on RAIx was similar, as was the effect on aortic AIx@75, which would be expected given the lack of effect of sildenafil on HR. The reduction in aortic AIx indicates that sildenafil reduced peripheral artery wave reflection. However, the relatively small magnitude of this effect is no more than would be expected simply as a result of the reduction in systemic BP

(Wilkinson *et al*., 2001b), suggesting that sildenafil did not act specifically to reduce large artery stiffness. This conclusion may also be supported by the lack of effect of sildenafil on CF-PWV, a more direct measure of large artery stiffness. On the other hand, although the effects were not statistically significant there was a trend to a progressive reduction in CF-PWV (-0.2 m/s at 1 hour, -0.5 m/s on day 16 before sildenafil and -0.8 m/s on day 16 after sildenafil). The *P* value for the comparison between baseline and day 16 after sildenafil was 0.053, very nearly statistically significant. Thus, the possibility of a real effect on CF-PWV should not be dismissed; a larger sample size or a longer treatment period may have demonstrated a significant effect. Given that distending pressure is conventionally considered to an important influence on arterial stiffness, the acute reduction in MAP that occurred with sildenafil might, in itself, have been expected to significantly reduce CF-PWV. However, CF-PWV was not acutely affected by sildenafil. Although this observation may seem surprising, it is consistent with data recently reported by Stewart *et al* (2006). They investigated the effect of acute changes in BP on CF-PWV in normotensive and hypertensive subjects. As expected, in the normotensive subjects increasing BP with angiotensin II increased CF-PWV and decreasing BP with GTN decreased CF-PWV. However, in the hypertensive subjects, despite reducing MAP by 22 mmHg to the same level as in the normotensive subjects, GTN had no effect on CF-PWV. These data suggest that the increased aortic stiffness characteristic of hypertension is the result of structural changes in the arterial wall rather than elevated BP *per se*. It is intriguing to speculate that the seemingly progressive reduction in CF-PWV observed with regular sildenafil in the present study was the result of progressive improvement of the intrinsic stiffness of the aortic wall.

With chronic treatment, sildenafil reduced central BP with a similar magnitude to the effect on peripheral BP. Measurement of central BP did not, therefore, provide any additional information on the hypotensive action of sildenafil over and above the measurement of peripheral BP. Acutely, there was a slightly greater effect on central systolic BP than on central diastolic BP, which manifested as reduced central PP, but this was not sustained with regular treatment.

6.4.5 Effects on endothelium-dependent vasomotor function

Mean FMD at baseline of the subjects in the present study was 2.1 to 2.6% (placebo and sildenafil phases respectively). Even though there was no healthy control group, these values are low compared to published values in healthy subjects and, consistent with most previous studies in hypertensives (Ghiadoni *et al.*, 2003; Gokce *et al.*, 2001; Panza *et al.*, 1990; Taddei *et al.*, 1998), likely indicate impaired endothelium-dependent vasomotion.

Sildenafil had no effect on FMD, indicating that it did not modulate endothelium-dependent vasomotion, either acutely or after chronic treatment. As outlined in the introduction (section 1.5.5), there are conflicting data on the acute effects of sildenafil on endothelium-dependent vasomotor responses, with some reports of an improvement and other reports showing no effect. While these previous studies have recruited different patients and used different methodologies there are no apparent consistent differences to explain the different effects observed. For example, there are positive and negative studies that used forearm plethysmography (Kimura *et al.*, 2003; Robinson *et al.*, 2006) or FMD (Dishy *et al.*, 2004; Vlachopoulos *et al.*, 2004), and positive and negative studies that recruited smokers (Dishy *et al.*, 2004; Vlachopoulos *et al.*, 2004) or patients with CAD (Halcox *et al.*, 2002a; Robinson *et al.*, 2006). One of these studies found that sildenafil did not affect FMD when measured as peak dilatation of the brachial artery but did prolong the period of vasodilatation after reactive hyperaemia (Halcox *et al.*, 2002a). However, in the present study sildenafil had no effect on either peak dilatation or the period of dilatation. Given that FMD is largely NO-mediated it would seem logical that sildenafil, by preserving NO-stimulated cGMP, would improve FMD. Possible explanations for the lack of any effect on FMD include insufficient sensitivity of the methodology, insufficient dose of sildenafil or predominant action of sildenafil in the resistance circulation rather than in conduit arteries (see section 6.4.7), although the previous positive studies using FMD argue against each of these. An alternative explanation is that, compared to healthy subjects (Doshi *et al.*, 2001; Joannides *et al.*, 1995), NO contributes relatively little to shear-stress induced vasodilatation at the brachial artery in hypertensives. In support of this possibility it has previously been

shown that although vasodilatation to bradykinin in the forearm is NO-mediated in healthy subjects, in hypertensives it is not only reduced but is also mediated by a different pathway, possibly involving endothelium-dependent hyperpolarisation (Taddei *et al.*, 1999). Although not previously investigated, if similar changes occur in the brachial artery sildenafil might be expected to have little or no effect on FMD in hypertension.

The lack of any effect of sildenafil on FMD despite reduction in BP suggests that BP reduction *per se* is not sufficient to improve endothelium-dependent vasomotor function, at least within 16 days. The effect of antihypertensive treatment on FMD has been investigated previously. In one study, FMD improved after 6 and 12 months of treatment with various antihypertensives and also after 2 months of monotherapy with nifedipine but not hydrochlorothiazide (Muiesan *et al.*, 1999). In a further study, 6 months of treatment with perindopril improved FMD but there was no effect on FMD after 6 months of treatment with telmisartan, nifedipine (directly contradicting the previous study), amlodipine, atenolol or nebivolol, despite these drugs similarly reducing BP (Ghiadoni *et al.*, 2003). Thus, it appears that BP reduction, in itself, may not be sufficient to improve endothelium-dependent vasomotion and, in this respect, the data from the present study are consistent with the previous studies.

What are the implications of the finding that sildenafil does not affect FMD in terms of its potential use as an antihypertensive in clinical practice? There is evidence that cardiovascular prognosis is better in hypertensive patients in whom there is an improvement in FMD with treatment (Modena *et al.*, 2002). In this study both BP reduction and the antihypertensives used to reduce BP were similar in the group in which there was an improvement in FMD to the group in which there was no improvement in FMD. This may suggest that drugs that consistently improve FMD might be more effective in reducing cardiovascular events in patients with hypertension. On the other hand, with the possible exception of ß-blockers (Lindholm *et al.*, 2005), there is good evidence that the major antihypertensive drug classes are substantially equivalent in reducing cardiovascular events (Blood Pressure Lowering Trialists Collaboration, 2003). This calls into question the clinical

relevance of the studies that have demonstrated differences between these agents in their effects on FMD. Therefore, the lack of effect of sildenafil on FMD, especially after just 16 days, should not, in itself, detract from its potential as an antihypertensive in clinical practice.

6.4.6 Tolerability

Side effects from regular sildenafil treatment were experienced relatively commonly, especially indigestion/heart burn, headache and low back/buttock/leg muscle ache. Dyspepsia and headache are common side effects of sildenafil. In the seminal clinical trial in which sildenafil was established as an effective treatment for PED (Goldstein *et al.*, 1998), headache was reported by 21% of subjects taking sildenafil 50 mg and 30% of subjects taking sildenafil 100 mg, similar to the frequency in the current study (32%). In a study recently reported by Galiè *et al* (2005), in which sildenafil was given regularly for 12 weeks to patients with pulmonary hypertension, headache occurred in 42% of those taking 40 mg three times daily and in 49% of those taking 80 mg three times daily. Dyspepsia was experienced by 40% of subjects in the current study. This is more frequent than when used intermittently for PED (16% with 100 mg sildenafil; Goldstein *et al.*, 1998), and is also more frequent than was reported in the pulmonary hypertension study (13% of subjects taking 80 mg 3 times daily; Galiè *et al.*, 2005).

Low back/buttock/leg muscle ache was reported by 28% of subjects in the current study. These sorts of symptoms were not reported as side effects of sildenafil in the clinical trial by Goldstein *et al*, although this may be because only those symptoms that occurred in 5% or more subjects were reported at all. Indeed, in a further study in which sildenafil was given for PED, back pain was reported by 2.5% of 367 men (Eardley *et al.*, 2005). Other studies have reported myalgia as a side effect of sildenafil (Olsson *et al.*, 2000; Osegbe *et al.*, 2003), for example occurring in 3% of 58 men in a study from Nigeria (Osegbe *et al.*, 2003). In the pulmonary hypertension study, myalgia occurred in 14% of subjects taking 80 mg 3 times daily (Galiè *et al.*, 2005). Thus, myalgia appears to occur more frequently with chronic regular use compared to with intermittent use. The lack of any rise in plasma creatine kinase

suggests that the muscle aches experienced by some subjects are not due to an underlying myositis.

6.4.7 Nitrate-like action of sildenafil?

Although sildenafil acts on the NO-cGMP pathway, the data from the present study suggest that it is not particularly "nitrate-like" in its haemodynamic action. For example, even in very small doses nitrates dilate conduit arteries. This can be observed directly at the brachial artery and indirectly as a reduction in peripheral artery wave reflection, even at doses that do not affect systemic BP (see section 0). In contrast, sildenafil did not affect brachial artery diameter (or FMD at this site) and had no greater effect on peripheral artery wave reflection than would be expected from its effect on systemic BP, suggesting that it acts predominantly in the resistance circulation rather than at conduit arteries.

6.4.8 PDE5 inhibition – monotherapy for hypertension in clinical practice?

Given that sildenafil has a persistent effect on BP after 2 weeks of regular treatment, it seems likely that its antihypertensive action will also be sustained over longer treatment periods. Indeed, it is possible that the maximum effect on BP had not occurred by 2 weeks and that additional antihypertensive effect would be observed over longer periods of time. A longer term study is needed to address these questions.

The dose of sildenafil in the current study was 50 mg 3 times daily. In single dose studies, the effect of sildenafil on BP does not appear to be strongly dose-related (Jackson *et al.*, 1999; Zusman *et al.*, 1999). However, exploration of the hypotensive effects of other doses in *chronic* therapy should be investigated. While there may be no additional antihypertensive effect in some subjects with doses of more than 150 mg/day, higher doses may be more effective in other subjects. Alternatively, lower doses, such as 25 mg 3 times daily, may effectively lower BP but with fewer side effects. A study in which hypertensives are initially started at a low dose that is then titrated upwards according to BP response would be particularly informative in elucidating a clinical dosing strategy. Once the most appropriate doses of PDE5

inhibitors have been established for the treatment of hypertension, head-to-head comparison with established treatments of antihypertensive efficacy and tolerability would be of value in determining their potential place in clinical practice.

Hypertension guidelines recommend particular drugs for the first-line treatment of hypertension. For example, both NICE (North of England Hypertension Guideline Development Group, 2004) and JNC 7 (Chobanian *et al.*, 2003b) guidelines recommend low dose thiazide diuretics. However, it is also recognised that there are often compelling reasons to use alternative agents. For example, ß-blockers in patients with CAD, inhibitors of the renin-angiotensin system in heart failure or alpha-adrenoreceptor antagonists in men with symptomatic benign prostatic hypertrophy (Williams *et al.*, 2004). A potential valuable indication for the use of PDE5 inhibitors as antihypertensives is in men with PED. However, it would first be necessary to demonstrate that the effect of PDE5 inhibition on PED is maintained in the long term with the frequent, regular dosing pattern that would be required for the treatment of hypertension. As discussed above, there are encouraging animal data suggesting that no tolerance develops to the effects on erectile function with continuous use of sildenafil for up to 8 weeks (Behr-Roussel *et al.*, 2005; Musicki *et al.*, 2005), but human data are currently lacking.

Antihypertensives are frequently prescribed to patients who do not feel unwell and, as a consequence, gain no symptomatic benefit from treatment (in contrast to the treatment of angina or heart failure, for example). Furthermore, most hypertensives are not generally at immediate risk of a cardiovascular event and treatment is aimed at reducing risk in the long term. Thus, the benefits of antihypertensive treatment may not be all that tangible to an individual patient. It is clearly desirable for any chronic drug treatment to be as free as possible from side effects but, for these reasons, concordance with treatment, and consequently BP control, are likely to be maximised when antihypertensives with a low incidence of side effects are used. Side effects from regular sildenafil were relatively frequent in the current study and may limit the potential for widespread use as an antihypertensive. However, the major side effects, dyspepsia, headache and myalgia, generally lasted a few days.

Thus, if patients were warned that these might occur at the outset of treatment but that they are then likely to subside, the long-term acceptability might be good.

A number of questions arise from the pattern of side effects experienced with regular sildenafil. A possible mechanism for the dyspeptic symptoms is that sildenafil relaxes the smooth muscle of the lower oesophageal sphincter (Eherer *et al.*, 2002) so that gastric contents reflux into the oesophagus. This might increase the risk of chronic reflux oesophagitis and even Barrett's oesophagus and oesophageal adenocarcinoma. Given this possibility, investigation of the effects of chronic PDE5 inhibition on the lower oesophagus would be warranted. Plasma creatine kinase was not elevated in 4 of the subjects who experienced muscle ache in the current study. While this suggests that there was no underlying myositis in these subjects, the possibility that myositis might occur in some subjects with regular treatment, even if only rarely, should continue to be borne in mind. Routine monitoring of plasma creatine kinase concentrations should be considered as part of future studies of chronic sildenafil, or other PDE5 inhibitor, treatment.

6.4.9 Summary

In untreated hypertensives oral sildenafil 50 mg given 3 times daily reduces BP. It also reduces arterial wave reflection but to no greater an extent than would be expected from its effect on BP. It does not affect CF-PWV or brachial artery FMD.

CHAPTER 7

POTENTIAL OF COMBINED ORGANIC NITRATE AND PHOSPHODIESTERASE TYPE 5 INHIBITOR IN THE MANAGEMENT OF TREATMENT-RESISTANT HYPERTENSION

7.1 INTRODUCTION

7.1.1 Treatment-resistant hypertension

The current US national guideline on hypertension (JNC 7; Chobanian *et al.*, 2003b) defines treatment-resistant hypertension (TRH) as the failure to achieve goal BP in patients who are adhering to full doses of an appropriate 3-drug regimen that includes a diuretic. The current BHS guideline (BHS IV; Williams *et al.*, 2004) does not give a specific definition, but states that one of the indications for specialist referral is "resistance to multi-drug regimen, that is, ≥3 drugs". It is probably reasonable to not include the need for a diuretic as one of the 3 drugs in the definition, especially given the recent ASCOT data showing that BP control was better with a CCB/ACE inhibitor combination than a diuretic/ß-blocker combination (Dahlöf *et al.*, 2005). In terms of published studies on TRH there is considerable variation in the definition. This, along with the problems of identifying truly treatment-resistant patients, means that there are no accurate estimates of the prevalence of TRH in the hypertensive population. However, apparent TRH is a common reason for referral to specialist hypertension clinics and it is likely that TRH will become increasingly common as a result of factors such as an ageing population and increasing obesity and diabetes.

TRH must be confirmed with out-of-office measurement of BP. In a study, of 286 patients referred for TRH on the basis of an office BP of ≥140/90 mmHg, 56% were found to have true TRH, as defined as an average 24-hour ABPM of ≥135/85 mmHg (Muxfeldt *et al.*, 2003). In this study, patients with true TRH had higher rates of target organ damage (nephropathy and LVH) than patients with 'white coat' TRH (i.e. uncontrolled office BP but normal 24-hour ABPM). Relatively high rates of target organ damage in TRH, also documented in other studies (Cuspidi *et al.*, 2001; Muxfeldt *et al.*, 2005), suggest that TRH is associated with increased cardiovascular risk, which would be expected on the basis of the known association between BP and cardiovascular events (Prospective Studies Collaboration, 2002). While there are few specific data on the prognosis of TRH, a recent prospective study reported that true

TRH independently predicted cardiovascular events over a 5-year follow-up period (Pierdomenico *et al*., 2005).

Once true TRH has been established, potential contributing factors should be evaluated and managed (Taler, 2005). Such factors include obesity, excess sodium or alcohol intake, and the use of oral contraceptives or non-steroidal anti-inflammatory drugs. It may also be appropriate to investigate for identifiable causes of hypertension, including renal, renovascular or endocrine abnormalities, or obstructive sleep apnoea. There has been a lot of interest recently in the potential contribution of aldosterone excess to TRH. Calhoun *et al* (2002) found that primary hyperaldosteronism, diagnosed as a low plasma renin activity and high urinary aldosterone concentration in the setting of high sodium intake, was present in 20% of patients with TRH and Eide *et al* (2004) found that a low plasma renin activity was present in 67% of TRH patients.

The recognition that aldosterone excess is relatively common in patients with TRH has led to increased use of aldosterone antagonists to reduce BP in this condition. For example, in the study by Calhoun *et al* BP fell significantly in all patients with hyperaldosteronism who were given sprionolactone, while in the study by Eide *et al* 89% of low renin subjects given amiloride with hydrochlorothiazide, had substantially reduced BP compared to with placebo. These effects on BP were additional to existing therapy, often including ACE inhibitors or ARAs. Other common pharmacological approaches to TRH are the intensification of diuretic treatment, to counteract intravascular volume expansion, or the addition of α-adrenoreceptor antagonists such as doxazosin. Drugs such as the centrally acting moxonidine (Martin *et al*., 2005) or the vasodilator minoxidil (Sica, 2004), which can cause hypertrichosis and must be given concomitantly with a ß-blocker to prevent reflex tachycardia and a loop diuretic to prevent fluid retention, may also be considered, although less commonly.

7.1.2 Combined organic nitrate and PDE5 inhibition as a possible treatment for TRH

The haemodynamic interaction between organic nitrates and PDE5 inhibitors has been discussed extensively in section 1.3.3 and in chapter 5, with an emphasis on the potentially harmful systemic hypotension that can result when these drugs are administered in combination. Although, for this reason, co-administration of these drugs is currently contraindicated, the unique antihypertensive potency of the combination may actually be therapeutically useful in controlling BP in patients with TRH, at least in the setting of a specialist hypertension clinic. However, the extent to which these drugs in combination reduce BP in TRH is not known. The effect may be similar to that in healthy men or men with angina (Webb *et al.*, 1999; Webb *et al.*, 2000). Alternatively, there may either be a greater reduction in BP, because of higher baseline BP, or a lesser reduction in BP, because patients are generally resistant to antihypertensive treatment.

7.1.3 Aim

As the initial investigation of the potential of combined organic nitrate and PDE5 inhibitor in the management TRH, the aim of the present study was to characterise the effect on BP of a combination of single doses of sildenafil and ISMN in patients with TRH.

7.2 METHODS

7.2.1 Subjects

7.2.1.1 Identification of subjects

Potentially suitable subjects were identified from patients attending the WGH Cardiovascular Risk Clinic.

7.2.1.2 Inclusion criteria

- Male or female.
- Essential hypertension.
- Office BP >140/85 mmHg despite treatment with 3 or more antihypertensive drugs.
- Daytime average ambulatory BP >130/80[1] mmHg on the current antihypertensive drug regime, within 6 months of the start of the study.
- Resistance to treatment proven by directly observed therapy (to exclude poor compliance as a cause of uncontrolled BP, see below).

7.2.1.3 Exclusion criteria

- Identifiable underlying cause for hypertension (e.g. Conn's or Cushing's syndromes, renal artery stenosis, phaeochromocytoma or coarctation of the aorta).
- Clinically evident coronary artery or cerebrovascular disease.
- Taking regular organic nitrates or nicorandil.
- Significant renal or liver impairment.
- Pregnant.
- Current alcohol or drug abuse.
- Previous serious drug allergy.

7.2.2 Screening visit

Potentially suitable subjects who agreed to be considered for the study attended a morning screening visit at the CRC. At this visit the study was explained fully and

[1]An daytime average ambulatory BP of 130/80 is considered equivalent to an office BP of 140/85 mmHg (Williams *et al.*, 2004)

written consent was obtained. A medical history was taken, a physical examination and 12-lead ECG were performed, and a non-fasting screening blood sample was taken. A 24-hour ambulatory BP monitor was fitted if necessary.

During the screening visit the BP response to directly observed therapy was recorded. This was performed to ensure that the patients recruited had true TRH, rather than uncontrolled BP due to poor adherence to treatment. In addition, it was important to exclude patients whose uncontrolled BP was related to poor adherence for safety reasons. If such patients had been administered sildenafil and ISMN concurrently with their usual antihypertensives there would have been a significant risk of severe hypotension. Subjects were asked not to take their normal morning medicines but to bring them to the CRC. Following 30 minutes rest, baseline semi-erect BP was recorded in duplicate. Subjects then took all of their usual medicines under direct observation and single measures of BP were repeated every 15 minutes for 4 hours. The average systolic and diastolic BPs over the last 2 hours of the observation period were calculated and if either was >40 mmHg lower than the average at baseline the subject was not eligible for the study.

7.2.3 Study design

Randomised, placebo-controlled, double blind, 4-way crossover.

7.2.4 Protocol

All studies were performed first thing in the morning. On each study day patients took their usual medicines at home before coming to the CRC. They were asked to ensure that they took them at the same time for each visit. On separate visits, at least 5 days apart, subjects received the following orally:

- Placebo sildenafil and placebo ISMN
- Sildenafil 50 mg and ISMN placebo
- Placebo sildenafil and ISMN 10 mg
- Sildenafil 50 mg and ISMN 10 mg

The 2 drugs were administered simultaneously. The protocol is outlined in Figure 7.1. Subjects were studied supine. All measures were made in duplicate and mean values were entered into the analyses.

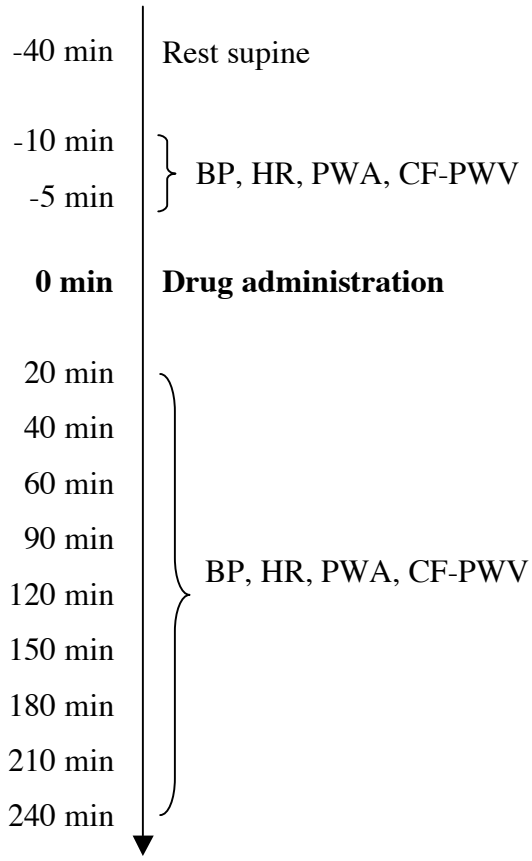

Figure 7.1. Study protocol.
BP, HR, PWA and CF-PWV were measured in the order shown.

7.2.5 Analyses

For each parameter, the AUC of the change from baseline to 4 hours after drug administration was calculated. The mean AUCs were compared between study phases by paired Student's *t*-tests.

7.3 RESULTS

7.3.1 Subjects

Thirteen subjects attended for screening to assess eligibility for the study and, of these, 7 were recruited. Of the 5 patients not recruited, 3 had well controlled BP at ABPM, 1 did not take part for personal reasons, and it emerged that another had recently had a minor stroke. On initial review of the data 1 subject had consistently well-controlled BP at baseline (117/72 mmHg, 121/72 mmHg, 117/63 mmHg 106/62 mmHg at visits 1, 2, 3 and 4 respectively) and, as a result, this subject's data were excluded from analysis. Therefore, the data presented are from the remaining 6 subjects. The baseline characteristics of the subjects are given in Table 7.1.

Table 7.1. Baseline characteristics of the subjects.
Means (SDs) are given for continuous variables. Values for RAIx, aortic AIx, aortic AIx@75, CF-PWV and central BP are means of the baselines of all 4 visits.

	All subjects (n = 7)	**Subjects analysed (n = 6)**
Males/females	3/4	3/3
Age (years)	61 (10)	61 (11)
Screening office BP (mmHg):		
Systolic	167 (13)	169 (12)
Diastolic	91 (14)	93 (14)
Ambulatory BP (mmHg):		
24 hour systolic	139 (13)	141 (13)
24 hour diastolic	77 (9)	77 (9)
Daytime systolic	145 (11)	146 (11)
Daytime diastolic	81 (10)	81 (11)
Night time systolic	124 (18)	125 (19)
Night time diastolic	64 (7)	63 (7)
Central BP (mmHg):		
Systolic	131 (11)	135 (4)
Diastolic	80 (13)	82 (13)

Continued on next page

	All subjects (n = 7)	Subjects analysed (n = 6)
RAIx (%)	86 (5)	86 (5)
Aortic AIx (%)	32 (2)	32 (3)
Aortic AIx@75 (%)	24 (4)	24 (4)
CF-PWV (m/s)	11.0 (3.1)	11.8 (2.5)
BMI (Kg/m^2)	31 (5)	30 (4)
LVH	0	0
Plasma glucose (mmol/L)	5.7 (1.3)	6.0 (1.2)
Serum cholesterol:		
Total (mmol/L)	5.2 (0.8)	5.1 (0.9)
LDL (mmol/L)	3.1 (0.7)	3.2 (0.8)
HDL (mmol/L)	1.4 (0.3)	1.3 (0.2)
Total:HDL ratio	3.8 (0.7)	4.0 (0.6)
Triglyceride (mmol/L)	1.4 (0.8)	1.5 (0.8)
Number of antihypertensives:		
3	5	4
4	2	2
Antihypertensive drugs:		
Thiazide diuretic	4	3
Loop diuretic	2	2
CCB	4	3
ACE inhibitor	4	3
ß-blocker	4	4
ARA	2	2
Doxazosin	1	1
Minoxidil	2	2
Aspirin	2	2
Statin	1	1

Table 7.1
Continued from previous page.

7.3.2 Effects on BP, HR, arterial wave reflection and CF-PWV

The data for peripheral BP and MAP are shown in Figure 7.2. Sildenafil alone, ISMN alone and the combination all reduced systolic BP, diastolic BP and MAP compared to placebo. The effects of sildenafil alone and ISMN alone were not significantly different for systolic or diastolic BP, but ISMN caused a greater fall in MAP. The combination of sildenafil and ISMN produced a greater fall in systolic BP than either drug given alone but the effect on diastolic BP and MAP was not significantly different. Compared to placebo, from 1 to 3 hours after drug administration BP was on average 13/10 mmHg lower with sildenafil alone, 18/14 mmHg lower with ISMN alone and 26/18 mmHg lower with combined sildenafil and ISMN.

The data for measures of arterial wave reflection are shown in Figure 7.3. The relative effects of sildenafil alone, ISMN alone and the combination of sildenafil and ISMN on each of aortic AIx, aortic AIx@75 and RAIx were the same. There appeared to be a small but statistically non-significant reduction in arterial wave reflection with sildenafil. In contrast, ISMN alone and in combination with sildenafil reduced arterial wave reflection, compared both to placebo and to sildenafil alone. There was a slight trend to a greater reduction in arterial wave reflection with the combination of ISMN and sildenafil compared to ISMN alone, but this was not statistically significant. Compared to placebo, from 1 to 3 hours after drug administration aortic AIx, aortic AIx@75 and RAIx were, respectively, on average 3%, 2% and 4% lower with sildenafil alone, 16%, 15% and 22% lower with ISMN alone and 18%, 17% and 26% lower with combined sildenafil and ISMN.

The data for central BP, HR and CF-PWV are shown in Figure 7.4. Sildenafil alone, ISMN alone and the combination all reduced central systolic BP and central diastolic BP compared to placebo. Central systolic BP was reduced more by ISMN alone than by sildenafil alone, although the effects of the 2 drugs given alone were not significantly different for central diastolic BP. The combination of sildenafil and ISMN produced a greater fall in central systolic BP than either drug given alone and a greater fall in central diastolic BP than sildenafil alone. The effect of the

combination on central diastolic BP was not significantly different to the effect of ISMN alone. Compared to placebo, from 1 to 3 hours after drug administration central BP was on average 15/10 mmHg lower with sildenafil alone, 28/14 mmHg lower with ISMN alone and 36/19 mmHg lower with combined sildenafil and ISMN. There was an early trend to a small increase in HR with sildenafil alone and the combination of sildenafil and ISMN compared to either placebo or ISMN alone, but there was no overall significant difference between any of the study phases. There was no effect on CF-PWV of either sildenafil alone, ISMN alone, or the combination of sildenafil and ISMN.

7.3.3 Side effects

Two subjects experienced transient side effects. One experienced flushing, headache and dizziness with the combination of sildenafil and ISMN and the other a headache with sildenafil alone.

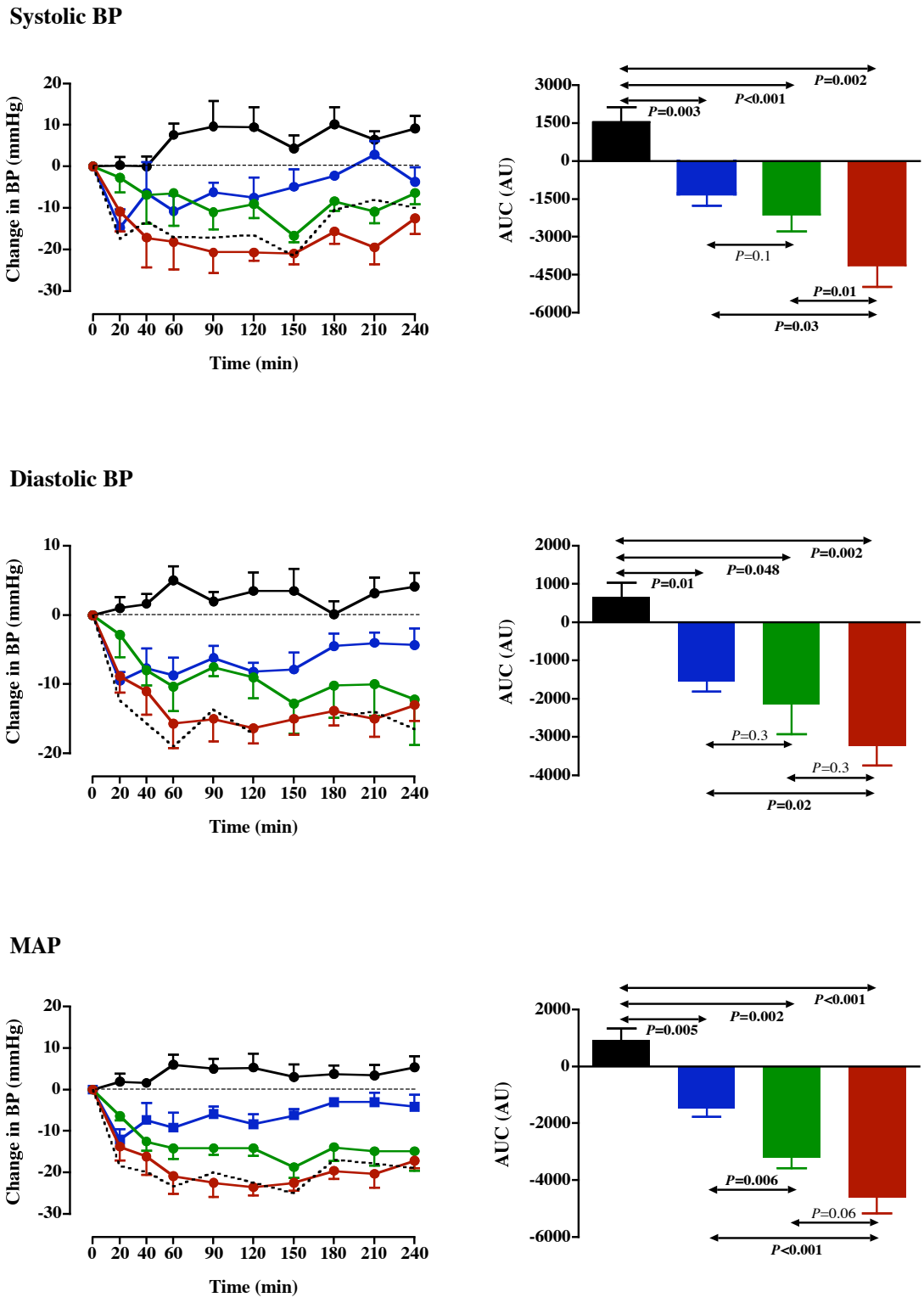

Figure 7.2. Effects on peripheral BP and MAP.
Data expressed as mean changes from baseline. Error bars are SEMs. Effects at each time point on the left and AUCs of these curves on the right. Placebo in black, sildenafil in blue, ISMN in green and combined sildenafil and ISMN in red. The dotted lines represent the sum of the effects of sildenafil alone and ISMN alone at each time point.

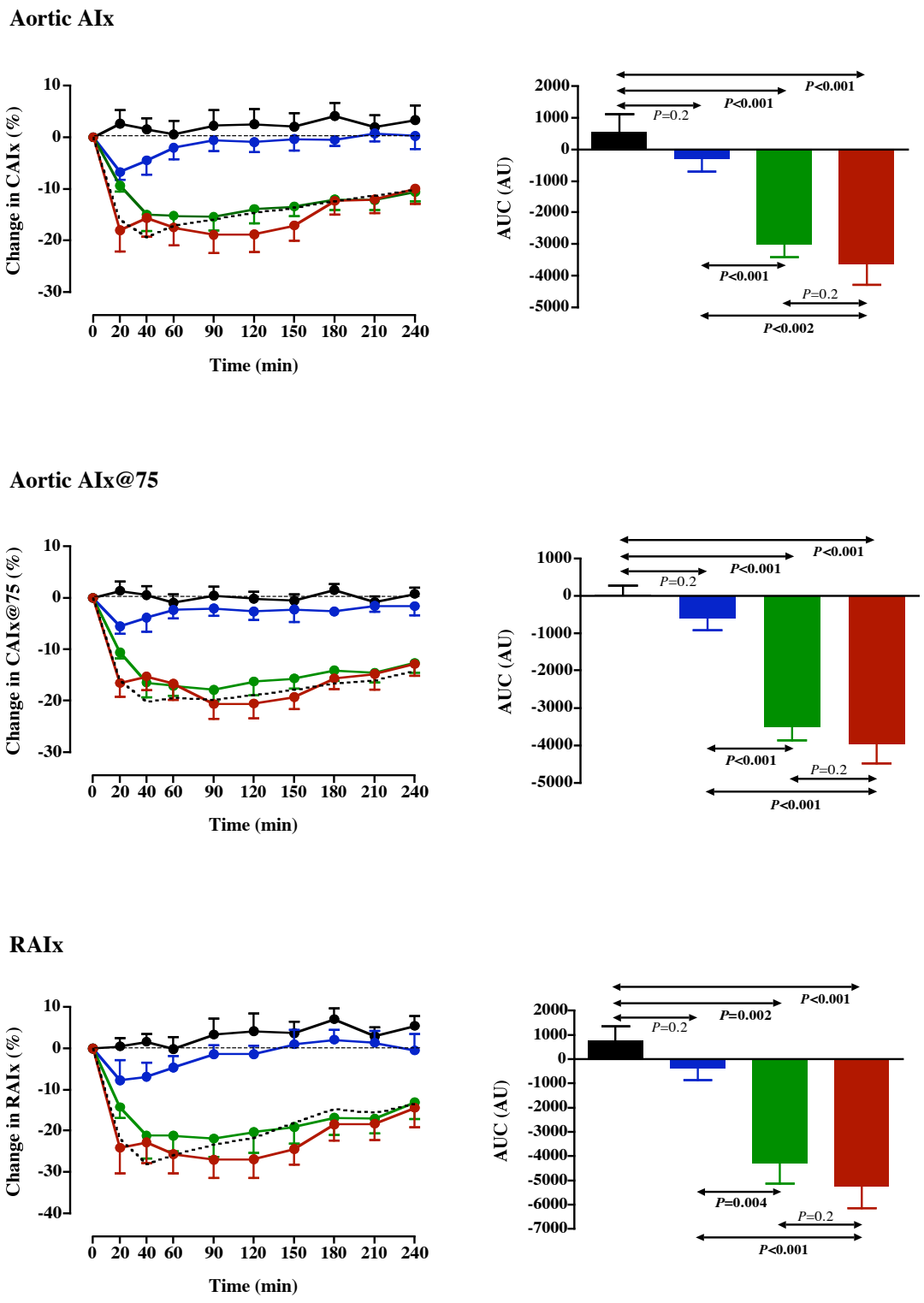

Figure 7.3. Effects on arterial wave reflection.
Data expressed as mean changes from baseline. Error bars are SEMs. Effects at each time point on the left and AUCs of these curves on the right. Placebo in black, sildenafil in blue, ISMN in green and combined sildenafil and ISMN in red. The dotted lines represent the sum of the effects of sildenafil alone and ISMN alone at each time point.

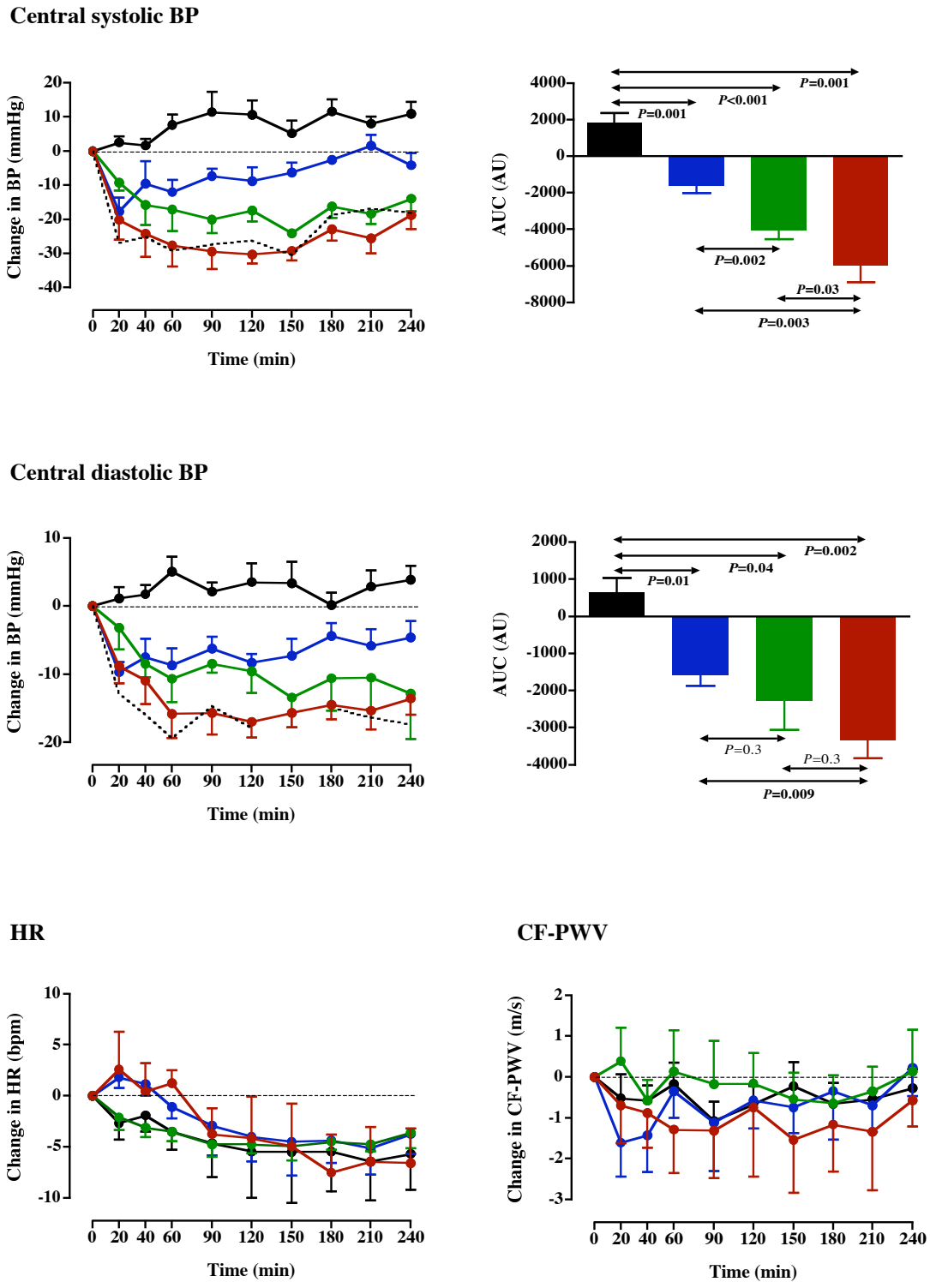

Figure 7.4. Effects on central BP, HR and CF-PWV.
Data expressed as mean changes from baseline. Error bars are SEMs. For central BP, effects at each time point are on the left and AUCs of these curves on the right. AUCs are not shown for HR or PWV because none of the comparisons were not statistically significant for these parameters. Placebo in black, sildenafil in blue, ISMN in green and combined sildenafil and ISMN in red. The dotted lines represent the sum of the effects of sildenafil alone and ISMN alone at each time point.

7.4 DISCUSSION

7.4.1 Main findings

The main findings from the present study are that in patients with TRH who were maintained on their usual antihypertensives both sildenafil and ISMN given alone acutely reduced BP and there was additional BP reduction when these drugs were given in combination.

7.4.2 The effect of placebo

During the placebo phase of the study there was a general trend to an increase in BP throughout the monitoring period. Subjects took their usual antihypertensives on waking, generally a couple of hours before starting the study. Thus, it is likely that baseline measures were made at around the time of the peak effect of their normal regimens. The subsequent increase in BP with placebo may, therefore, be the result of waning of this peak effect. In keeping with this, HR tended to decline with time, which might be expected as a baroreceptor-mediated response to increasing BP. The alternative explanation, that the subjects became increasingly restless, seems less likely because HR would be expected to increase under these circumstances.

7.4.3 The effects of sildenafil, ISMN and combined sildenafil and ISMN

7.4.3.1 Peripheral BP

The effect of PDE5 inhibition on BP in patients with TRH has not been investigated previously. The observed acute reduction in BP with sildenafil of around 13/10 mmHg compared to placebo would likely be of real clinical benefit if it were sustained with regular treatment. Given that sildenafil alone reduces BP in uncomplicated hypertensives when taken regularly (chapter 6), it seems likely that it would also continue to reduce BP with regular treatment in TRH, although this would have to be investigated specifically.

Once daily extended-release ISMN has recently been shown to reduce systolic BP, but not diastolic BP, in patients with treatment-resistant systolic hypertension (Stokes *et al*., 2005). In the present study, in addition to reducing systolic BP, ISMN

also reduced diastolic BP, suggesting that the benefit of organic nitrates may not be restricted to systolic BP when a more heterogeneous TRH population is studied. Compared to placebo, ISMN reduced BP acutely by around 18/14 mmHg and this was achieved at a very low dose of 10 mg. Whether this effect can be sustained with regular treatment warrants further investigation. With chronic treatment the development of nitrate tolerance might attenuate the degree of BP reduction that can be achieved. However, nitrate tolerance can be avoided by ensuring that there is a regular nitrate-free or nitrate-low period in each 24-hours (Thadani *et al.*, 1992). Indeed, a preparation that allowed for this was used in the study that demonstrated chronic efficacy in resistant systolic hypertension. The downside of requiring a nitrate-free or nitrate-low period is that, during this time, BP may be less well controlled. However, reducing BP for a substantial portion of the day may be preferable to not reducing it at all. Further advantages of organic nitrates are that they are very familiar to physicians, being widely used in CAD, and that they are relatively inexpensive.

The primary aim of the present study was to investigate the effect of combined sildenafil and ISMN on BP in TRH. When given together these drugs substantially reduced BP, by around 26/18 mmHg compared to placebo. The effect on systolic BP was significantly greater than the effect of either drug given alone and the effect on diastolic BP was significantly greater than sildenafil alone. There was also a trend to a greater reduction in diastolic BP than with ISMN alone, and the lack of statistical significance is probably due the small size of the study. As can be seen in Figure 7.2, the effect of the combination on BP was no greater than would be expected from the sum of the effects of each drug given individually. Indeed, the effects in comparison to placebo appear to be less than additive (reductions of 13/10 mmHg with sildenafil and 18/14 with ISMN compared to 26/18 mmHg with the combination). The lack of any synergism in the interaction of these drugs on BP may indicate that they could be used to reduce BP in TRH in a controlled manner. However, while the present study demonstrates the potential of an organic nitrate and PDE5 inhibitor combination to reduce BP in TRH, further research is needed to establish the longer-term efficacy and safety of such an approach.

The rationale for investigating the effect of combined sildenafil and ISMN on BP in TRH was that the unique antihypertensive potency of this drug combination might be useful in reducing BP in this therapeutically challenging population. However, as discussed above, while these drugs produced substantial reduction in BP when given together, they also each significantly reduced BP when given alone. This is interesting because neither drug alone is considered to be a particularly powerful antihypertensive, at least in the doses used, yet clinically significant BP reductions occurred even though subjects were treatment-resistant and were also taking at least 3 other antihypertensives. One possible explanation for this is that the NO-cGMP pathway is not the primary target of any of the usual antihypertensives that the subjects were taking. As a consequence, the potential for BP reduction with drugs that act on this pathway might be greater than with drugs that act on pathways that are already targeted by other drugs. It should also be borne in mind that the term "resistant hypertension" refers to a failure to reach a target BP rather than necessarily a general insensitivity to the effects of antihypertensives. For example, a patient with a BP of 210/110 mmHg which is reduced to 160/95 mmHg with 3 antihypertensives may be defined as treatment-resistant even though there has been a substantial drug-related BP reduction. Thus, even though the patients recruited had TRH, there was no particular reason to suppose that they would necessarily be relatively insensitive to drugs targeting the NO-cGMP pathway as part of a more generalised insensitivity to antihypertensives. The administration of the study drugs several hours after the patients had taken their usual antihypertensives may have had the consequence of maximising the observed effects on BP, in that the potential for BP reduction may have been greater if the effects of the regular drugs had passed their peaks, whereas if all drugs had been administered simultaneously there may have been less scope for additional BP reduction. Although this is speculative, if it proved to be correct it would suggest that staggering administration times throughout the day might provide better overall control of BP in TRH.

7.4.3.2 Arterial wave reflection, central BP and PWV

While ISMN alone had a slightly greater effect on BP than did sildenafil alone, ISMN substantially reduced arterial wave reflection whereas sildenafil had very little

effect. In addition, there was very little additional effect on wave reflection when sildenafil was given with ISMN. These data further support the concept (discussed in section 6.4.7) that PDE5 inhibitors are not "nitrate-like" in their haemodynamic action, even though they act on the same biochemical pathway. ISMN 10 mg most likely dilated peripheral conduit arteries, as well as resistance arterioles, whereas sildenafil probably mainly dilated arterioles.

Sildenafil alone had a similar effect on central systolic BP (reduction of ~13 mmHg vs placebo) as it did on peripheral systolic BP (reduction of ~15 mmHg vs placebo). In contrast, the effect of ISMN alone on systolic BP was greater centrally (reduction of ~28 mmHg vs placebo) than peripherally (reduction of ~18 mmHg vs placebo). Given that peripheral systolic BP was greater than central systolic BP at baseline, the proportional difference in the effect of ISMN between the 2 sites was even larger. As central systolic BP is augmented by the arterial pressure wave returning from the periphery, this finding is expected given the observed effects on arterial wave reflection. There is recent evidence that reducing central BP may be more important than reducing peripheral BP in the preventing cardiovascular events (Williams *et al.*, 2006). Therefore, the substantial effect of ISMN on central systolic BP in TRH may be of particular benefit if it were sustained with regular treatment. Even further benefit might be gained with the addition of sildenafil, given that the combination reduced central systolic BP by ~36 mmHg.

Even though BP is considered to be a major confounding factor of arterial stiffness, PWV was unaffected by any treatment in the current study, despite very substantial acute changes in BP. Sildenafil alone also did not affect PWV acutely when given to untreated hypertensives, although the effects of BP were milder than in the present study (see sections 6.3.6 and 6.4.4). As discussed in section 6.4.4, these findings are consistent with the possibility that, at least in hypertension, structural changes to large arteries occur such that the stiffness of their walls remains relatively constant despite changes in distending pressure.

7.4.3.3 HR

There were no significant changes in HR during any phase of the study despite significant reductions in BP, especially with the combination of sildenafil and ISMN. Four of the subjects were taking a ß-blocker and this may have limited any increase in HR that might otherwise have occurred. In addition, the subjects remained rested supine throughout the study and it is possible that HR might have increased if the subjects had been ambulatory. Nevertheless, the lack of any increase in HR is encouraging in terms of potential clinical use, especially when compared to the vasodilator minoxidil which, although often used for TRH, must be prescribed with a ß-blocker to prevent reflex tachycardia.

7.4.3.4 Side effects

Few side effects were experienced, even with the combination of sildenafil and ISMN. However, this was a small single dose study and the subjects were kept supine, which may have limited symptoms of hypotension. Any future study investigating the potential of combined PDE5 inhibitor and organic nitrate should closely monitor for side effects.

7.4.4 Summary

In patients with TRH maintained on their usual antihypertensives sildenafil given alone and ISMN given alone both acutely reduce BP. There is additional BP reduction when these drugs are given in combination.

CHAPTER 8

CONCLUSIONS AND FUTURE WORK

8.1 NON-INVASIVE ASSESSMENT OF ENDOTHELIAL VASOMOTOR FUNCTION

8.1.1 The appropriate dose of GTN to use as an endothelium-independent control

Relatively low doses of sublingual GTN, in the order of 8 to 50 μg, are generally equivalent to the effects of the endothelium-dependent stimuli that are used to assess endothelial vasomotor function non-invasively. For FMD, the dose of sublingual GTN that is equivalent to the effect of post-reactive hyperaemia-induced increased shear stress on brachial artery diameter is between 8 and 35 μg. Given that values for FMD in normal healthy subjects vary in the published literature, it is not possible to define a dose that is precisely equivalent to the normal FMD response in different populations. However, the dose-response data clearly indicate that 400 μg, a dose still used by many investigators (Pena *et al.*, 2004; Woodman *et al.*, 2004; Wu *et al.*, 2004; Zilkens *et al.*, 2003), dilates the brachial artery to a much greater extent than does post-reactive hyperaemia-induced increased shear stress. Moreover, this dose causes significant systemic haemodynamic changes and may not even be sufficient to cause maximum NO-mediated dilatation. Therefore, it seems rational to use a lower dose of GTN in FMD studies and 25 μg, already used by some investigators (Bennett-Richards *et al.*, 2002; Cross *et al.*, 2003; Ghiadoni *et al.*, 2001; Ghiadoni *et al.*, 2003), would generally be appropriate.

The dose-response study data suggested that the dose of GTN that was equivalent to the effect of salbutamol 400 μg on aortic AIx was 50 μg. This was based on previously published data in which the mean maximum change in aortic AIx with salbutamol was ~11% in healthy subjects (Wilkinson *et al.*, 2002a). When this dose was used in the study investigating the effect of age and gender on salbutamol responses, it resulted in a greater effect on aortic AIx that did salbutamol (-14% vs -6%), suggesting that a smaller dose would have been more appropriate. GTN also had a much greater effect on RAIx (-18% vs -8% with salbutamol). In contrast to FMD, which has been used extensively the last 10 to 15 years, there is currently little published data on the effect of salbutamol on arterial wave reflection, However, it is

likely that the methodology will be used with increasing frequency and as further data are published there will be greater appreciation of the range of normal responses. It will then be possible, by referring to the dose-response relationships characterised here, to choose with greater confidence an appropriate dose of GTN to use as a control for the effects of salbutamol.

8.1.2 Reduction in arterial wave reflection with salbutamol: the effects of age, gender and correlation with FMD

There were no effects of either age or gender on the degree of reduction in arterial wave reflection with inhaled salbutamol. Given the well-documented age-related decline in both FMD and muscarinic agonist-induced vasodilatation in the forearm, the lack of any effect of age on salbutamol responses is of particular interest and is well worthy of further investigation. Although the study reported here was a reasonable size overall, in any given decade of age (20s, 30s etc) there were only around 10 subjects. Moreover, there were only 3 subjects in their 60s and 1 subject in their 70s. A similar study recruiting greater numbers of healthy subjects of different ages, especially over 60 years, would be of value. Consideration should also be given to controlling for aerobic fitness in such a study. Given the evidence that the age-related decline in endothelium-dependent vasomotor function is agonist-specific (DeSouza *et al*., 2002), the effect of age on salbutamol-induced vasodilatation in the forearm of healthy subjects should be investigated. If there is no effect of age on salbutamol-induced vasodilatation, this would be consistent with the data on systemic salbutamol responses. If, on the other hand, there is a decline in salbutamol-induced vasodilatation in the forearm with age, this would suggest that measurement of arterial wave reflection is not sufficiently sensitive to detect this effect.

There was no correlation between brachial artery FMD and the extent of reduction in arterial wave reflection with salbutamol in healthy subjects. Although this may initially seem surprising, because both are measures of NO-dependent vasodilatation, it is in keeping with recent data from the much larger PIVUS study (Lind *et al.*, 2005). The possibility that measurement of resistance artery endothelial vasomotor function might provide information on cardiovascular risk that is additional to

information obtained by measuring conduit artery endothelial vasomotor function is intriguing. In this respect, the longitudinal data from the PIVUS study will be very interesting. Other prospective studies on the value of measuring the effect of inhaled salbutamol on arterial wave reflection will determine the potential of this methodology for cardiovascular risk assessment. These studies will also generate data on baseline arterial wave reflection and should resolve whether additional prognostic information can be gained from the derived central waveform over the directly-measured peripheral waveform. Moreover, it will also be possible to determine whether challenge with salbutamol provides additional prognostic information over simple measurement of baseline arterial wave reflection.

8.2 ENDOTHELIAL FUNCTION IN HEALTHY SMOKERS AND THE EFFECT OF SILDENAFIL

Compared to non-smokers, there was a reduced systemic vascular response to inhaled salbutamol in smokers. While this is consistent with previous studies that have demonstrated that smoking is associated with impaired endothelium-dependent vasomotor function, the reduced effect of salbutamol was only shown when the response was quantified as the AUC of the change in aortic AIx. This may indicate that the AUC of the change in aortic AIx is more sensitive for the detection for endothelial vasomotor dysfunction than the maximum change in aortic AIx or either the AUC of the change or the maximum change in RAIx, in which there were statistically non-significant trends to reduced responses with salbutamol in smokers. Future studies using this methodology, in smokers and in other at risk populations, should address this question.

There was a trend to an improvement in the response to salbutamol in smokers with sildenafil. If this was a real effect the study was underpowered to detect it with statistical confidence. Therefore, a larger study would be required to definitely address this question. Power calculations suggested that around 60 smokers would be needed for such a study. It is possible that subjects with a more extensive smoking history would exhibit greater impairment of salbutamol responses at baseline and

also greater improvement in salbutamol responses with sildenafil. Therefore, recruiting subjects with a more extensive smoking history might reduce the required sample size. A similar study in which endothelial vasomotor function was also measured by other methodologies, such as brachial artery FMD or response to ACh in the foreman, would allow for a comparison between methodologies in the assessment of both baseline endothelium-dependent vasomotor function and the effect on this of treatment with sildenafil.

8.3 THE TIME COURSE OF THE HYPOTENSIVE INTERACTION BETWEEN SILDENAFIL AND GTN

In men with stable angina the interaction on BP reduction between sildenafil 100 mg and GTN 400 μg lasted for at least 8 hours after sildenafil administration, but was, on average, no more than additive from 6 hours after sildenafil. These data have direct clinical relevance in that they will inform clinical judgement on whether GTN could be considered for a patient with angina who has taken sildenafil more than 6 hours previously. Importantly, the study does not exclude the possibility of significant hypotension if GTN is administered between 6 and 8 hours after sildenafil but suggests that this would be substantially less likely than if it were given within 6 hours of sildenafil. Therefore, if GTN is considered appropriate it should be used cautiously in patients who are haemodynamically stable and closely monitored. A much larger study would be required to determine with some confidence the frequency with which significant hypotension occurs when GTN is given more than 6 hours after sildenafil. However, it is not likely that such a study will be performed. GTN is also frequently given intravenously when patients are admitted to hospital with acute angina. The data on the time course of the interaction with sublingual GTN may not easily be extrapolated to GTN given intravenously, but a similar study to that reported here could be performed with intravenous GTN.

The time course of the hypotensive interactions between GTN and the other PDE5 inhibitors in clinical use have not been characterised in detail in patients with CAD. Unpublished data in healthy subjects have apparently shown that vardenafil 10 mg

does not potentiate the BP lowering effect of sublingual GTN 400 μg given after 1 to 24 hours, but that vardenafil 20 mg does potentiate the BP lowering effect of sublingual GTN 400 μg given after 1 and 4 hours, though not after 24 hours (Summary of product characteristics for Levitra, 2005). Vardenafil has a similar pharmacokinetic profile to sildenafil (see Table 1.1) and it is possible that the time course of its interaction with sublingual GTN in angina patients would also be similar to that with sildenafil. However, this would need to be investigated directly. Tadalafil has a much longer duration of action than sildenafil and in 166 men, mostly healthy but some of whom were diabetic or hypertensive, an interaction with sublingual GTN 400 μg was evident at 24 hours but not at 48 hours after tadalafil administration (Kloner *et al.*, 2003a).

8.4 THE EFFECT OF PDE5 INHIBITION IN HYPERTENSION

8.4.1 PDE5 inhibitors for the initial treatment of hypertension

In otherwise untreated hypertensives sildenafil given 3 times daily for 16 days reduced daytime ambulatory BP by 10/6 mmHg compared to placebo. This is the first study to investigate the effects of chronic PDE5 inhibition on BP in hypertension and indicates that PDE5 inhibitors have potential in the long-term treatment of this condition. While there was some attenuation of the acute effect on BP, this was relatively small and the persistent hypotensive effect at 16 days suggests that the effects on BP will be maintained in the long term. However, longer studies would be needed to confirm this.

Sildenafil did not reduce arterial wave reflection to any greater extent than would be expected from its effects on BP. However, the trend to an improvement in CF-PWV after 16 days of regular sildenafil is worth investigating further. Specifically, does longer treatment with a PDE5 inhibitor reduce PWV? Given the strong relationship between PWV and cardiovascular events, reduction in PWV may be an important mechanism through which treatment improves clinical outcome. The dissociation between the effect of sildenafil on BP and its potential effect on PWV may suggest a

progressive reduction in the intrinsic stiffness of large arteries. This, itself, might be a result of chronic reduction in BP, although reduction in PWV has not been shown consistently with chronic treatment with antihypertensives of different classes (Oliver *et al*., 2003).

Sildenafil also had no effect on conduit artery endothelium-dependent vasomotor function, measured as brachial artery FMD, in hypertensives. This finding does not discount the possibility that PDE5 inhibition would improve endothelium-dependent vasomotor function in other vascular territories. For example, the effects of chronic treatment with a PDE5 inhibitor on endothelium-dependent vasomotion in the forearm vascular bed or on the systemic vascular response to salbutamol could be investigated in future studies.

Drugs with a duration of action long enough to allow once-daily administration are preferred in the management of hypertension (or, indeed, any condition requiring chronic treatment) to maximise patient convenience and adherence to treatment. In this respect sildenafil, which has a relatively short duration action and must be given 3 times daily, is not ideal. However, tadalafil might be more suitable as an antihypertensive, given that it has a much longer duration of action and can be given once daily. Therefore, wherever possible, tadalafil should probably be used in future studies investigating the potential of PDE5 inhibitors for the treatment of hypertension. Alternatively, if modified release preparations of sildenafil or vardenafil were developed these might also be suitable. A priority for further research on the role of PDE5 inhibitors as treatments for hypertension is to characterise appropriate dosing strategies for use in regular therapy. For example, what are the smallest doses that reduce BP and how much additional hypotensive effect can be achieved by increasing dose? Subsequently, studies comparing both antihypertensive efficacy and tolerability of PDE5 inhibitors with established antihypertensives would help to determine their place in clinical practice.

Side effects were reported relatively commonly with regular sildenafil administration and these might limit the potential for widespread clinical use in hypertension. Given

that tadalafil is likely to be the most suitable PDE5 inhibitor for the chronic treatment of hypertension, at least of those currently available, any future chronic studies using this drug should carefully evaluate its side effect profile. There is a suggestion from the literature that back pain and myalgia may be more common with tadalafil (Govier *et al.*, 2003; Hatzimouratidis *et al.*, 2005; Setter *et al.*, 2005) and future studies should monitor plasma creatine kinase concentrations. Dyspepsia was reported relatively commonly with regular sildenafil. If the PDE5 inhibitors are pursued as treatments for hypertension there should be very careful evaluation of their chronic effects on the lower oesophagus. The possibility of chronic reflux oesophagitis should be taken very seriously given the possibility of an increased risk in oesophageal neoplasia.

8.4.2 PDE5 inhibitors and organic nitrates for TRH

In patients with TRH who were maintained on their usual antihypertensives, a combination of sildenafil and ISMN produced substantial acute reduction in BP. However, each of these drugs given alone also significantly reduced BP, albeit to a lesser degree than in combination. Although this study was very small the clear effects on BP that were demonstrated provide a sufficient base from which to progress to regular dosing studies. Further investigation of the effects of a PDE5 inhibitor alone or a long-acting nitrate alone on BP in TRH could be performed relatively easily in an outpatient-based study. For further investigation of the effects of combined PDE5 inhibitor and organic nitrate, one possible study design would be to give patients with TRH either regular sildenafil or regular ISMN alone initially and, if BP is not controlled, to then also give the alternative drug. Given the potential for substantial BP reduction with the combination, subjects would need to be monitored closely. The relatively short duration of action of sildenafil would make it suitable for such initial chronic dosing studies, because profound hypotension might last substantially longer if a longer acting drug, such as tadalafil, were used. However, after these studies, the effect of tadalafil in combination with an organic nitrate, such as ISMN, would be of particular interest.

There are certain groups of patients in whom BP control is considered particularly important but often more difficult to achieve, including patients with diabetes or renal failure. Moreover, because of their increased cardiovascular risk, target BPs are lower for these patients than for hypertensives in general (Williams *et al.*, 2004). Aggressive BP lowering therapy in the form of combined PDE5 inhibitor and organic nitrate could be particularly useful in these situations and studies specifically recruiting renal failure or diabetes patients should be considered.

If future research confirms the potential of combined PDE5 inhibitor and organic nitrate in TRH, the substantial BP reduction that might be achieved may even allow for cessation of other antihypertensives. This would allow for simplification of medication regimens, but would have to be investigated directly.

The antihypertensive drug nebivolol is a $ß_1$-adrenoreceptor antagonist that also acts as a vasodilator by stimulating the production of endothelial NO (Dessy *et al.*, 2005; Mason *et al.*, 2005). By virtue of this action, PDE5 inhibitors might be expected to potentiate nebivolol's effects on BP. There are no published data on the combined effects of PDE5 inhibitors and nebivolol on BP, but the potential of such a drug combination, either in uncomplicated or treatment-resistant hypertension, would be worth studying.

CHAPTER 9

REFERENCES

ADAMS, M.R., ROBINSON, J., MCCREDIE, R., SEALE, J.P., SORENSEN, K.E., DEANFIELD, J.E. & CELERMAJER, D.S. (1998). Smooth muscle dysfunction occurs independently of impaired endothelium-dependent dilation in adults at risk of atherosclerosis. *J Am Coll Cardiol*, **32,** 123-7.

AIRD, W.C. (2004). Endothelium as an organ system. *Crit Care Med*, **32,** S271-9.

ALFAKIH, K., WALTERS, K., JONES, T., RIDGWAY, J., HALL, A.S. & SIVANANTHAN, M. (2004). New gender-specific partition values for ECG criteria of left ventricular hypertrophy. *Hypertension*, **44,** 1-5.

ALLEN, J.D., WILSON, J.B., TULLEY, R.T., LEFEVRE, M. & WELSCH, M.A. (2000). Influence of age and normal plasma fibrinogen levels on flow-mediated dilation in healthy adults. *Am J Cardiol*, **86,** 703-5.

ALLI, C., AVANZINI, F., BETTELLI, G., COLOMBO, F., TORRI, V. & TOGNONI, G. (1999). The long-term prognostic significance of repeated blood pressure measurements in the elderly: SPAA (Studio sulla Pressione Arteriosa nell'Anziano) 10-year follow-up. *Arch Intern Med*, **159,** 1205-12.

ANDERSON, T.J., UEHATA, A., GERHARD, M.D., MEREDITH, I.T., KNAB, S., DELAGRANGE, D., LIEBERMAN, E.H., GANZ, P., CREAGER, M.A., YEUNG, A.C. & ET AL. (1995). Close relation of endothelial function in the human coronary and peripheral circulations. *J Am Coll Cardiol*, **26,** 1235-41.

ANTIKAINEN, R., JOUSILAHTI, P. & TUOMILEHTO, J. (1998). Systolic blood pressure, isolated systolic hypertension and risk of coronary heart disease, strokes, cardiovascular disease and all-cause mortality in the middle-aged population. *J Hypertens*, **16,** 577-83.

APTER, J.T. (1967). Correlation of visco-elastic properties with microscopic structure of large arteries. IV. Thermal responses of collagen, elastin, smooth muscle, and intact arteries. *Circ Res*, **21,** 901-18.

ARMSTRONG, P.W., ARMSTRONG, J.A. & MARKS, G.S. (1979). Blood levels after sublingual nitroglycerin. *Circulation*, **59,** 585-8.

ARORA, R.R., TIMONEY, M. & MELILLI, L. (1999). Acute myocardial infarction after the use of sildenafil. *N Engl J Med*, **341,** 700.

ARRUDA-OLSON, A.M., MAHONEY, D.W., NEHRA, A., LECKEL, M. & PELLIKKA, P.A. (2002). Cardiovascular effects of sildenafil during exercise in men with known or probable coronary artery disease: a randomized crossover trial. *JAMA*, **287,** 719-25.

ASMAR, R.G., LONDON, G.M., O'ROURKE, M.E. & SAFAR, M.E. (2001). Improvement in blood pressure, arterial stiffness and wave reflections with a

very-low-dose perindopril/indapamide combination in hypertensive patient: a comparison with atenolol. *Hypertension*, **38,** 922-6.

AVOLIO, A., JONES, D. & TAFAZZOLI-SHADPOUR, M. (1998). Quantification of alterations in structure and function of elastin in the arterial media. *Hypertension*, **32,** 170-5.

BANK, A.J., KAISER, D.R., RAJALA, S. & CHENG, A. (1999). In vivo human brachial artery elastic mechanics: effects of smooth muscle relaxation. *Circulation*, **100,** 41-7.

BARENBROCK, M., KOSCH, M., JOSTER, E., KISTERS, K., RAHN, K.H. & HAUSBERG, M. (2002). Reduced arterial distensibility is a predictor of cardiovascular disease in patients after renal transplantation. *J Hypertens*, **20,** 79-84.

BANK, A.J., WANG, H., HOLTE, J.E., MULLEN, K., SHAMMAS, R. & KUBO, S.H. (1996). Contribution of collagen, elastin, and smooth muscle to in vivo human brachial artery wall stress and elastic modulus. *Circulation*, **94,** 3263-70.

BASHIR, A., LEWIS, M.J. & HENDERSON, A.H. (1982). Pharmacokinetic studies of various preparations of glyceryl trinitrate. *Br J Clin Pharmacol*, **14,** 779-84.

BAUMGART, P. & KAMP, J. (1998). Accuracy of the SpaceLabs Medical 90217 ambulatory blood pressure monitor. *Blood Press Monit*, **3,** 303-7.

BEAVO, J.A. (1995). Cyclic nucleotide phosphodiesterases: functional implications of multiple isoforms. *Physiol Rev*, **75,** 725-48.

BEHR-ROUSSEL, D., GORNY, D., MEVEL, K., CAISEY, S., BERNABE, J., BURGESS, G., WAYMAN, C., ALEXANDRE, L. & GIULIANO, F. (2005). Chronic sildenafil improves erectile function and endothelium-dependent cavernosal relaxations in rats: lack of tachyphylaxis. *Eur Urol*, **47,** 87-91.

BENETOS, A., ADAMOPOULOS, C., BUREAU, J.M., TEMMAR, M., LABAT, C., BEAN, K., THOMAS, F., PANNIER, B., ASMAR, R., ZUREIK, M., SAFAR, M. & GUIZE, L. (2002). Determinants of accelerated progression of arterial stiffness in normotensive subjects and in treated hypertensive subjects over a 6-year period. *Circulation*, **105,** 1202-7.

BENNETT-RICHARDS, K., KATTENHORN, M., DONALD, A., OAKLEY, G., VARGHESE, Z., REES, L. & DEANFIELD, J.E. (2002). Does oral folic acid lower total homocysteine levels and improve endothelial function in children with chronic renal failure? *Circulation*, **105,** 1810-5.

BERKELS, R., KLOTZ, T., STICHT, G., ENGLEMANN, U. & KLAUS, W. (2001). Modulation of human platelet aggregation by the phosphodiesterase type 5 inhibitor sildenafil. *J Cardiovasc Pharmacol*, **37,** 413-21.

BETIK, A.C., LUCKHAM, V.B. & HUGHSON, R.L. (2004). Flow-mediated dilation in human brachial artery after different circulatory occlusion conditions. *Am J Physiol Heart Circ Physiol*, **286,** H442-8.

BLACHER, J., ASMAR, R., DJANE, S., LONDON, G.M. & SAFAR, M.E. (1999a). Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension*, **33,** 1111-7.

BLACHER, J., GUERIN, A.P., PANNIER, B., MARCHAIS, S.J., SAFAR, M.E. & LONDON, G.M. (1999b). Impact of aortic stiffness on survival in end-stage renal disease. *Circulation*, **99,** 2434-9.

BLACHER, J., PANNIER, B., GUERIN, A.P., MARCHAIS, S.J., SAFAR, M.E. & LONDON, G.M. (1998). Carotid arterial stiffness as a predictor of cardiovascular and all-cause mortality in end-stage renal disease. *Hypertension*, **32,** 570-4.

BLACK, H.R., ELLIOTT, W.J., NEATON, J.D., GRANDITS, G., GRAMBSCH, P., GRIMM, R.H., JR., HANSSON, L., LACOUCIERE, Y., MULLER, J., SLEIGHT, P., WEBER, M.A., WHITE, W.B., WILLIAMS, G., WITTES, J., ZANCHETTI, A., FAKOUHI, T.D. & ANDERS, R.J. (2001). Baseline characteristics and early blood pressure control in the CONVINCE trial. *Hypertension*, **37,** 12-18.

BLAND, J.M. & ALTMAN, D.G. (2003). Applying the right statistics: analyses of measurement studies. *Ultrasound Obstet Gynecol*, **22,** 85-93.

BLOOD PRESSURE LOWERING TRIALISTS COLLABORATION (2003). Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet*, **362,** 1527-35.

BOGREN, H.G., MOHIADDIN, R.H., KLIPSTEIN, R.K., FIRMIN, D.N., UNDERWOOD, R.S., REES, S.R. & LONGMORE, D.B. (1989). The function of the aorta in ischemic heart disease: a magnetic resonance and angiographic study of aortic compliance and blood flow patterns. *Am Heart J*, **118,** 234-47.

BONETTI, P.O., LERMAN, L.O. & LERMAN, A. (2003). Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol*, **23,** 168-75.

BOOLELL, M., ALLEN, M.J., BALLARD, S.A., GEPI-ATTEE, S., MUIRHEAD, G.J., NAYLOR, A.M., OSTERLOH, I.H. & GINGELL, C. (1996). Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. *Int J Impot Res*, **8,** 47-52.

Borlaug, B.A., Melenovsky, V., Marhin, T., Fitzgerald, P. & Kass, D.A. (2005). Sildenafil inhibits β-adrenergic-stimulated cardiac contractility in humans. Circulation, 112, 2642-9.

BOSHIER, A., WILTON, L.V. & SHAKIR, S.A. (2004). Evaluation of the safety of sildenafil for male erectile dysfunction: experience gained in general practice use in England in 1999. *BJU Int*, **93,** 796-801.

BOUTOUYRIE, P., TROPEANO, A.I., ASMAR, R., GAUTIER, I., BENETOS, A., LACOLLEY, P. & LAURENT, S. (2002). Aortic stiffness is an independent predictor of primary coronary events in hypertensive patients: a longitudinal study. *Hypertension*, **39,** 10-5.

BRAMWELL, J.C. & HILL, A.V. (1922). The velocity of the pulse wave in man. *Proc R Soc Lond B Biol Sci*, **93,** 298-306.

BRAMWELL, J.C., HILL, A.V. & MCSWINEY, B.A. (1923). The velocity of the pulse wave in man in relation to age as measured by the hot wire sphygmograph. *Heart*, **10,** 233-55.

BREMER, Y.A., SALLOUM, F., OCKAILI, R., CHOU, E., MOSKOWITZ, W.B. & KUKREJA, R.C. (2005). Sildenafil citrate (viagra) induces cardioprotective effects after ischemia/reperfusion injury in infant rabbits. *Pediatr Res*, **57,** 22-7.

BREVETTI, G., SILVESTRO, A., SCHIANO, V. & CHIARIELLO, M. (2003). Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. *Circulation*, **108,** 2093-8.

BRUNNER, H., COCKCROFT, J.R., DEANFIELD, J., DONALD, A., FERRANNINI, E., HALCOX, J., KIOWSKI, W., LUSCHER, T.F., MANCIA, G., NATALI, A., OLIVER, J.J., PESSINA, A.C., RIZZONI, D., ROSSI, G.P., SALVETTI, A., SPIEKER, L.E., TADDEI, S. & WEBB, D.J. (2005). Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. *J Hypertens*, **23,** 233-46.

BUGIARDINI, R., MANFRINI, O., PIZZI, C., FONTANA, F. & MORGAGNI, G. (2004). Endothelial function predicts future development of coronary artery disease: a study of women with chest pain and normal coronary angiograms. *Circulation*, **109,** 2518-23.

CALHOUN, D.A., NISHIZAKA, M.K., ZAMAN, M.A., THAKKAR, R.B. & WEISSMANN, P. (2002). Hyperaldosteronism among black and white subjects with resistant hypertension. *Hypertension*, **40,** 892-6.

CAMERON, J.D., MCGRATH, B.P. & DART, A.M. (1998). Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. *J Am Coll Cardiol*, **32,** 1214-20.

CARSON, C.C. & LUE, T.F. (2005). Phosphodiesterase type 5 inhibitors for erectile dysfunction. *BJU Int*, **96,** 257-80.

CELERMAJER, D.S., ADAMS, M.R., CLARKSON, P., ROBINSON, J., MCCREDIE, R., DONALD, A. & DEANFIELD, J.E. (1996). Passive smoking and impaired endothelium-dependent arterial dilatation in healthy young adults. *N Engl J Med*, **334,** 150-4.

CELERMAJER, D.S., SORENSEN, K.E., GEORGAKOPOULOS, D., BULL, C., THOMAS, O., ROBINSON, J. & DEANFIELD, J.E. (1993). Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation*, **88,** 2149-55.

CELERMAJER, D.S., SORENSEN, K.E., GOOCH, V.M., SPIEGELHALTER, D.J., MILLER, O.I., SULLIVAN, I.D., LLOYD, J.K. & DEANFIELD, J.E. (1992). Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet*, **340,** 1111-5.

CELERMAJER, D.S., SORENSEN, K.E., SPIEGELHALTER, D.J., GEORGAKOPOULOS, D., ROBINSON, J. & DEANFIELD, J.E. (1994). Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. *J Am Coll Cardiol*, **24,** 471-6.

CHAN, S.Y., MANCINI, G.B.J., KURAMOTO, L., SCHULZER, M., FROHLICH, J. & IGNASZEWSKI, A. (2003). The prognostic importance of endothelial dysfunction and carotid atheromaburden in patients with coronary artery disease. *J Am Coll Cardiol*, **42,** 1037-1043.

CHANG, J.J., RABINOWITZ, D. & SHEA, S. (2003). Sources of variability in blood pressure measurement using the Dinamap PRO 100 automated oscillometric device. *Am J Epidemiol*, **158,** 1218-26.

CHEITLIN, M.D., HUTTER, A.M., JR., BRINDIS, R.G., GANZ, P., KAUL, S., RUSSELL, R.O., JR. & ZUSMAN, R.M. (1999). ACC/AHA expert consensus document. Use of sildenafil (Viagra) in patients with cardiovascular disease. American College of Cardiology/American Heart Association. *J Am Coll Cardiol*, **33,** 273-82.

CHEN, C.H., NEVO, E., FETICS, B., PAK, P.H., YIN, F.C., MAUGHAN, W.L. & KASS, D.A. (1997). Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. Validation of generalized transfer function. *Circulation*, **95,** 1827-36.

CHEN, C.H., TING, C.T., LIN, S.J., HSU, T.L., YIN, F.C., SIU, C.O., CHOU, P., WANG, S.P. & CHANG, M.S. (1995). Different effects of fosinopril and atenolol on wave reflections in hypertensive patients. *Hypertension*, **25,** 1034-41.

CHIA, S., QADAN, M., NEWTON, R., LUDLAM, C.A., FOX, K.A. & NEWBY, D.E. (2003). Intra-arterial tumor necrosis factor-alpha impairs endothelium-dependent

vasodilatation and stimulates local tissue plasminogen activator release in humans. *Arterioscler Thromb Vasc Biol*, **23,** 695-701.

CHIRINOS, J.A., ZAMBRANO, J.P., CHAKKO, S., VEERANI, A., SCHOB, A., WILLENS, H.J., PEREZ, G. & MENDEZ, A.J. (2005). Aortic pressure augmentation predicts adverse cardiovascular events in patients with established coronary artery disease. *Hypertension*, **45,** 980-5.

CHOBANIAN, A.V., BAKRIS, G.L., BLACK, H.R., CUSHMAN, W.C., GREEN, L.A., IZZO, J.L., JR, JONES, D.W., MATERSON, B.J., OPARIL, S., WRIGHT, J.T., JR & ROCCELLA, E.J. (2003a). The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: The JNC 7 report. *JAMA*, **289,** 2560-72.

CHOBANIAN, A.V., BAKRIS, G.L., BLACK, H.R., CUSHMAN, W.C., GREEN, L.A., IZZO, J.L., JR., JONES, D.W., MATERSON, B.J., OPARIL, S., WRIGHT, J.T., JR. & ROCCELLA, E.J. (2003b). Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*, **42,** 1206-52.

CHOWIENCZYK, P.J., KELLY, R.P., MACCALLUM, H., MILLASSEAU, S.C., ANDERSSON, T.L., GOSLING, R.G., RITTER, J.M. & ANGGARD, E.E. (1999). Photoplethysmographic assessment of pulse wave reflection: blunted response to endothelium-dependent beta2-adrenergic vasodilation in type II diabetes mellitus. *J Am Coll Cardiol*, **34,** 2007-14.

CLEMENT, D.L., DE BUYZERE, M.L., DE BACQUER, D.A., DE LEEUW, P.W., DUPREZ, D.A., FAGARD, R.H., GHEERAERT, P.J., MISSAULT, L.H., BRAUN, J.J., SIX, R.O., VAN DER NIEPEN, P. & O'BRIEN, E. (2003). Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *N Engl J Med*, **348,** 2407-15.

CORBIN, J.D., BLOUNT, M.A., WEEKS, J.L., 2ND, BEASLEY, A., KUHN, K.P., HO, Y.S., SAIDI, L.F., HURLEY, J.H., KOTERA, J. & FRANCIS, S.H. (2003). [3H]sildenafil binding to phosphodiesterase-5 is specific, kinetically heterogeneous, and stimulated by cGMP. *Mol Pharmacol*, **63,** 1364-72.

CORBIN, J.D., TURKO, I.V., BEASLEY, A. & FRANCIS, S.H. (2000). Phosphorylation of phosphodiesterase-5 by cyclic nucleotide-dependent protein kinase alters its catalytic and allosteric cGMP-binding activities. *Eur J Biochem*, **267,** 2760-7.

CORRETTI, M.C., ANDERSON, T.J., BENJAMIN, E.J., CELERMAJER, D., CHARBONNEAU, F., CREAGER, M.A., DEANFIELD, J., DREXLER, H., GERHARD-HERMAN, M., HERRINGTON, D., VALLANCE, P., VITA, J. & VOGEL, R. (2002). Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*, **39,** 257-65.

COVIC, A., GOLDSMITH, D.J., FLOREA, L., GUSBETH-TATOMIR, P. & COVIC, M. (2004). The influence of dialytic modality on arterial stiffness, pulse wave reflections, and vasomotor function. *Perit Dial Int*, **24,** 365-72.

COVIC, A., GOLDSMITH, D.J., GUSBETH-TATOMIR, P., BUHAESCU, I. & COVIC, M. (2003). Successful renal transplantation decreases aortic stiffness and increases vascular reactivity in dialysis patients. *Transplantation*, **76,** 1573-7.

CROSS, J.M., DONALD, A.E., NUTTALL, S.L., DEANFIELD, J.E., WOOLFSON, R.G. & MACALLISTER, R.J. (2003). Vitamin C improves resistance but not conduit artery endothelial function in patients with chronic renal failure. *Kidney Int*, **63,** 1433-42.

CRUICKSHANK, K., RISTE, L., ANDERSON, S.G., WRIGHT, J.S., DUNN, G. & GOSLING, R.G. (2002). Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? *Circulation*, **106,** 2085-90.

CURRAN, M. & KEATING, G. (2003). Tadalafil. *Drugs*, **63,** 2203-12.

CUSHMAN, W.C., FORD, C.E., CUTLER, J.A., MARGOLIS, K.L., DAVIS, B.R., GRIMM, R.H., BLACK, H.R., HAMILTON, B.P., HOLLAND, J., NWACHUKU, C., PAPADEMETRIOU, V., PROBSTFIELD, J., WRIGHT, J.T., JR., ALDERMAN, M.H., WEISS, R.J., PILLER, L., BETTENCOURT, J. & WALSH, S.M. (2002). Success and predictors of blood pressure control in diverse North American settings: the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). *J Clin Hypertens*, **4,** 393-404.

CUSPIDI, C., MACCA, G., SAMPIERI, L., MICHEV, I., SALERNO, M., FUSI, V., SEVERGNINI, B., MEANI, S., MAGRINI, F. & ZANCHETTI, A. (2001). High prevalence of cardiac and extracardiac target organ damage in refractory hypertension. *J Hypertens*, **19,** 2063-70.

DAHLÖF, B., SEVER, P.S., POULTER, N.R., WEDEL, H., BEEVERS, D.G., CAULFIELD, M., COLLINS, R., KJELDSEN, S.E., KRISTINSSON, A., MCINNES, G.T., MEHLSEN, J., NIEMINEN, M., O'BRIEN, E. & OSTERGREN, J. (2005). Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required, in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA): a multicentre randomised controlled trial. *Lancet*, **366,** 895-906.

DAWES, M., CHOWIENCZYK, P.J. & RITTER, J.M. (1997). Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilation caused by β-adrenergic agonists in human forearm. *Circulation*, **95,** 2293-7.

DEANFIELD, J., DONALD, A., FERRI, C., GIANNATTASIO, C., HALCOX, J., HALLIGAN, S., LERMAN, A., MANCIA, G., OLIVER, J.J., PESSINA, A.C., RIZZONI, D., ROSSI,

G.P., SALVETTI, A., SCHIFFRIN, E.L., TADDEI, S. & WEBB, D.J. (2005). Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens*, **23,** 7-17.

DEARY, A.J., SCHUMANN, A.L., MURFET, H., HAYDOCK, S.F., FOO, R.S. & BROWN, M.J. (2002). Double-blind, placebo-controlled crossover comparison of five classes of antihypertensive drugs. *J Hypertens*, **20,** 771-7.

DESOUZA, C.A., CLEVENGER, C.M., GREINER, J.J., SMITH, D.T., HOETZER, G.L., SHAPIRO, L.F. & STAUFFER, B.L. (2002). Evidence for agonist-specific endothelial vasodilator dysfunction with ageing in healthy humans. *J Physiol*, **542,** 255-62.

DESOUZA, C.A., SHAPIRO, L.F., CLEVENGER, C.M., DINENNO, F.A., MONAHAN, K.D., TANAKA, H. & SEALS, D.R. (2000). Regular aerobic exercise prevents and restores age-related declines in endothelium-dependent vasodilation in healthy men. *Circulation*, **102,** 1351-7.

DESSY, C., SALIEZ, J., GHISDAL, P., DANEAU, G., LOBYSHEVA, II, FRERART, F., BELGE, C., JNAOUI, K., NOIRHOMME, P., FERON, O. & BALLIGAND, J.L. (2005). Endothelial beta3-adrenoreceptors mediate nitric oxide-dependent vasorelaxation of coronary microvessels in response to the third-generation beta-blocker nebivolol. *Circulation*, **112,** 1198-205.

DICKERSON, J.E.C., HINGORANI, A.D., ASHBY, M.J., PALMER, C.R. & BROWN, M.J. (1999). Optimisation of antihypertensive treatment by crossover rotation of four major classes. *The Lancet*, **353,** 2008-13.

DISHY, V., HARRIS, P.A., PIERCE, R., PRASAD, H.C., SOFOWORA, G., BONAR, H.L., WOOD, A.J.J. & STEIN, C.M. (2004). Sildenafil does not improve nitric oxide-mediated endothelium-dependent vascular responses in smokers. *Br J Clin Pharmacol*, **57,** 209-12.

DISHY, V., SOFOWORA, G., HARRIS, P.A., KANDCER, M., ZHAN, F., WOOD, A.J. & STEIN, C.M. (2001). The effect of sildenafil on nitric oxide-mediated vasodilation in healthy men. *Clin Pharmacol Ther*, **70,** 270-9.

DOSHI, S.N., MCDOWELL, I.F., MOAT, S.J., PAYNE, N., DURRANT, H.J., LEWIS, M.J. & GOODFELLOW, J. (2002). Folic acid improves endothelial function in coronary artery disease via mechanisms largely independent of homocysteine lowering. *Circulation*, **105,** 22-6.

DOSHI, S.N., NAKA, K.K., PAYNE, N., JONES, C.J., ASHTON, M., LEWIS, M.J. & GOODFELLOW, J. (2001). Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. *Clin Sci*, **101,** 629-35.

EARDLEY, I., MIRONE, V., MONTORSI, F., RALPH, D., KELL, P., WARNER, M.R., ZHAO, Y. & BEARDSWORTH, A. (2005). An open-label, multicentre, randomized, crossover study comparing sildenafil citrate and tadalafil for treating erectile dysfunction in men naive to phosphodiesterase 5 inhibitor therapy. *BJU Int*, **96,** 1323-32.

EGASHIRA, K., INOU, T., HIROOKA, Y., KAI, H., SUGIMACHI, M., SUZUKI, S., KUGA, T., URABE, Y. & TAKESHITA, A. (1993). Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. *Circulation*, **88,** 77-81.

EHERER, A.J., SCHWETZ, I., HAMMER, H.F., PETNEHAZY, T., SCHEIDL, S.J., WEBER, K. & KREJS, G.J. (2002). Effect of sildenafil on oesophageal motor function in healthy subjects and patients with oesophageal motor disorders. *Gut*, **50,** 758-64.

EIDE, I.K., TORJESEN, P.A., DROLSUM, A., BABOVIC, A. & LILLEDAHL, N.P. (2004). Low-renin status in therapy-resistant hypertension: a clue to efficient treatment. *J Hypertens*, **22,** 2217-26.

ELKAYAM, U. (1991). Tolerance to organic nitrates: evidence, mechanisms, clinical relevance, and strategies for prevention. *Ann Intern Med*, **114,** 667-77.

EUROPEAN SOCIETY OF HYPERTENSION & EUROPEAN SOCIETY OF CARDIOLOGY (2003). 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. *J Hypertens*, **21,** 1011-53.

EZZATI, M., LOPEZ, A.D., RODGERS, A., HOORN, S.V. & MURRAY, C.J.L. (2002). Selected major risk factors and global and regional burden of disease. *Lancet*, **360,** 1347-60.

FATHI, R., HALUSKA, B., ISBEL, N., SHORT, L. & MARWICK, T.H. (2004). The relative importance of vascular structure and function in predicting cardiovascular events. *J Am Coll Cardiol*, **43,** 616-23.

FDA (2005). FDA updates labeling for erectile dysfunction drugs. *FDA Consum*, **39,** 3.

FEENSTRA, J., VAN DRIE-PIERIK, R.J., LACLE, C.F. & STRICKER, B.H. (1998). Acute myocardial infarction associated with sildenafil. *Lancet*, **352,** 957-8.

FICHTLSCHERER, S., BREUER, S. & ZEIHER, A.M. (2004). Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the "vulnerable" patient. *Circulation*, **110,** 1926-32.

FITCHETT, D.H., SIMKUS, G.J., BEAUDRY, J.P. & MARPOLE, D.G. (1988). Reflected pressure waves in the ascending aorta: effect of glyceryl trinitrate. *Cardiovasc Res*, **22,** 494-500.

FLOWER, R. (2004). Lifestyle drugs: pharmacology and the social agenda. *Trends Pharmacol Sci*, **25,** 182-5.

FRANKLIN, S.S., GUSTIN, W., 4TH, WONG, N.D., LARSON, M.G., WEBER, M.A., KANNEL, W.B. & LEVY, D. (1997). Hemodynamic patterns of age-related changes in blood pressure. The Framingham Heart Study. *Circulation*, **96,** 308-15.

FRANKLIN, S.S., JACOBS, M.J., WONG, N.D., L'ITALIEN, G.J. & LAPUERTA, P. (2001a). Predominance of isolated systolic hypertension among middle-aged and elderly US hypertensives: analysis based on National Health and Nutrition Examination Survey (NHANES) III. *Hypertension*, **37,** 869-74.

FRANKLIN, S.S., LARSON, M.G., KHAN, S.A., WONG, N.D., LEIP, E.P., KANNEL, W.B. & LEVY, D. (2001b). Does the relation of blood pressure to coronary heart disease risk change with aging? The Framingham Heart Study. *Circulation*, **103,** 1245-9.

FRICK, M., SUESSENBACHER, A., ALBER, H.F., DICHTL, W., ULMER, H., PACHINGER, O. & WEIDINGER, F. (2005). Prognostic value of brachial artery endothelial function and wall thickness. *J Am Coll Cardiol*, **46,** 1006-10.

FRIES, R., SHARIAT, K., VON WILMOWSKY, H. & BOHM, M. (2005). Sildenafil in the treatment of Raynaud's phenomenon resistant to vasodilatory therapy. *Circulation*, **112,** 2980-5.

FUKAO, M., MASON, H.S., BRITTON, F.C., KENYON, J.L., HOROWITZ, B. & KEEF, K.D. (1999). Cyclic GMP-dependent protein kinase activates cloned BKCa channels expressed in mammalian cells by direct phosphorylation at serine 1072. *J Biol Chem*, **274,** 10927-35.

FUNG, E., FISCUS, R.R., YIM, A.P., ANGELINI, G.D. & ARIFI, A.A. (2005). The potential use of type-5 phosphodiesterase inhibitors in coronary artery bypass graft surgery. *Chest*, **128,** 3065-73.

FURCHGOTT, R.F. & ZAWADZKI, J.V. (1980). The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*, **288,** 373-6.

GALIè, N., GHOFRANI, H.A., TORBICKI, A., BARST, R.J., RUBIN, L.J., BADESCH, D., FLEMING, T., PARPIA, T., BURGESS, G., BRANZI, A., GRIMMINGER, F., KURZYNA, M. & SIMONNEAU, G. (2005). Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med*, **353,** 2148-57.

GALLEY, H.F. & WEBSTER, N.R. (2004). Physiology of the endothelium. *Br J Anaesth*, **93,** 105-13.

GATZKA, C.D., CAMERON, J.D., DART, A.M., BERRY, K.L., KINGWELL, B.A., DEWAR, E.M., REID, C.M. & JENNINGS, G.L.R. (2001). Correction of carotid augmentation index for heart rate in elderly essential hypertensives. *Am J Hypertens*, **14,** 573-7.

GERHARD, M., RODDY, M.A., CREAGER, S.J. & CREAGER, M.A. (1996). Aging progressively impairs endothelium-dependent vasodilation in forearm resistance vessels of humans. *Hypertension*, **27,** 849-53.

GHIADONI, L., DONALD, A.E., CROPLEY, M., MULLEN, M.J., OAKLEY, G., TAYLOR, M., O'CONNOR, G., BETTERIDGE, J., KLEIN, N., STEPTOE, A. & DEANFIELD, J.E. (2000). Mental stress induces transient endothelial dysfunction in humans. *Circulation*, **102,** 2473-8.

GHIADONI, L., HUANG, Y., MAGAGNA, A., BURALLI, S., TADDEI, S. & SALVETTI, A. (2001). Effect of acute blood pressure reduction on endothelial function in the brachial artery of patients with essential hypertension. *J Hypertens*, **19,** 547-51.

GHIADONI, L., MAGAGNA, A., VERSARI, D., KARDASZ, I., HUANG, Y., TADDEI, S. & SALVETTI, A. (2003). Different effect of antihypertensive drugs on conduit artery endothelial function. *Hypertension*, **41,** 1281-6.

GILES, T. (2005). Relevance of blood pressure variation in the circadian onset of cardiovascular events. *J Hypertens Suppl*, **23,** S35-9.

GOKCE, N., HOLBROOK, M., DUFFY, S.J., DEMISSIE, S., CUPPLES, L.A., BIEGELSEN, E., KEANEY, J.F., JR., LOSCALZO, J. & VITA, J.A. (2001). Effects of race and hypertension on flow-mediated and nitroglycerin-mediated dilation of the brachial artery. *Hypertension*, **38,** 1349-54.

GOKCE, N., HOLBROOK, M., HUNTER, L.M., PALMISANO, J., VIGALOK, E., KEANEY, J., JOHN F. & VITA, J.A. (2002a). Acute effects of vasoactive drug treatment on brachial artery reactivity. *J Am Coll Cardiol*, **40,** 761-5.

GOKCE, N., KEANEY J F JR, HUNTER, L.M., WATKINS, M.T., MENZOIAN, J.O. & VITA, J.A. (2002b). Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation*, **105,** 1567-72.

GOKCE, N., KEANEY JF JR, HUNTER, L.M., WATKINS, M.T., NEDELJKOVIC, Z.S., MENZOIAN, J.O. & VITA, J.A. (2003). Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. *J Am Coll Cardiol*, **41,** 1769-75.

GOLDSTEIN, I., LUE, T.F., PADMA-NATHAN, H., ROSEN, R.C., STEERS, W.D. & WICKER, P.A. (1998). Oral sildenafil in the treatment of erectile dysfunction. *N Engl J Med*, **338,** 1397-404.

GORI, T., SICURO, S., DRAGONI, S., DONATI, G., FORCONI, S. & PARKER, J.D. (2005). Sildenafil prevents endothelial dysfunction induced by ischemia and reperfusion via opening of adenosine triphosphate-sensitive potassium channels: a human in vivo study. *Circulation*, **111,** 742-6.

GOVIER, F., POTEMPA, A.J., KAUFMAN, J., DENNE, J., KOVALENKO, P. & AHUJA, S. (2003). A multicenter, randomized, double-blind, crossover study of patient preference for tadalafil 20 mg or sildenafil citrate 50 mg during initiation of treatment for erectile dysfunction. *Clin Ther*, **25,** 2709-23.

GREENFIELD, J.C. & PATEL, D.J. (1962). Relation between pressure and diameter in the ascending aorta of man. *Circ Res*, **10,** 778-81.

GROVER, S.A., LOWENSTEYN, I., KAOUACHE, M., MARCHAND, S., COUPAL, L., DECAROLIS, E., ZOCCOLI, J. & DEFOY, I. (2006). The prevalence of erectile dysfunction in the primary care setting: importance of risk factors for diabetes and vascular disease. *Arch Intern Med*, **166,** 213-9.

GUAZZI, M., TUMMINELLO, G., DI MARCO, F., FIORENTINI, C. & GUAZZI, M.D. (2004a). The effects of phosphodiesterase-5 inhibition with sildenafil on pulmonary hemodynamics and diffusion capacity, exercise ventilatory efficiency, and oxygen uptake kinetics in chronic heart failure. *J Am Coll Cardiol*, **44,** 2339-48.

GUAZZI, M., TUMMINELLO, G., DI MARCO, F. & GUAZZI, M.D. (2004b). Influences of sildenafil on lung function and hemodynamics in patients with chronic heart failure. *Clin Pharmacol Ther*, **76,** 371-8.

GUERIN, A.P., BLACHER, J., PANNIER, B., MARCHAIS, S.J., SAFAR, M.E. & LONDON, G.M. (2001). Impact of aortic stiffness attenuation on survival of patients in end-stage renal failure. *Circulation*, **103,** 987-92.

GUPTA, M., KOVAR, A. & MEIBOHM, B. (2005). The clinical pharmacokinetics of phosphodiesterase-5 inhibitors for erectile dysfunction. *J Clin Pharmacol*, **45,** 987-1003.

HALCOX, J., NOUR, K., ZALOS, G., MINCEMOYER, R., WACLAWIW, M., RIVERA, C., WILLIE, G., ELLAHHAM, S. & QUYYUMI, A. (2002a). The effect of sildenafil on human vascular function, platelet activation, and myocardial ischemia. *J Am Coll Cardiol*, **40,** 1232-40.

HALCOX, J.P.J., SCHENKE, W.H., ZALOS, G., MINCEMOYER, R., PRASAD, A., WACLAWIW, M.A., NOUR, K.R.A. & QUYYUMI, A.A. (2002b). Prognostic value of coronary vascular endothelial dysfunction. *Circulation*, **106,** 653-8.

HALLOCK, P. & BENSON, I.C. (1937). Studies on the elastic properties of human isolated aorta. *J Clin Invest*, **16,** 595-602.

HANSEN, T.W., JEPPESEN, J., RASMUSSEN, S., IBSEN, H. & TORP-PEDERSEN, C. (2005). Ambulatory blood pressure and mortality: a population-based study. *Hypertension*, **45,** 499-504.

HATZIMOURATIDIS, K. & HATZICHRISTOU, D.G. (2005). A comparative review of the options for treatment of erectile dysfunction: which treatment for which patient? *Drugs*, **65,** 1621-50.

HAYNES, W.G., NOON, J.P., WALKER, B.R. & WEBB, D.J. (1993a). Inhibition of nitric-oxide synthesis increases blood pressure in healthy humans. *J Hypertens*, **11,** 1375-80.

HAYNES, W.G., NOON, J.P., WALKER, B.R. & WEBB, D.J. (1993b). L-NMMA increases blood pressure in man. *Lancet*, **342,** 931-2.

HAYWARD, C.S., AVOLIO, A.P., O'ROURKE, M.F., LANTELME, P., MESTRE, C., LIEVRE, M., GRESSARD, A. & MILON, H. (2002a). Arterial pulse wave velocity and heart rate. *Hypertension*, **40,** 8e-9e.

HAYWARD, C.S. & KELLY, R.P. (1997). Gender-related differences in the central arterial pressure waveform. *J Am Coll Cardiol*, **30,** 1863-71.

HAYWARD, C.S., KRAIDLY, M., WEBB, C.M. & COLLINS, P. (2002b). Assessment of endothelial function using peripheral waveform analysis: A clinical application. *J Am Coll Cardiol*, **40,** 521-8.

HEITZER, T., SCHLINZIG, T., KROHN, K., MEINERTZ, T. & MUNZEL, T. (2001). Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation*, **104,** 2673-8.

HEITZER, T., YLA-HERTTUALA, S., LUOMA, J., KURZ, S., MUNZEL, T., JUST, H., OLSCHEWSKI, M. & DREXLER, H. (1996). Cigarette smoking potentiates endothelial dysfunction of forearm resistance vessels in patients with hypercholesterolemia. Role of oxidized LDL. *Circulation*, **93,** 1346-53.

HERRMANN, H.C., CHANG, G., KLUGHERZ, B.D. & MAHONEY, P.D. (2000). Hemodynamic effects of sildenafil in men with severe coronary artery disease. *N Engl J Med*, **342,** 1622-6.

HIRATA, K., ADJI, A., VLACHOPOULOS, C. & O'ROURKE, M.F. (2005). Effect of sildenafil on cardiac performance in patients with heart failure. *Am J Cardiol*, **96,** 1436-40.

HOFMANN, F., AMMENDOLA, A. & SCHLOSSMANN, J. (2000). Rising behind NO: cGMP-dependent protein kinases. *J Cell Sci*, **113,** 1671-6.

HOPE, S.A., MEREDITH, I.T. & CAMERON, J.D. (2004). Effect of non-invasive calibration of radial waveforms on error in transfer-function-derived central aortic waveform characteristics. *Clin Sci*, **107,** 205-11.

HOPE, S.A., TAY, D.B., MEREDITH, I.T. & CAMERON, J.D. (2002). Comparison of generalized and gender-specific transfer functions for the derivation of aortic waveforms. *Am J Physiol*, **283,** H1150-6.

HRAFNKELSDÓTTIR, T., OTTOSSON, P., GUDNASON, T., SAMUELSSON, O. & JERN, S. (2004). Impaired endothelial release of tissue-type plasminogen activator in patients with chronic kidney disease and hypertension. *Hypertension*, **44,** 300-4.

HRAFNKELSDÓTTIR, T., WALL, U., JERN, C. & JERN, S. (1998). Impaired capacity for endogenous fibrinolysis in essential hypertension. *Lancet*, **352,** 1597-8.

HRYNIEWICZ, K., DIMAYUGA, C., HUDAIHED, A., ANDRONE, A.S., ZHENG, H., JANKOWSKI, K. & KATZ, S.D. (2005). Inhibition of angiotensin-converting enzyme and phosphodiesterase type 5 improves endothelial function in heart failure. *Clin Sci*, **108,** 331-8.

HULTHEN, U.L., BOLLI, P., AMANN, F.W., KIOWSKI, W. & BUHLER, F.R. (1982). Enhanced vasodilatation in essential hypertension by calcium channel blockade with verapamil. *Hypertension*, **4,** 26-31.

JACKSON, G., BENJAMIN, N., JACKSON, N. & ALLEN, M.J. (1999). Effects of sildenafil citrate on human hemodynamics. *Am J Cardiol*, **83,** 13C-20C.

JACKSON, G., KELTAI, M., CSANADY, M., EDES, I., BELLAMY, G.R., WIDIMSKY, P., LISA, L. & GILLIES, H. (2005). Hemodynamic effects of sildenafil citrate and isosorbide mononitrate in men with coronary artery disease and erectile dysfunction. *J Sex Med*, **2,** 407-14.

JACKSON, G., KLONER, R.A., COSTIGAN, T.M., WARNER, M.R. & EMMICK, J.T. (2004). Update on clinical trials of tadalafil demonstrates no increased risk of cardiovascular adverse events. *J Sex Med*, **1,** 161-7.

JARVISALO, M.J., LEHTIMAKI, T. & RAITAKARI, O.T. (2004). Determinants of arterial nitrate-mediated dilatation in children: role of oxidized low-density lipoprotein, endothelial function, and carotid intima-media thickness. *Circulation*, **109,** 2885-9.

JETTER, A., KINZIG-SCHIPPERS, M., WALCHNER-BONJEAN, M., HERING, U., BULITTA, J., SCHREINER, P., SORGEL, F. & FUHR, U. (2002). Effects of grapefruit juice on the pharmacokinetics of sildenafil. *Clin Pharmacol Ther*, **71,** 21-9.

JIANG, X.J., O'ROURKE, M.F., JIN, W.Q., LIU, L.S., LI, C.W., TAI, P.C., ZHANG, X.C. & LIU, S.Z. (2002). Quantification of glyceryl trinitrate effect through analysis of the synthesised ascending aortic pressure waveform. *Heart*, **88,** 143-8.

JOANNIDES, R., HAEFELI, W.E., LINDER, L., RICHARD, V., BAKKALI, E.H., THUILLEZ, C. & LUSCHER, T.F. (1995). Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*, **91,** 1314-9.

KANG, K.K., YU, J.Y., YOO, M. & KWON, J.W. (2005). The effect of DA-8159, a novel PDE5 inhibitor, on erectile function in the rat model of hypercholesterolemic erectile dysfunction. *Int J Impot Res*, **17,** 409-16.

KANNEL, W.B., GORDON, T. & SCHWARTZ, M.J. (1971). Systolic versus diastolic blood pressure and risk of coronary heart disease. The Framingham study. *Am J Cardiol*, **27,** 335-46.

KARAMANOGLU, M., O'ROURKE, M.F., AVOLIO, A.P. & KELLY, R.P. (1993). An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J*, **14,** 160-7.

KARIO, K., PICKERING, T.G., UMEDA, Y., HOSHIDE, S., HOSHIDE, Y., MORINARI, M., MURATA, M., KURODA, T., SCHWARTZ, J.E. & SHIMADA, K. (2003a). Morning surge in blood pressure as a predictor of silent and clinical cerebrovascular disease in elderly hypertensives: a prospective study. *Circulation*, **107,** 1401-6.

KARIO, K., SHIMADA, K. & PICKERING, T.G. (2003b). Clinical implication of morning blood pressure surge in hypertension. *J Cardiovasc Pharmacol*, **42 Suppl 1,** S87-91.

KATZ, S.D., HRYNIEWICZ, K., HRILJAC, I., BALIDEMAJ, K., DIMAYUGA, C., HUDAIHED, A. & YASSKIY, A. (2005). Vascular endothelial dysfunction and mortality risk in patients with chronic heart failure. *Circulation*, **111,** 310-4.

KELLY, R., HAYWARD, C., AVOLIO, A. & O'ROURKE, M. (1989). Noninvasive determination of age-related changes in the human arterial pulse. *Circulation*, **80,** 1652-9.

KELLY, R.P., GIBBS, H.H., O'ROURKE, M.F., DALEY, J.E., MANG, K., MORGAN, J.J. & AVOLIO, A.P. (1990). Nitroglycerin has more favourable effects on left ventricular afterload than apparent from measurement of pressure in a peripheral artery. *Eur Heart J*, **11,** 138-44.

KELLY, R.P., MILLASSEAU, S.C., RITTER, J.M. & CHOWIENCZYK, P.J. (2001). Vasoactive drugs influence aortic augmentation index independently of pulse-wave velocity in healthy men. *Hypertension*, **37,** 1429-33.

KIMURA, M., HIGASHI, Y., HARA, K., NOMA, K., SASAKI, S., NAKAGAWA, K., GOTO, C., OSHIMA, T., YOSHIZUMI, M. & CHAYAMA, K. (2003). PDE5 inhibitor

sildenafil citrate augments endothelium-dependent vasodilation in smokers. *Hypertension*, **41,** 1106-10.

KINGWELL, B.A., WADDELL, T.K., MEDLEY, T.L., CAMERON, J.D. & DART, A.M. (2002). Large artery stiffness predicts ischemic threshold in patients with coronary artery disease. *J Am Coll Cardiol*, **40,** 773-9.

KLONER, R.A. (2005). Pharmacology and drug interaction effects of the phosphodiesterase 5 inhibitors: focus on alpha-blocker interactions. *Am J Cardiol*, **96,** 42M-46M.

KLONER, R.A., HUTTER, A.M., EMMICK, J.T., MITCHELL, M.I., DENNE, J. & JACKSON, G. (2003a). Time course of the interaction between tadalafil and nitrates. *J Am Coll Cardiol*, **42,** 1855-60.

KLONER, R.A., MITCHELL, M. & EMMICK, J.T. (2003b). Cardiovascular effects of tadalafil. *Am J Cardiol*, **92,** 37M-46M.

KLONER, R.A., MITCHELL, M. & EMMICK, J.T. (2003c). Cardiovascular effects of tadalafil in patients on common antihypertensive therapies. *Am J Cardiol*, **92,** 47-52.

KROEKER, E.J. & WOOD, E.H. (1955). Comparison of simultaneously recorded central and peripheral arterial pressure pulses during rest, exercise and tilted position in man. *Circ Res*, **3,** 623-32.

KUGIYAMA, K., YASUE, H., OHGUSHI, M., MOTOYAMA, T., KAWANO, H., INOBE, Y., HIRASHIMA, O. & SUGIYAMA, S. (1996). Deficiency in nitric oxide bioactivity in epicardial coronary arteries of cigarette smokers. *J Am Coll Cardiol*, **28,** 1161-7.

KUVIN, J.T. & KARAS, R.H. (2003). Clinical utility of endothelial function testing: ready for prime time? *Circulation*, **107,** 3243-7.

LANTELME, P., MESTRE, C., LIEVRE, M., GRESSARD, A. & MILON, H. (2002). Heart rate. An important confounder of pulse wave velocity assessment. *Hypertension*, **39,** 1083-7.

LAURENT, S., BOUTOUYRIE, P., ASMAR, R., GAUTIER, I., LALOUX, B., GUIZE, L., DUCIMETIERE, P. & BENETOS, A. (2001). Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension*, **37,** 1236-41.

LAURENT, S., KATSAHIAN, S., FASSOT, C., TROPEANO, A.-I., GAUTIER, I., LALOUX, B. & BOUTOUYRIE, P. (2003). Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke*, **34,** 1203-6.

LEHMANN, E.D., GOSLING, R.G. & SONKSEN, P.H. (1992a). Arterial wall compliance in diabetes. *Diabet Med*, **9,** 114-9.

LEHMANN, E.D., HOPKINS, K.D., RAWESH, A., JOSEPH, R.C., KONGOLA, K., COPPACK, S.W. & GOSLING, R.G. (1998). Relation between number of cardiovascular risk factors/events and noninvasive Doppler ultrasound assessments of aortic compliance. *Hypertension*, **32,** 565-9.

LEHMANN, E.D., WATTS, G.F. & GOSLING, R.G. (1992b). Aortic distensibility and hypercholesterolaemia. *Lancet*, **340,** 1171-2.

LIN, C.-S., XIN, Z.-C., LIN, G. & LUE, T.F. (2003). Phosphodiesterases as therapeutic targets. *Urology*, **61,** 685-91.

LINCOLN, T.M., DEY, N. & SELLAK, H. (2001). Invited review: cGMP-dependent protein kinase signaling mechanisms in smooth muscle: from the regulation of tone to gene expression. *J Appl Physiol*, **91,** 1421-30.

LIND, L., FORS, N., HALL, J., MARTTALA, K. & STENBORG, A. (2005). A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Arterioscler Thromb Vasc Biol*, **25,** 2368-75.

LINDHOLM, L.H., CARLBERG, B. & SAMUELSSON, O. (2005). Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. *Lancet*, **366,** 1545-53.

LLOYD-JONES, D.M., EVANS, J.C., LARSON, M.G., O'DONNELL, C.J. & LEVY, D. (1999). Differential impact of systolic and diastolic blood pressure level on JNC-VI staging. Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*, **34,** 381-5.

LONDON, G.M., BLACHER, J., PANNIER, B., GUERIN, A.P., MARCHAIS, S.J. & SAFAR, M.E. (2001). Arterial wave reflections and survival in end-stage renal failure. *Hypertension*, **38,** 434-8.

LUDMER, P.L., SELWYN, A.P., SHOOK, T.L., WAYNE, R.R., MUDGE, G.H., ALEXANDER, R.W. & GANZ, P. (1986). Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med*, **315,** 1046-51.

MAHMUD, A. & FEELY, J. (2003). Effect of smoking on arterial stiffness and pulse pressure amplification. *Hypertension*, **41,** 183-7.

MAHMUD, A., HENNESSY, M. & FEELY, J. (2001). Effect of sildenafil on blood pressure and arterial wave reflection in treated hypertensive men. *J Hum Hypertens*, **15,** 707-13.

MANFROI, W.C., CARAMORI, P.R., ZAGO, A.J., MELCHIOR, R., ZEN, V., ACCORDI, M., GUTIERRES, D. & NOER, C. (2003). Hemodynamic effects of sildenafil in patients with stable ischemic heart disease. *Int J Cardiol*, **90,** 153-7.

MARTIN, U., HILL, C. & O' MAHONY, D. (2005). Use of moxonidine in elderly patients with resistant hypertension. *J Clin Pharm Ther*, **30,** 433-7.

MASON, R.P., KALINOWSKI, L., JACOB, R.F., JACOBY, A.M. & MALINSKI, T. (2005). Nebivolol reduces nitroxidative stress and restores nitric oxide bioavailability in endothelium of black Americans. *Circulation*, **112,** 3795-801.

MATERSON, B.J., REDA, D.J., CUSHMAN, W.C., MASSIE, B.M., FREIS, E.D., KOCHAR, M.S., HAMBURGER, R.J., FYE, C., LAKSHMAN, R., GOTTDIENER, J., RAMIREZ, E.A. & HENDERSON, W.G. (1993). Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. *N Engl J Med*, **328,** 914-21.

MATSUMOTO, T., KOBAYASHI, T. & KAMATA, K. (2003). Phosphodiesterases in the vascular system. *J Smooth Muscle Res*, **39,** 67-86.

MATTACE-RASO, F.U.S., VAN DER CAMMEN, T.J.M., HOFMAN, A., VAN POPELE, N.M., BOS, M.L., SCHALEKAMP, M.A.D.H., ASMAR, R., RENEMAN, R.S., HOEKS, A.P.G., BRETELER, M.M.B. & WITTEMAN, J.C.M. (2006). Arterial stiffness and risk of coronary heart disease and stroke: the rotterdam study. *Circulation*, **113,** 657-63.

MCENIERY, C.M., YASMIN, HALL, I.R., QASEM, A., WILKINSON, I.B. & COCKCROFT, J.R. (2005). Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity. The Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol*, **46,** 1753-60.

MEAUME, S., BENETOS, A., HENRY, O.F., RUDNICHI, A. & SAFAR, M.E. (2001). Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. *Arterioscler Thromb Vasc Biol*, **21,** 2046-50.

MEYER, B., MORTL, D., STRECKER, K., HULSMANN, M., KULEMANN, V., NEUNTEUFL, T., PACHER, R. & BERGER, R. (2005). Flow-mediated vasodilation predicts outcome in patients with chronic heart failure: comparison with B-type natriuretic peptide. *J Am Coll Cardiol*, **46,** 1011-8.

MICHELAKIS, E., TYMCHAK, W., LIEN, D., WEBSTER, L., HASHIMOTO, K. & ARCHER, S. (2002). Oral sildenafil is an effective and specific pulmonary vasodilator in patients with pulmonary arterial hypertension: comparison with inhaled nitric oxide. *Circulation*, **105,** 2398-403.

MICHELAKIS, E.D., TYMCHAK, W., NOGA, M., WEBSTER, L., WU, X.-C., LIEN, D., WANG, S.-H., MODRY, D. & ARCHER, S.L. (2003). Long-term treatment with oral sildenafil is safe and improves functional capacity and hemodynamics in patients with pulmonary arterial hypertension. *Circulation*, **108,** 2066-9.

MILLASSEAU, S.C., PATEL, S.J., REDWOOD, S.R., RITTER, J.M. & CHOWIENCZYK, P.J. (2003). Pressure wave reflection assessed from the peripheral pulse: Is a transfer function necessary? *Hypertension*, **41,** 1016-20.

MILLIGAN, P.A., MARSHALL, S.F. & KARLSSON, M.O. (2002). A population pharmacokinetic analysis of sildenafil citrate in patients with erectile dysfunction. *Br J Clin Pharmacol*, **53,** 45S-52S.

MITTLEMAN, M.A., MACLURE, M. & GLASSER, D.B. (2005). Evaluation of acute risk for myocardial infarction in men treated with sildenafil citrate. *Am J Cardiol*, **96,** 443-6.

MODENA, M.G., BONETTI, L., COPPI, F., BURSI, F. & ROSSI, R. (2002). Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol*, **40,** 505-10.

MUIESAN, M.L., SALVETTI, M., MONTEDURO, C., RIZZONI, D., ZULLI, R., CORBELLINI, C., BRUN, C. & AGABITI-ROSEI, E. (1999). Effect of treatment on flow-dependent vasodilation of the brachial artery in essential hypertension. *Hypertension*, **33,** 575-80.

MUIRHEAD, G.J., FAULKNER, S., HARNESS, J.A. & TAUBEL, J. (2002a). The effects of steady-state erythromycin and azithromycin on the pharmacokinetics of sildenafil in healthy volunteers. *Br J Clin Pharmacol*, **53,** 37S-43S.

MUIRHEAD, G.J., WILNER, K., COLBURN, W., HAUG-PIHALE, G. & ROUVIEX, B. (2002b). The effects of age and renal and hepatic impairment on the pharmacokinetics of sildenafil. *Br J Clin Pharmacol*, **53,** 21S-30S.

MUIRHEAD, G.J., WULFF, M.B., FIELDING, A., KLEINERMANS, D. & BUSS, N. (2000). Pharmacokinetic interactions between sildenafil and saquinavir/ritonavir. *Br J Clin Pharmacol*, **50,** 99-107.

MULLER, J.E., MITTLEMAN, M.A., MACLURE, M., SHERWOOD, J.B. & TOFLER, G.H. (1996). Triggering myocardial infarction by sexual activity. Low absolute risk and prevention by regular physical exertion. Determinants of Myocardial Infarction Onset Study Investigators. *JAMA*, **275,** 1405-9.

MULLER, J.E., STONE, P.H., TURI, Z.G., RUTHERFORD, J.D., CZEISLER, C.A., PARKER, C., POOLE, W.K., PASSAMANI, E., ROBERTS, R., ROBERTSON, T. & ET AL. (1985). Circadian variation in the frequency of onset of acute myocardial infarction. *N Engl J Med*, **313,** 1315-22.

MULLERSHAUSEN, F., RUSSWURM, M., THOMPSON, W.J., LIU, L., KOESLING, D. & FRIEBE, A. (2001). Rapid nitric oxide-induced desensitization of the cGMP response is caused by increased activity of phosphodiesterase type 5 paralleled by phosphorylation of the enzyme. *J Cell Biol*, **155,** 271-8.

MUNZEL, T., DAIBER, A. & MULSCH, A. (2005). Explaining the phenomenon of nitrate tolerance. *Circ Res*, **97,** 618-28.

MUNZEL, T., HEITZER, T., KURZ, S., HARRISON, D.G., LUHMAN, C., PAPE, L., OLSCHEWSKI, M. & JUST, H. (1996). Dissociation of coronary vascular tolerance and neurohormonal adjustments during long-term nitroglycerin therapy in patients with stable coronary artery disease. *J Am Coll Cardiol*, **27,** 297.

MUSICKI, B., CHAMPION, H.C., BECKER, R.E., KRAMER, M.F., LIU, T., SEZEN, S.F. & BURNETT, A.L. (2005). In vivo analysis of chronic phosphodiesterase-5 inhibition with sildenafil in penile erectile tissues: no tachyphylaxis effect. *J Urol*, **174,** 1493-6.

MUXFELDT, E.S., BLOCH, K.V., NOGUEIRA ADA, R. & SALLES, G.F. (2005). True resistant hypertension: is it possible to be recognized in the office? *Am J Hypertens*, **18,** 1534-40.

MUXFELDT, E.S., BLOCH, K.V., NOGUEIRA, A.R. & SALLES, G.F. (2003). Twenty-four hour ambulatory blood pressure monitoring pattern of resistant hypertension. *Blood Press Monit*, **8,** 181-5.

NASEEM, K.M. (2005). The role of nitric oxide in cardiovascular diseases. *Mol Aspects Med*, **26,** 33-65.

NEUNTEUFL, T., HEHER, S., KATZENSCHLAGER, R., WOLFL, G., KOSTNER, K., MAURER, G. & WEIDINGER, F. (2000). Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain. *Am J Cardiol*, **86,** 207-10.

NEWBY, D.E., BOON, N.A. & WEBB, D.J. (1997). Comparison of forearm vasodilatation to substance P and acetylcholine: contribution of nitric oxide. *Clin Sci*, **92,** 133-8.

NEWBY, D.E., MCLEOD, A.L., UREN, N.G., FLINT, L., LUDLAM, C.A., WEBB, D.J., FOX, K.A. & BOON, N.A. (2001). Impaired coronary tissue plasminogen activator release is associated with coronary atherosclerosis and cigarette smoking: direct link between endothelial dysfunction and atherothrombosis. *Circulation*, **103,** 1936-41.

NEWBY, D.E., WITHEROW, F.N., WRIGHT, R.A., BLOOMFIELD, P., LUDLAM, C.A., BOON, N.A., FOX, K.A. & WEBB, D.J. (2002). Hypercholesterolaemia and lipid lowering treatment do not affect the acute endogenous fibrinolytic capacity in vivo. *Heart*, **87,** 48-53.

NEWBY, D.E., WRIGHT, R.A., LABINJOH, C., LUDLAM, C.A., FOX, K.A., BOON, N.A. & WEBB, D.J. (1999). Endothelial dysfunction, impaired endogenous fibrinolysis, and cigarette smoking: a mechanism for arterial thrombosis and myocardial infarction. *Circulation*, **99,** 1411-5.

NICHOLS, D.J., MUIRHEAD, G.J. & HARNESS, J.A. (2002). Pharmacokinetics of sildenafil after single oral doses in healthy male subjects: absolute bioavailability, food effects and dose proportionality. *Br J Clin Pharmacol*, **53 (Suppl 1),** 5S-12S.

NICHOLS, W.W. & O'ROURKE, M.F. (1998). Chapter 9. Wave reflections. In *McDonald's Blood Flow in Arteries: Theoretic, Experimental and Critical Principles*. pp. 201-22. London: Arnold.

NIELSEN, W.B., VESTBO, J. & JENSEN, G.B. (1995). Isolated systolic hypertension as a major risk factor for stroke and myocardial infarction and an unexploited source of cardiovascular prevention: a prospective population-based study. *J Hum Hypertens*, **9,** 175-80.

NORTH OF ENGLAND HYPERTENSION GUIDELINE DEVELOPMENT GROUP (2004). Essential hypertension: managing adults in primary care. National Institute for Heath and Clinical Excellence, report 111.

NYBERG, G. & WESTLING, H. (1981). Circulatory effects of sublingual and oral sustained-release nitroglycerin in healthy young men. *Eur J Clin Pharmacol*, **19,** 245-9.

O'BRIEN, E., MEE, F., ATKINS, N. & THOMAS, M. (1996). Evaluation of three devices for self-measurement of blood pressure according to the revised British Hypertension Society Protocol: the Omron HEM-705CP, Philips HP5332, and Nissei DS-175. *Blood Press Monit*, **1,** 55-61.

O'ROURKE, M.F., STAESSEN, J.A., VLACHOPOULOS, C., DUPREZ, D. & PLANTE, G.E. (2002). Clinical applications of arterial stiffness; definitions and reference values. *Am J Hypertens*, **15,** 426-44.

OHTSUKA, S., KAKIHANA, M., WATANABE, H. & SUGISHITA, Y. (1994). Chronically decreased aortic distensibility causes deterioration of coronary perfusion during increased left ventricular contraction. *J Am Coll Cardiol*, **24,** 1406-14.

OLIVER, J.J., BOWLER, A., BEUDEKER, Q., TEN CATE, T. & WEBB, D.J. (2005a). Dose-response relationship of sublingual nitroglycerin with brachial artery dilatation and change in central and peripheral augmentation index. *Clin Pharmacol Ther*, **77,** 337-8.

OLIVER, J.J. & WEBB, D.J. (2003). Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arterioscler Thromb Vasc Biol*, **23,** 554-66.

OLIVER, J.J., WEBB, D.J. & NEWBY, D.E. (2005b). Stimulated tissue plasminogen activator release as a marker of endothelial function in humans. *Arterioscler Thromb Vasc Biol*, **25,** 2470-9.

OLSSON, A.M., SPEAKMAN, M.J., DINSMORE, W.W., GIULIANO, F., GINGELL, C., MAYTOM, M., SMITH, M.D. & OSTERLOH, I. (2000). Sildenafil citrate (Viagra) is effective and well tolerated for treating erectile dysfunction of psychogenic or mixed aetiology. *Int J Clin Pract*, **54,** 561-6.

OSEGBE, D.N., SHITTU, O.B., AGHAJI, A.E., ONYEMELUKWE, G.C., DOGO, D. & DIKKO, A.A. (2003). Sildenafil citrate (Viagra) for the treatment of erectile dysfunction in Nigerian men. *Int J Impot Res*, **15 Suppl 1,** S15-8.

PALMER, R.M., FERRIGE, A.G. & MONCADA, S. (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*, **327,** 524-6.

PANNIER, B., GUERIN, A.P., MARCHAIS, S.J., SAFAR, M.E. & LONDON, G.M. (2005). Stiffness of capacitive and conduit arteries: prognostic significance for end-stage renal disease patients. *Hypertension*, **45,** 592-6.

PANNIER, B.M., GUERIN, A.P., MARCHAIS, S.J. & LONDON, G.M. (2001). Different aortic reflection wave responses following long-term angiotensin-converting enzyme inhibition and beta-blocker in essential hypertension. *Clin Exp Pharmacol Physiol*, **28,** 1074-7.

PANZA, J.A., QUYYUMI, A.A., BRUSH, J.E., JR. & EPSTEIN, S.E. (1990). Abnormal endothelium-dependent vascular relaxation in patients with essential hypertension. *N Engl J Med*, **323,** 22-7.

PARK, S.H. & SHIM, K.W. (2005). Reduction in visceral adiposity is highly related to improvement in vascular endothelial dysfunction among obese women: an assessment of endothelial function by radial artery pulse wave analysis. *Yonsei Med J*, **46,** 511-8.

PASYK, K.A. & JAKOBCZAK, B.A. (2004). Vascular endothelium: recent advances. *Eur J Dermatol*, **14,** 209-13.

PATTERSON, D., KLONER, R., EFFRON, M., EMMICK, J., BEDDING, A., WARNER, M., MITCHELL, M., BRAAT, S. & MACDONALD, T. (2005). The effect of tadalafil on the time to exercise-induced myocardial ischaemia in subjects with coronary artery disease. *Br J Clin Pharmacol*, **60,** 459-468.

PATTI, G., PASCERI, V., MELFI, R., GOFFREDO, C., CHELLO, M., D'AMBROSIO, A., MONTESANTI, R. & DI SCIASCIO, G. (2005). Impaired flow-mediated dilation and risk of restenosis in patients undergoing coronary stent implantation. *Circulation*, **111,** 70-5.

PENA, S.A., WILTSHIRE, E., GENT, R., HIRTE, C. & COUPER, J. (2004). Folic acid improves endothelial function in children and adolescents with type 1 diabetes. *J Pediatr*, **144,** 500-4.

PERTICONE, F., CERAVOLO, R., PUJIA, A., VENTURA, G., IACOPINO, S., SCOZZAFAVA, A., FERRARO, A., CHELLO, M., MASTROROBERTO, P., VERDECCHIA, P. & SCHILLACI, G. (2001). Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation*, **104,** 191-6.

PIERDOMENICO, S.D., LAPENNA, D., BUCCI, A., DI TOMMASO, R., DI MASCIO, R., MANENTE, B.M., CALDARELLA, M.P., NERI, M., CUCCURULLO, F. & MEZZETTI, A. (2005). Cardiovascular outcome in treated hypertensive patients with responder, masked, false resistant, and true resistant hypertension. *Am J Hypertens*, **18,** 1422-8.

POMARA, G., MORELLI, G., POMARA, S., TADDEI, S., GHIADONI, L., DINELLI, N., TRAVAGLINI, F., DICUIO, M., MONDAINI, N., SALVETTI, A. & SELLI, C. (2004). Cardiovascular parameter changes in patients with erectile dysfunction using PDE-5 inhibitors: a study with sildenafil and vardenafil. *J Androl*, **25,** 625-9.

POMERANZ, H.D. (2006). Can erectile dysfunction drug use lead to ischaemic optic neuropathy? *Br J Ophthalmol*, **90,** 127-8.

POTTS, A., GRACE, V., GAVEY, N. & VARES, T. (2004). "Viagra stories": challenging 'erectile dysfunction'. *Soc Sci Med*, **59,** 489-99.

POULTER, N.R., WEDEL, H., DAHLOF, B., SEVER, P.S., BEEVERS, D.G., CAULFIELD, M., KJELDSEN, S.E., KRISTINSSON, A., MCINNES, G.T., MEHLSEN, J., NIEMINEN, M., O'BRIEN, E., OSTERGREN, J. & POCOCK, S. (2005). Role of blood pressure and other variables in the differential cardiovascular event rates noted in the Anglo-Scandinavian Cardiac Outcomes Trial-Blood Pressure Lowering Arm (ASCOT-BPLA). *Lancet*, **366,** 907-13.

PRETORIUS, M., ROSENBAUM, D.A., LEFEBVRE, J., VAUGHAN, D.E. & BROWN, N.J. (2002). Smoking impairs bradykinin-stimulated t-PA release. *Hypertension*, **39,** 767-71.

PROSPECTIVE STUDIES COLLABORATION (2002). Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*, **360,** 1903-13.

RAITAKARI, O.T., ADAMS, M.R., MCCREDIE, R.J., GRIFFITHS, K.A. & CELERMAJER, D.S. (1999). Arterial endothelial dysfunction related to passive smoking is potentially reversible in healthy young adults. *Ann Intern Med*, **130,** 578-81.

ROACH, M.R. & BURTON, A.C. (1957). The reason for the shape of the distensibility curves of arteries. *Can J Biochem Physiol*, **35,** 681-90.

ROBINSON, S.D., LUDLAM, C.A., BOON, N.A. & NEWBY, D.E. (2006). Phosphodiesterase type 5 inhibition does not reverse endothelial dysfunction in patients with coronary heart disease. *Heart*, **92,** 170-6.

ROSANO, G.M.C., AVERSA, A., VITALE, C., FABBRI, A., FINI, M. & SPERA, G. (2005). Chronic treatment with tadalafil improves endothelial function in men with increased cardiovascular risk. *Eur Urol*, **47,** 214-20.

ROSSI, R., CHIURLIA, E., NUZZO, A., CIONI, E., ORIGLIANI, G. & MODENA, M.G. (2004). Flow-mediated vasodilation and the risk of developing hypertension in healthy postmenopausal women. *J Am Coll Cardiol*, **44,** 1636-40.

RYBALKIN, S.D., RYBALKINA, I.G., FEIL, R., HOFMANN, F. & BEAVO, J.A. (2002). Regulation of cGMP-specific phosphodiesterase (PDE5) phosphorylation in smooth muscle cells. *J Biol Chem*, **277,** 3310-7.

RYBALKIN, S.D., RYBALKINA, I.G., SHIMIZU-ALBERGINE, M., TANG, X.B. & BEAVO, J.A. (2003a). PDE5 is converted to an activated state upon cGMP binding to the GAF A domain. *EMBO J*, **22,** 469-78.

RYBALKIN, S.D., YAN, C., BORNFELDT, K.E. & BEAVO, J.A. (2003b). Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circ Res*, **93,** 280-91.

RYLISKYTE, L., GHIADONI, L., PLANTINGA, Y., JANAVICIENE, S., PETRULIONIENE, Z., LAUCEVICIUS, A. & GINTAUTAS, J. (2004). High-frequency ultrasonographic imaging of the endothelium-dependent flow-mediated dilatation (FMD) in a brachial artery: normative ranges in a group of low CV risk subjects of different ages. *Proc West Pharmacol Soc*, **47,** 67-8.

SAFAR, M.E., BLACHER, J., PANNIER, B., GUERIN, A.P., MARCHAIS, S.J., GUYONVARC'H, P.-M. & LONDON, G.M. (2002). Central pulse pressure and mortality in end-stage renal disease. *Hypertension*, **39,** 735-8.

SAVOLAINEN, A., KETO, P., POUTANEN, V.P., HEKALI, P., STANDERTSKJOLD-NORDENSTAM, C.G., RAMES, A. & KUPARI, M. (1996). Effects of angiotensin-converting enzyme inhibition versus beta-adrenergic blockade on aortic stiffness in essential hypertension. *J Cardiovasc Pharmacol*, **27,** 99-104.

SCHACHINGER, V., BRITTEN, M.B. & ZEIHER, A.M. (2000). Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation*, **101,** 1899-906.

SCHALCHER, C., SCHAD, K., BRUNNER-LA ROCCA, H.P., SCHINDLER, R., OECHSLIN, E., SCHARF, C., SUETSCH, G., BERTEL, O. & KIOWSKI, W. (2002). Interaction of sildenafil with cAMP-mediated vasodilation in vivo. *Hypertension*, **40,** 763-7.

SCHOFIELD, R.S., EDWARDS, D.G., SCHULER, B.T., ESTRADA, J., ARANDA, J., JUAN M., PAULY, D.F., HILL, J.A., AGGARWAL, R. & NICHOLS, W.W. (2003). Vascular effects of sildenafil in hypertensive cardiac transplant recipients. *Am J Hypertens*, **16,** 874-7.

SCHMITT, M., AVOLIO, A., QASEM, A., MCENIERY, C.M., BUTLIN, M., WILKINSON, I.B. & COCKCROFT, J.R. (2005). Basal NO locally modulates human iliac artery function in vivo. *Hypertension*, **46,** 227-231.

SEGERS, P., CARLIER, S., PASQUET, A., RABBEN, S.I., HELLEVIK, L.R., REMME, E., DE BACKER, T., DE SUTTER, J., THOMAS, J.D. & VERDONCK, P. (2000). Individualizing the aorto-radial pressure transfer function: feasibility of a model-based approach. *Am J Physiol*, **279,** H542-9.

SEGERS, P., QASEM, A., DE BACKER, T., CARLIER, S., VERDONCK, P. & AVOLIO, A. (2001). Peripheral "oscillatory" compliance is associated with aortic augmentation index. *Hypertension*, **37,** 1434-9.

SETTER, S.M., ILTZ, J.L., FINCHAM, J.E., CAMPBELL, R.K. & BAKER, D.E. (2005). Phosphodiesterase 5 inhibitors for erectile dysfunction. *Ann Pharmacother*, **39,** 1286-95.

SHAKIR, S.A., WILTON, L.V., BOSHIER, A., LAYTON, D. & HEELEY, E. (2001). Cardiovascular events in users of sildenafil: results from first phase of prescription event monitoring in England. *BMJ*, **322,** 651-2.

SHEN, D., O'MALLEY, K., GIBALDI, M. & MCNAY, J.L. (1975). Pharmacodynamics of minoxidil as a guide for individualizing dosage regimens in hypertension. *Clin Pharmacol Ther*, **17,** 593-8.

SHEP COOPERATIVE RESEARCH GROUP (1991). Prevention of stroke by antihypertensive drug treatment in older persons with isolated systolic hypertension. Final results of the Systolic Hypertension in the Elderly Program (SHEP). *JAMA*, **265,** 3255-64.

SHEPHERD, A.M. (1988). Determinants of antihypertensive response to calcium antagonists in systemic hypertension. *Am J Cardiol*, **62,** 92G-96G.

SHEPHERD, A.M., LEFORCE, C., PARK, G.D., HOOP, R.S. & WEIR, S. (1991). The determinants of response to diltiazem in hypertension. *Clin Pharmacol Ther*, **50,** 338-49.

SHOJI, T., EMOTO, M., SHINOHARA, K., KAKIYA, R., TSUJIMOTO, Y., KISHIMOTO, H., ISHIMURA, E., TABATA, T. & NISHIZAWA, Y. (2001). Diabetes mellitus, aortic stiffness, and cardiovascular mortality in end-stage renal disease. *J Am Soc Nephrol*, **12,** 2117-24.

SHOKAWA, T., IMAZU, M., YAMAMOTO, H., TOYOFUKU, M., TASAKI, N., OKIMOTO, T., YAMANE, K. & KOHNO, N. (2005). Pulse wave velocity predicts cardiovascular mortality: findings from the Hawaii-Los Angeles-Hiroshima study. *Circ J*, **69,** 259-64.

SICA, D.A. (2004). Minoxidil: an underused vasodilator for resistant or severe hypertension. *J Clin Hypertens*, **6,** 283-7.

SILBER, H.A., OUYANG, P., BLUEMKE, D.A., GUPTA, S.N., FOO, T.K. & LIMA, J.A. (2005). Why is flow-mediated dilation dependent on arterial size? Assessment of the shear stimulus using phase-contrast magnetic resonance imaging. *Am J Physiol Heart Circ Physiol*, **288,** H822-8.

SINGH, N., PRASAD, S., SINGER, D.R. & MACALLISTER, R.J. (2002). Ageing is associated with impairment of nitric oxide and prostanoid dilator pathways in the human forearm. *Clin Sci*, **102,** 595-600.

SMULYAN, H., MARCHAIS, S.J., PANNIER, B., GUERIN, A.P., SAFAR, M.E. & LONDON, G.M. (1998). Influence of body height on pulsatile arterial hemodynamic data. *J Am Coll Cardiol*, **31,** 1103-1109.

SODERSTROM, S., NYBERG, G., O'ROURKE, M.F., SELLGREN, J. & PONTEN, J. (2002). Can a clinically useful aortic pressure wave be derived from a radial pressure wave? *Br J Anaesth*, **88,** 481-8.

STAESSEN, J.A. & BIRKENHAGER, W.H. (2005). Evidence that new antihypertensives are superior to older drugs. *Lancet*, **366,** 869-71.

STAESSEN, J.A., FAGARD, R., THIJS, L., CELIS, H., ARABIDZE, G.G., BIRKENHAGER, W.H., BULPITT, C.J., DE LEEUW, P.W., DOLLERY, C.T., FLETCHER, A.E., FORETTE, F., LEONETTI, G., NACHEV, C., O'BRIEN, E.T., ROSENFELD, J., RODICIO, J.L., TUOMILEHTO, J. & ZANCHETTI, A. (1997). Randomised double-blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. The Systolic Hypertension in Europe (Syst-Eur) Trial Investigators. *Lancet*, **350,** 757-64.

STAMLER, J.S., LOH, E., RODDY, M.A., CURRIE, K.E. & CREAGER, M.A. (1994). Nitric oxide regulates basal systemic and pulmonary vascular resistance in healthy humans. *Circulation*, **89,** 2035-40.

STEFANADIS, C., DERNELLIS, J., TSIAMIS, E., STRATOS, C., DIAMANTOPOULOS, L., MICHAELIDES, A. & TOUTOUZAS, P. (2000). Aortic stiffness as a risk factor for recurrent acute coronary events in patients with ischaemic heart disease. *Eur Heart J*, **21,** 390-6.

STEWART, A.D., JIANG, B., MILLASSEAU, S.C., RITTER, J.M. & CHOWIENCZYK, P.J. (2006). Acute reduction of blood pressure by nitroglycerin does not normalize large artery stiffness in essential hypertension. *Hypertension*, **48,** 404-10.

STEWART, D.J., ELSNER, D., SOMMER, O., HOLTZ, J. & BASSENGE, E. (1986). Altered spectrum of nitroglycerin action in long-term treatment: nitroglycerin-specific venous tolerance with maintenance of arterial vasodepressor potency. *Circulation*, **74,** 573-82.

STOKES, G.S., BUNE, A.J., HUON, N. & BARIN, E.S. (2005). Long-term effectiveness of extended-release nitrate for the treatment of systolic hypertension. *Hypertension*, **45,** 380-4.

STONE, J.R. & MARLETTA, M.A. (1995). Heme stoichiometry of heterodimeric soluble guanylate cyclase. *Biochemistry*, **34,** 14668-74.

STUEHR, D.J. (1999). Mammalian nitric oxide synthases. *Biochim Biophys Acta*, **1411,** 217-30.

SUH, H.S., PARK, Y.W., KANG, J.H., LEE, S.H., LEE, H.S. & SHIM, K.W. (2005). Vascular endothelial dysfunction tested by blunted response to endothelium-dependent vasodilation by salbutamol and its related factors in uncomplicated pre-menopausal obese women. *Int J Obes Relat Metab Disord*, **29,** 217-22.

SUMMARY OF PRODUCT CHARACTERISTICS FOR LEVITRA (2005). Electronic medicines compendium. Accessed January 30, 2006. http://emc.medicines.org.uk/emc/assets/c/html/displaydoc.asp?documentid=11775.

SURKS, H.K., MOCHIZUKI, N., KASAI, Y., GEORGESCU, S.P., TANG, K.M., ITO, M., LINCOLN, T.M. & MENDELSOHN, M.E. (1999). Regulation of myosin phosphatase by a specific interaction with cGMP-dependent protein kinase Iα. *Science*, **286,** 1583-7.

SUTTON-TYRRELL, K., NAJJAR, S.S., BOUDREAU, R.M., VENKITACHALAM, L., KUPELIAN, V., SIMONSICK, E.M., HAVLIK, R., LAKATTA, E.G., SPURGEON, H., KRITCHEVSKY, S., PAHOR, M., BAUER, D. & NEWMAN, A. (2005). Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation*, **111,** 3384-90.

SUWAIDI, J.A., HAMASAKI, S., HIGANO, S.T., NISHIMURA, R.A., HOLMES D R JR & LERMAN, A. (2000). Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation*, **101,** 948-54.

TADDEI, S., GALETTA, F., VIRDIS, A., GHIADONI, L., SALVETTI, G., FRANZONI, F., GIUSTI, C. & SALVETTI, A. (2000). Physical activity prevents age-related impairment in nitric oxide availability in elderly athletes. *Circulation*, **101,** 2896-901.

TADDEI, S., GHIADONI, L., VIRDIS, A., BURALLI, S. & SALVETTI, A. (1999). Vasodilation to bradykinin is mediated by an ouabain-sensitive pathway as a compensatory mechanism for impaired nitric oxide availability in essential hypertensive patients. *Circulation*, **100,** 1400-5.

TADDEI, S., VIRDIS, A., GHIADONI, L., MAGAGNA, A. & SALVETTI, A. (1998). Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation*, **97,** 2222-9.

TADDEI, S., VIRDIS, A., GHIADONI, L., SALVETTI, G., BERNINI, G., MAGAGNA, A. & SALVETTI, A. (2001). Age-related reduction of NO availability and oxidative stress in humans. *Hypertension*, **38,** 274-9.

TADDEI, S., VIRDIS, A., MATTEI, P., GHIADONI, L., FASOLO, C.B., SUDANO, I. & SALVETTI, A. (1997). Hypertension causes premature aging of endothelial function in humans. *Hypertension*, **29,** 736-43.

TADDEI, S., VIRDIS, A., MATTEI, P., GHIADONI, L., GENNARI, A., FASOLO, C.B., SUDANO, I. & SALVETTI, A. (1995). Aging and endothelial function in normotensive subjects and patients with essential hypertension. *Circulation*, **91,** 1981-7.

TALER, S.J. (2005). Treatment of resistant hypertension. *Curr Hypertens Rep*, **7,** 323-9.

TARGONSKI, P.V., BONETTI, P.O., PUMPER, G.M., HIGANO, S.T., HOLMES DR JR & LERMAN, A. (2003). Coronary endothelial dysfunction is associated with an increased risk of cerebrovascular events. *Circulation*, **107,** 2805-9.

TERAGAWA, H., UEDA, K., MATSUDA, K., KIMURA, M., HIGASHI, Y., OSHIMA, T., YOSHIZUMI, M. & CHAYAMA, K. (2005). Relationship between endothelial function in the coronary and brachial arteries. *Clin Cardiol*, **28,** 460-6.

THADANI, U. & DE VANE, P.J. (1992). Efficacy of isosorbide mononitrate in angina pectoris. *Am J Cardiol*, **70,** 67G-71G.

THADANI, U., SMITH, W., NASH, S., BITTAR, N., GLASSER, S., NARAYAN, P., STEIN, R.A., LARKIN, S., MAZZU, A. & TOTA, R. (2002). The effect of vardenafil, a potent and highly selective phosphodiesterase-5 inhibitor for the treatment of erectile dysfunction, on the cardiovascular response to exercise in patients with coronary artery disease. *J Am Coll Cardiol*, **40,** 2006-12.

VAITKEVICIUS, P.V., FLEG, J.L., ENGEL, J.H., O'CONNOR, F.C., WRIGHT, J.G., LAKATTA, L.E., YIN, F.C. & LAKATTA, E.G. (1993). Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation*, **88,** 1456-62.

VALLANCE, P., COLLIER, J. & MONCADA, S. (1989). Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet*, **2,** 997-1000.

VALLANCE, P. & LEIPER, J. (2004). Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol*, **24,** 1023-30.

VARDI, Y., KLEIN, L., NASSAR, S., SPRECHER, E. & GRUENWALD, I. (2002). Effects of sildenafil citrate (viagra) on blood pressure in normotensive and hypertensive men. *Urology*, **59,** 747-52.

VERBEKE, F., SEGERS, P., HEIREMAN, S., VANHOLDER, R., VERDONCK, P. & VAN BORTEL, L.M. (2005). Noninvasive assessment of local pulse pressure: importance of brachial-to-radial pressure amplification. *Hypertension*, **46,** 244-8.

VLACHOPOULOS, C., HIRATA, K. & O'ROURKE, M.F. (2003). Effect of sildenafil on arterial stiffness and wave reflection. *Vasc Med*, **8,** 243-8.

VLACHOPOULOS, C., TSEKOURA, D., ALEXOPOULOS, N., PANAGIOTAKOS, D., AZNAOURIDIS, K. & STEFANADIS, C. (2004). Type 5 phosphodiesterase inhibition by sildenafil abrogates acute smoking-induced endothelial dysfunction. *Am J Hypertens*, **17,** 1040-4.

VOGEL, R.A. (2003). Heads and hearts: The endothelial connection. *Circulation*, **107,** 2766-8.

VON MERING, G.O., ARANT, C.B., WESSEL, T.R., MCGORRAY, S.P., BAIREY MERZ, C.N., SHARAF, B.L., SMITH, K.M., OLSON, M.B., JOHNSON, B.D., SOPKO, G., HANDBERG, E., PEPINE, C.J. & KERENSKY, R.A. (2004). Abnormal coronary vasomotion as a prognostic indicator of cardiovascular events in women: results from the National Heart, Lung, and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation (WISE). *Circulation*, **109,** 722-5.

WALKER, D.K., ACKLAND, M.J., JAMES, G.C., MUIRHEAD, G.J., RANCE, D.J., WASTALL, P. & WRIGHT, P.A. (1999). Pharmacokinetics and metabolism of sildenafil in mouse, rat, rabbit, dog and man. *Xenobiotica*, **29,** 297-310.

WALLIS, R.M., CORBIN, J.D., FRANCIS, S.H. & ELLIS, P. (1999). Tissue distribution of phosphodiesterase families and the effects of sildenafil on tissue cyclic nucleotides, platelet function, and the contractile responses of trabeculae carneae and aortic rings in vitro. *Am J Cardiol*, **83,** 3C-12C.

WARRINGTON, J.S., VON MOLTKE, L.L., SHADER, R.I. & GREENBLATT, D.J. (2002). In vitro biotransformation of sildenafil (Viagra) in the male rat: the role of CYP2C11. *Drug Metab Dispos*, **30,** 655-7.

WEBB, D.J., FREESTONE, S., ALLEN, M.J. & MUIRHEAD, G.J. (1999). Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist. *Am J Cardiol*, **83,** 21C-28C.

WEBB, D.J., MUIRHEAD, G.J., WULFF, M., SUTTON, J.A., LEVI, R. & DINSMORE, W.W. (2000). Sildenafil citrate potentiates the hypotensive effects of nitric oxide donor drugs in male patients with stable angina. *J Am Coll Cardiol*, **36,** 25-31.

WEBER, T., AUER, J., O'ROURKE M, F., KVAS, E., LASSNIG, E., LAMM, G., STARK, N., RAMMER, M. & EBER, B. (2005). Increased arterial wave reflections predict severe cardiovascular events in patients undergoing percutaneous coronary interventions. *Eur Heart J*, **26,** 2657-63.

WEBER, T., AUER, J., O'ROURKE, M.F., KVAS, E., LASSNIG, E., BERENT, R. & EBER, B. (2004). Arterial stiffness, wave reflections, and the risk of coronary artery disease. *Circulation*, **109,** 184-9.

WENN, C.M. & NEWMAN, D.L. (1990). Arterial tortuosity. *Australas Phys Eng Sci Med*, **13,** 67-70.

WIERZBICKI, A.S., SOLOMON, H., LUMB, P.J., LYTTLE, K., LAMBERT-HAMMILL, M. & JACKSON, G. (2006). Asymmetric dimethyl arginine levels correlate with cardiovascular risk factors in patients with erectile dysfunction. *Atherosclerosis*, **185,** 421-5.

WILKINSON, I.B. & COCKCROFT, J.R. (2004a). Estimation of central aortic pressure: shedding new light or clouding the issue? *Clin Sci*, **106,** 433-4.

WILKINSON, I.B., FRANKLIN, S.S. & COCKCROFT, J.R. (2004b). Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. *Hypertension*, **44,** 112-6.

WILKINSON, I.B., FRANKLIN, S.S., HALL, I.R., TYRRELL, S. & COCKCROFT, J.R. (2001a). Pressure amplification explains why pulse pressure is unrelated to risk in young subjects. *Hypertension*, **38,** 1461-6.

WILKINSON, I.B., FUCHS, S.A., JANSEN, I.M., SPRATT, J.C., MURRAY, G.D., COCKCROFT, J.R. & WEBB, D.J. (1998). Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens*, **16,** 2079-84.

WILKINSON, I.B., HALL, I.R., MACCALLUM, H., MACKENZIE, I.S., MCENIERY, C.M., VAN DER AREND, B.J., SHU, Y.E., MACKAY, L.S., WEBB, D.J. & COCKCROFT, J.R. (2002a). Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. *Arterioscler Thromb Vasc Biol*, **22,** 147-52.

WILKINSON, I.B., MACCALLUM, H., FLINT, L., COCKCROFT, J.R., NEWBY, D.E. & WEBB, D.J. (2000a). The influence of heart rate on augmentation index and central arterial pressure in humans. *J Physiol*, **525,** 263-70.

WILKINSON, I.B., MACCALLUM, H., HUPPERETZ, P.C., VAN THOOR, C.J., COCKCROFT, J.R. & WEBB, D.J. (2001b). Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. *J Physiol*, **530,** 541-50.

WILKINSON, I.B., MACCALLUM, H., ROOIJMANS, D.F., MURRAY, G.D., COCKCROFT, J.R., MCKNIGHT, J.A. & WEBB, D.J. (2000b). Increased augmentation index and systolic stress in type 1 diabetes mellitus. *QJM*, **93,** 441-8.

WILKINSON, I.B., PRASAD, K., HALL, I.R., THOMAS, A., MACCALLUM, H., WEBB, D.J., FRENNEAUX, M.P. & COCKCROFT, J.R. (2002b). Increased central pulse pressure and augmentation index in subjects with hypercholesterolemia. *J Am Coll Cardiol*, **39,** 1005-11.

WILKINSON, I.B., QASEM, A., MCENIERY, C.M., WEBB, D.J., AVOLIO, A.P. & COCKCROFT, J.R. (2002c). Nitric oxide regulates local arterial distensibility in vivo. *Circulation*, **105,** 213-217.

WILKINSON, I.B. & WEBB, D.J. (2001c). Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol*, **52,** 631-46.

WILLIAMS, B., LACY, P.S., THOM, S.M., CRUICKSHANK, K., STANTON, A., COLLIER, D., HUGHES, A.D., THURSTON, H. & O'ROURKE, M. (2006). Differential impact of blood pressure-lowering drugs on central aortic pressure and clinical outcomes: principal results of the Conduit Artery Function Evaluation (CAFE) study. *Circulation*, **113,** 1213-25.

WILLIAMS, B., POULTER, N.R., BROWN, M.J., DAVIS, M., MCINNES, G.T., POTTER, J.F., SEVER, P.S. & THOM, S.M. (2004). Guidelines for management of hypertension: report of the fourth working party of the British Hypertension Society, 2004-BHS IV. *J Hum Hypertens*, **18,** 139-85.

WILLICH, S.N., GOLDBERG, R.J., MACLURE, M., PERRIELLO, L. & MULLER, J.E. (1992). Increased onset of sudden cardiac death in the first three hours after awakening. *Am J Cardiol*, **70,** 65-8.

WILLUM HANSEN, T., STAESSEN, J.A., TORP-PEDERSEN, C., RASMUSSEN, S., THIJS, L., IBSEN, H. & JEPPESEN, J. (2006). Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. *Circulation*, **113,** 664-70.

WILNER, K., LABOY, L. & LEBEL, M. (2002). The effects of cimetidine and antacid on the pharmacokinetic profile of sildenafil citrate in healthy male volunteers. *Br J Clin Pharmacol*, **53,** 31S-36S.

WOLF-MAIER, K., COOPER, R.S., BANEGAS, J.R., GIAMPAOLI, S., HENSE, H.-W., JOFFRES, M., KASTARINEN, M., POULTER, N., PRIMATESTA, P., RODRIGUEZ-ARTALEJO, F., STEGMAYR, B., THAMM, M., TUOMILEHTO, J., VANUZZO, D. & VESCIO, F. (2003). Hypertension prevalence and blood pressure levels in 6 European countries, Canada, and the United States. *JAMA*, **289,** 2363-9.

WOODMAN, R.J., CELERMAJER, D.E., THOMPSON, P.L. & HUNG, J. (2004). Folic acid does not improve endothelial function in healthy hyperhomocysteinaemic subjects. *Clin Sci*, **106,** 353-8.

WROE, S.J., SANDERCOCK, P., BAMFORD, J., DENNIS, M., SLATTERY, J. & WARLOW, C. (1992). Diurnal variation in incidence of stroke: Oxfordshire community stroke project. *BMJ*, **304,** 155-7.

WU, H.D., BERGLUND, L., DIMAYUGA, C., JONES, J., SCIACCA, R.R., DI TULLIO, M.R. & HOMMA, S. (2004). High lipoprotein(a) levels and small apolipoprotein(a) sizes are associated with endothelial dysfunction in a multiethnic cohort. *J Am Coll Cardiol*, **43,** 1828-33.

WYKRETOWICZ, A., GUZIK, P., BARTKOWIAK, G., KRAUZE, T., KASINOWSKI, R., DZIARMAGA, M., WESSELING, K.H. & WYSOCKI, H. (2005). Endothelial function and baroreflex sensitivity according to the oral glucose tolerance test in patients with coronary artery disease and normal fasting glucose levels. *Clin Sci*, **109,** 397-403.

YAGINUMA, T., AVOLIO, A., O'ROURKE, M., NICHOLS, W., MORGAN, J.J., ROY, P., BARON, D., BRANSON, J. & FENELEY, M. (1986). Effect of glyceryl trinitrate on peripheral arteries alters left ventricular hydraulic load in man. *Cardiovasc Res*, **20,** 153-60.

YASUE, H., MATSUYAMA, K., MATSUYAMA, K., OKUMURA, K., MORIKAMI, Y. & OGAWA, H. (1990). Responses of angiographically normal human coronary arteries to intracoronary injection of acetylcholine by age and segment. Possible role of early coronary atherosclerosis. *Circulation*, **81,** 482-90.

ZEIHER, A.M., SCHACHINGER, V. & MINNERS, J. (1995). Long-term cigarette smoking impairs endothelium-dependent coronary arterial vasodilator function. *Circulation*, **92,** 1094-100.

ZILKENS, R.R., RICH, L., BURKE, V., BEILIN, L.J., WATTS, G.F. & PUDDEY, I.B. (2003). Effects of alcohol intake on endothelial function in men: a randomized controlled trial. *J Hypertens*, **21,** 97-103.

ZUSMAN, R.M., MORALES, A., GLASSER, D.B. & OSTERLOH, I.H. (1999). Overall cardiovascular profile of sildenafil citrate. *Am J Cardiol*, **83,** 35C-44C.

ZUSMAN, R.M., PRISANT, L.M. & BROWN, M.J. (2000). Effect of sildenafil citrate on blood pressure and heart rate in men with erectile dysfunction taking concomitant antihypertensive medication. Sildenafil Study Group. *J Hypertens*, **18,** 1865-9.

APPENDIX

COPIES OF PUBLISHED PAPERS

1. OLIVER, J.J., BOWLER, A., BEUDEKER, Q., TEN CATE, T. & WEBB, D.J. (2005). Dose-response relationship of sublingual nitroglycerin with brachial artery dilatation and change in central and peripheral augmentation index. *Clin Pharmacol Ther*, **77,** 337-8.

2. OLIVER, J.J., MELVILLE, V.P. & WEBB, D.J (2006). Effect of regular phosphodiesterase type 5 inhibition in hypertension. *Hypertension*, **48,** 622-7.

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Dose-response relationship of sublingual nitroglycerin with brachial artery dilatation and change in central and peripheral augmentation index

To the Editor:

Flow-mediated dilatation (FMD) of the brachial artery (BA)[1] and arterial pressure waveform analysis are used to assess nitric oxide (NO)–mediated endothelial vasomotor function noninvasively, the latter by measuring the change in augmentation index after inhaled albuterol (INN, salbutamol), at either the central aorta (AAIx)[2] or the radial artery (RAIx).[3] To differentiate impaired endothelial function from generalized vascular dysfunction, the endothelium-dependent responses are usually compared with the effects of sublingual nitroglycerin (NTG), an endothelium-independent vasodilator. In FMD studies 400 μg NTG has been used most commonly, although lower doses, which may dilate the BA to an extent similar to the shear stress stimulus, may be more appropriate.[4] However, the dose-response relationships of sublingual NTG to change in BA diameter, AAIx, and RAIx are not known. To help define the doses of NTG that should be used, we characterized these dose-response relationships.

In this 9-way, double-blind, randomized, crossover study, 17 healthy men, with a mean age of 40 years (SEM, 4 years), received sublingual 80-μL solutions of placebo (water on 2 occasions) and 7 doses of NTG (5-400 μg). BA diameter was measured continuously by ultrasound, and AAIx and RAIx were assessed by use of the SphygmoCor system (AtCor Medical, West Ryde, New South Wales, Australia). Subjects were seen on 3 separate days. On each day, FMD was measured first, followed by the responses to 3 separate doses of NTG or placebo, each given 2 hours apart. After drug administration, the BA was scanned for 5 minutes and the peak change in diameter was recorded. Heart rate and blood pressure were measured after 4 minutes, and AAIx and RAIx were measured after 5 minutes.

At baseline, the mean values were as follows, with SEM in parentheses: blood pressure, 124 mm Hg (3 mm Hg)/77 mm Hg (3 mm Hg); FMD, 3.9% (0.6%); AAIx, 14% (2%); and RAIx, 64% (3%). Fig 1 illustrates the dose-response relation-

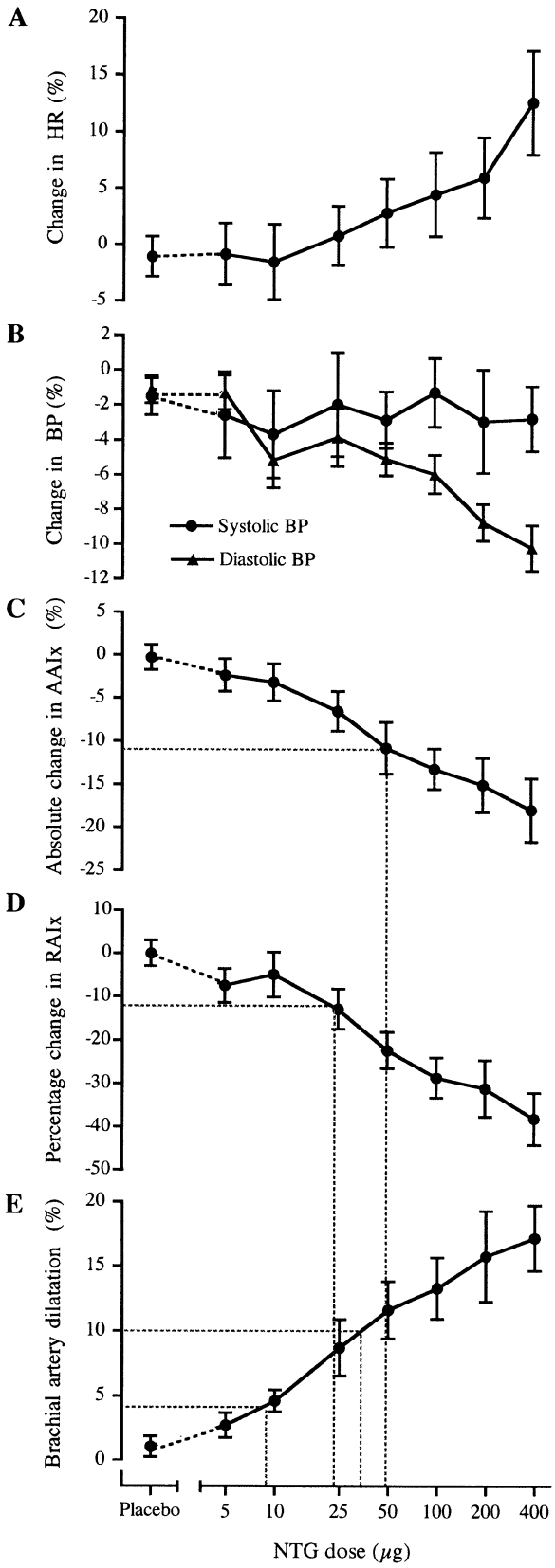

Fig 1. Log dose-response relationships of sublingual nitroglycerin (NTG) to change in heart rate (HR) (**A**), systolic and diastolic blood pressure (BP) (**B**), central aorta augmentation index (AAIx) (**C**), radial artery augmentation index (RAIx) (**D**), and brachial artery diameter (**E**). Data are given as mean changes (±95% confidence intervals). For AAIx and RAIx, the NTG doses that are equivalent to the normal responses to albuterol are indicated. For brachial artery diameter, the ranges of NTG doses that are equivalent to the normal flow-mediated dilatation response (approximately 4% to 10% dilatation) are indicated.

ships. The dose range of NTG that yielded vasodilatation equivalent to that of an accepted normal BA FMD of approximately 4% to 10% was approximately 8 to 35 μg. The doses equivalent to the effects of 400 μg albuterol on AAIx and RAIx were approximately 50 μg (absolute change in AAIx of approximately 11%)[2] and approximately 25 μg (percentage change in RAIx of approximately 12%).[3]

Thus relatively low doses of NTG, in the range from 8 to 50 μg, change BA diameter, AAIx, and RAIx to a degree similar to that of the endothelium-dependent stimuli in healthy subjects. Therefore, with the use of these doses, if a group of subjects has an impaired response to the endothelium-dependent stimulus but not to NTG, one can conclude that the defect lies at the level of the endothelium. If the NTG response is also reduced, more generalized vascular dysfunction is likely. Using higher NTG doses, on the plateau of the dose-response relationship, would theoretically assess the maximum capacity for NO-mediated dilatation, a different measure of vascular function and potential damage. Indeed, lower doses are not appropriate for assessing maximal responsiveness to NO. Of note, however, is that even with 400 μg NTG there was no evidence that a maximum response of change in BA diameter, AAIx, or RAIx was approached. Therefore assessing maximum NO-mediated dilatation would likely be confounded by the systemic hemodynamic effects of higher doses of NTG.

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References

1. Guazzi M, Tumminello G, Di Marco F, Guazzi MD. Influences of sildenafil on lung function and hemodynamics in patients with chronic heart failure. Clin Pharmacol Ther 2004;76:371-8.
2. Wilkinson IB, Hall IR, MacCallum H, Mackenzie IS, McEniery CM, van der Arend BJ, et al. Pulse-wave analysis: clinical evaluation of a noninvasive, widely applicable method for assessing endothelial function. Arterioscler Thromb Vasc Biol 2002;22:147-52.
3. Hayward CS, Kraidly M, Webb CM, Collins P. Assessment of endothelial function using peripheral waveform analysis: a clinical application. J Am Coll Cardiol 2002;40:521-8.
4. Ghiadoni L, Donald AE, Cropley M, Mullen MJ, Oakley G, Taylor M, et al. Mental stress induces transient endothelial dysfunction in humans. Circulation 2000;102:2473-8.

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Effect of Regular Phosphodiesterase Type 5 Inhibition in Hypertension

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Abstract—There are no published controlled clinical trials of regular phosphodiesterase type 5 inhibitor therapy as a long-term treatment of hypertension. In a randomized, double-blind, 2-way crossover study, 25 otherwise untreated hypertensive subjects were administered 50 mg of sildenafil or matched placebo 3 times daily for 16 days, and the effects on ambulatory blood pressure (BP), clinic BP, arterial wave reflection, carotid-femoral pulse wave velocity, and brachial artery flow-mediated dilatation were assessed. Three subjects were withdrawn because of adverse effects, and the data from the remaining 22 subjects were analyzed. Sildenafil reduced ambulatory BP (mean [SE] change from baseline for average daytime BP: systolic −8 [2] mm Hg versus 2 [2] mm Hg with placebo, $P<0.01$; diastolic −6 [1] mm Hg versus 0 [1] mm Hg, $P<0.01$) and clinic BP (change from baseline to 1 hour after drug administration on day 16: systolic −5 [2] mm Hg versus 4 [2] mm Hg, $P<0.01$; diastolic −5 [1] mm Hg versus 2 [2] mm Hg, $P<0.01$). Compared with baseline, sildenafil, but not placebo, reduced arterial wave reflection both acutely and after chronic treatment, but the chronic change in arterial wave reflection was not statistically different from the chronic change with placebo. Sildenafil did not affect pulse wave velocity or flow-mediated dilatation. The main adverse effects of sildenafil, which were generally transient and rated as mild or moderate in severity, were dyspepsia, headache, and myalgia. In conclusion, regular sildenafil constitutes effective antihypertensive therapy. Further studies are warranted to evaluate the role of longer-acting phosphodiesterase type 5 inhibitors as antihypertensive agents in clinical practice. **(*Hypertension*. 2006;48:622-627.)**

Key Words: phosphodiesterase 5 ■ sildenafil ■ hypertension ■ blood pressure ■ arterial stiffness

NO causes vasodilatation by stimulating vascular smooth muscle soluble guanylate cyclase to convert guanosine 5′-triphosphate to cGMP,[1] which leads to a reduction in intracellular calcium concentration.[2] cGMP is degraded by cGMP-specific, cGMP-binding phosphodiesterase 5 (PDE5), and intracellular concentrations of cGMP are tightly controlled by this enzyme via a number of negative feedback mechanisms.[3]

Inhibitors of PDE5 increase the intracellular concentration of cGMP, with the consequence that NO-mediated cellular responses, such as vascular smooth muscle relaxation, are promoted. By stimulating vascular relaxation within the corpora cavernosa during sexual stimulation, inhibitors of PDE5 promote penile erection and are effective treatments of male erectile dysfunction.[4] PDE5 inhibition also causes vasodilatation in the pulmonary vascular bed, and in pulmonary arterial hypertension the PDE5 inhibitor sildenafil substantially reduces pulmonary artery blood pressure (BP) and improves functional capacity.[5]

PDE5 inhibitors are also vasodilators in the systemic circulation.[6] In healthy subjects, single doses of sildenafil have been found to reduce BP acutely in some[6,7] but not all[8–10] studies. Similarly, some studies have found that sildenafil reduces BP acutely in patients with coronary artery disease (CAD),[6,11,12] whereas others have found no effect.[11,13] In hypertensive patients, acute reduction in BP with sildenafil has been demonstrated consistently, although only in studies in which other antihypertensive drugs were also being taken by the participants.[14–16] Although enhancement of the effects of endogenous NO through PDE5 inhibition may reduce BP in hypertension, the potential of regular PDE5 inhibitor therapy for the chronic treatment of hypertension has not been investigated previously in a controlled clinical trial.

Endothelium-dependent vasomotor function is generally assessed in vivo by methodologies that are essentially surrogate measures of endothelial NO generation. As a result, PDE5 inhibitors might be expected to improve these responses. Although this would not constitute an improvement in endothelial function as such, because release of NO and other elements of endothelial function would not be expected to change, it might nevertheless be of clinical benefit, given the general vasculoprotective actions of NO, which are predominantly mediated through simulation of cGMP.[17] Sildenafil has been found to improve endothelium-dependent vasodilatation in some previous studies[11,18–21] but not in all.[9–11,22] There are no published studies on the effect of

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PDE5 inhibition on endothelium-dependent vasomotor function in hypertension.

In single dose studies, sildenafil has been found to reduce central augmentation index (CAIx), a measure of the effect of peripheral arterial wave reflection on the pressure waveform of the central aorta, in treated hypertensives,[14] hypertensive cardiac transplant patients,[19] and patients with CAD.[23] It also acutely reduced carotid-femoral pulse wave velocity (CF-PWV), a measure of central arterial stiffness, in patients with CAD[23] and heart failure.[24] However, the effects of chronic PDE5 inhibition on arterial stiffness and arterial wave reflection have not been investigated previously. The aim of this study was to investigate the effects of chronic treatment with sildenafil on BP (the primary outcome measure), endothelium-dependent vasomotor function, arterial stiffness, and arterial wave reflection in otherwise untreated hypertensives.

Methods

The study was of randomized, placebo-controlled, double-blind, 2-way crossover design. It was approved by a local research ethics committee, was performed in accordance with the Declaration of Helsinki, and written informed consent was obtained from all of the subjects.

Subjects

Subjects were identified from primary care and hospital hypertension clinics. The inclusion criteria were: male or female, ≥3 separate clinic measurements of systolic BP ≥160 mm Hg or diastolic BP ≥100 mm Hg, not taking antihypertensives; hypertension confirmed on ambulatory BP monitoring (average daytime systolic BP ≥145 mm Hg or diastolic BP ≥95 mm Hg), within 3 months of the screening visit; and subjects with "borderline" hypertension (defined as an average awake systolic BP ≥135 and <145 mm Hg or diastolic BP ≥85 and <95 mm Hg) if their calculated 10-year risk of cardiovascular disease was >20% or they had evidence of target organ damage. The exclusion criteria were: history of other major cardiac, respiratory, neurologic, or renal disease; systolic BP consistently >210 mm Hg or diastolic BP consistently >120 mm Hg; current alcohol abuse; diabetes; taking any vasoactive drugs; previous serious drug allergy; and pregnancy.

Potentially suitable subjects attended a screening visit at which a medical history was taken and a physical examination and 12-lead ECG were performed. A nonfasting blood sample was also taken. A 24-hour ambulatory BP monitor was fitted if ambulatory monitoring had not been performed within 3 months.

Measurements

Clinic BP and heart rate (HR) were recorded, with an appropriate sized cuff, using a validated oscillometric sphygmomanometer, the Omron HEM-705CP.[25] Ambulatory BP was recorded at the brachial artery using a validated Spacelabs 90217 ambulatory BP monitor.[26] Measurements were taken every 30 minutes for 24 hours. BP variability was calculated as the within-subject SD of all of the systolic and diastolic daytime readings.

Radial artery waveforms, calibrated to brachial BP, were measured by applanation tonometry and the SphygmoCor apparatus. The radial augmentation index (RAIx) was derived from averaged radial artery waveforms. CAIx, CAIx adjusted to a standard HR of 75 bpm (CAIx@75), and central aortic BP were calculated from central aortic waveforms, which were derived by applying a generalized transfer function to the directly measured radial waveforms. True mean arterial BP was derived from integration of the radial waveform. The SphygmoCor apparatus was also used to measure CF-PWV. Brachial artery flow-mediated dilatation (FMD) was used to assess endothelium-dependent vasomotor function.[27] FMD was quantified both as the peak change from baseline and as the area under the curve of the change from baseline in brachial artery diameter after 5 minutes of forearm ischemia. The response to nitroglycerin, an endothelium-independent control, was not assessed because of the potential for significant hypotension when given with sildenafil.[28] Full details of the methodologies used are available in an online supplement at http://hyper.ahajournals.org.

Protocol

Subjects refrained from alcohol for ≥24 hours and caffeinated drinks, food, and smoking for ≥12 hours before each visit. Studies were conducted in a quiet room kept at 22°C to 24°C.

The study was composed of 2 periods that, except for the treatment received (sildenafil or placebo), were identical. On day 1, subjects attended the research unit at 8:00 AM. After 30 minutes, supine rest baseline measurements of BP and HR, radial waveforms, CF-PWV, and FMD were recorded in that order. Sildenafil 50 mg or matched placebo (both obtained from Pfizer, United Kingdom) was then administered orally, and the same measurements were repeated 1 hour after dose. Subjects were discharged with a supply of the same tablets to take 3 times daily (morning, early afternoon, and evening). An ambulatory BP monitor was fitted at 8:00 AM on day 15. On day 16, this was removed, and further measurements were made before and 1 hour after sildenafil or placebo, as on day 1. There was a washout period of ≥6 days between the periods. For all of the measures, except FMD, duplicate recordings were made and mean values entered into the analyses.

Subjects recorded the time they took each tablet on a diary card. During each period, they were provided with 46 tablets, 2 more than required, and were asked to return all of the unused tablets. Subjects were also issued with cards on which they were asked to rate any symptoms as mild, moderate, or severe.

Analyses

Data are given as means and SEs. Means were compared by paired Student *t* tests. Correlation coefficients were calculated using the Pearson method. The screening ambulatory BP was used as the baseline for both phases of the study.

Results

Subjects

Thirty-six subjects underwent screening (34 from primary care and 2 from the hospital clinic). Of those screened, 10 did not meet the entry criteria, and 1 withdrew before starting the study. Of the 25 subjects who started the study, 3 were withdrawn because of adverse effects. Analyses were performed on the data from the remaining 22 subjects. The baseline characteristics of the subjects are given in Table 1. At baseline, there was a nocturnal reduction of >10% in both systolic BP and diastolic BP in all of the subjects (ie, there were no nocturnal nondippers).

Vascular Effects

There were no differences in baseline measures of any parameter between placebo and sildenafil phases of the study. Sildenafil significantly reduced both systolic and diastolic ambulatory 24-hour, daytime, and nighttime BPs compared with both baseline and placebo (Table 2). Higher baseline ambulatory systolic BP but not diastolic BP was associated with a greater reduction with sildenafil (24-hour: $r=-0.55$, $P<0.01$; daytime: $r=-0.52$, $P<0.05$; nighttime: $r=-0.51$, $P<0.05$). There was no effect of either sildenafil or placebo on BP variability (systolic: 13.8 [0.6] mm Hg at baseline, 13.2 [0.6] mm Hg after placebo, and 13.2 [0.6] mm Hg after sildenafil; diastolic: 9.4 [0.6] mm Hg at baseline, 9.1 [0.7] mm Hg after placebo, and 9.1 [0.5] mm Hg after

TABLE 1. Subject Baseline Characteristics

Characteristic	Subjects Who Completed (n=22)	Subjects Withdrawn (n=3)
Male/female	18/4	1/2
Age, y	60 (3)	60 (10)
Weight, kg	89 (3)	87 (7)
BMI, kg/m^2	29 (1)	30 (3)
Left ventricular hypertrophy	1	0
Plasma glucose, mmol/L	5.3 (0.1)	5.9 (0.7)
Serum triglyceride, mmol/L	2.0 (0.3)	1.6 (0.1)
Serum cholesterol		
Total, mmol/L	5.4 (0.2)	5.9 (0.1)
LDL, mmol/L	3.2 (0.1)	3.9 (0.2)
HDL, mmol/L	1.3 (0.1)	1.3 (0.2)
Total:HDL ratio	4.5 (0.3)	5.0 (0.8)

For continuous variables data are means (SEs). BMI indicates body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

sildenafil; comparisons with baseline and between placebo and sildenafil are all statistically nonsignificant).

The effects of sildenafil and placebo on clinic BP, arterial wave reflection, CF-PWV, central BP, and FMD are shown in Table 3. Sildenafil reduced clinic systolic BP, diastolic BP, and mean arterial BP acutely (1 hour after administration), but by day 16, the magnitude of this reduction in BP was less than that on day 1. On day 16, BP was generally slightly lower 1 hour after sildenafil administration (time of peak effect) than just before sildenafil administration (time of trough effect). Sildenafil did not affect clinic pulse pressure or HR, either acutely or chronically (data not shown).

Sildenafil reduced CAIx, CAIx@75, and RAIx acutely but, as with the effects on clinic BP, the magnitude of these effects on day 16 were reduced compared with those on day 1. When recorded before sildenafil administration on day 16, these measures were not significantly different from baseline, but when recorded 1 hour after sildenafil administration on day 16, they were significantly lower than at baseline. The changes from baseline in CAIx, CAIx@75, and RAIx with sildenafil were significantly different from placebo on day 1 but were not significantly different from placebo either before or after sildenafil administration on day 16. Compared with baseline, sildenafil reduced central systolic BP and central diastolic BP, both at 1 hour and after 16 days of regular treatment. However, the changes in these parameters were not significantly different from the changes observed with placebo when measured before sildenafil administration on day 16. Sildenafil reduced central pulse pressure acutely but not after chronic treatment.

CF-PWV and FMD were unaffected by sildenafil, both acutely and chronically. Sildenafil also did not affect baseline brachial artery diameter or the extent of reactive hyperemia at any point (data not shown).

TABLE 2. Effects on Ambulatory BP (mm Hg)

	Absolute Values			Changes From Baseline	
BP Measure	Baseline	Sildenafil	Placebo	Sildenafil	Placebo
24-h					
Systolic BP	144 (1.5)	138 (1.7)*	147 (1.5)	−7 (1.9)	3 (2.1)*
Diastolic BP	85 (1.4)	80 (1.9)*	87 (2.2)	−5 (1.1)	1 (1.3)*
Daytime					
Systolic BP	153 (1.7)	145 (1.8)*	155 (1.5)	−8 (2.0)	2 (2.0)*
Diastolic BP	92 (1.4)	86 (1.8)*	92 (2.2)	−6 (1.1)	0 (1.3)*
Nighttime					
Systolic BP	124 (1.9)	119 (1.9)†	129 (2.1)	−5 (1.9)	5 (2.6)†
Diastolic BP	71 (1.6)	68 (2.0)†	74 (2.4)	−4 (1.3)	3 (1.7)†

For absolute values, comparisons are against baseline, and for changes from baseline, comparisons are between sildenafil and placebo.

*$P<0.01$; †$P<0.05$.

Adverse Effects

Two subjects were withdrawn while taking sildenafil, 1 because of severe headache (after 3 days) and the other because of back pain and feeling generally unwell (after 6 days), and 1 subject was withdrawn while taking placebo, because of joint pains, nausea, and headache (after 11 days). For a full summary of the symptoms experienced see the online supplement. Dyspepsia occurred in 10 subjects with sildenafil and lasted ≤5 days. Headaches occurred in 8 subjects and were generally mild and transient. Low back/buttock/leg muscle ache occurred in 7 subjects, was usually responsive to simple analgesia, and tended to settle within a few days. Plasma creatine kinase concentrations were measured in 4 of the subjects who experienced these symptoms, and all were within the normal laboratory reference range. Six of the 18 men in the study reported increased penile erection, which occurred only with sildenafil.

Discussion

Sildenafil reduced BP both acutely and after 16 days of regular administration. The reduction in average daytime BP was 8/6 mm Hg compared with baseline and ≈10/6 mm Hg compared with placebo. This degree of BP reduction is similar to the effect of other, commonly used antihypertensive drugs when they are given as monotherapy in hypertension.[29] The correlation between baseline systolic BP and the extent of reduction in systolic BP with sildenafil suggests that in a more hypertensive population, a greater absolute effect on BP may occur.

When given as 50 mg 3 times daily, sildenafil accumulates in the plasma. The accumulation ratio, based on the area under the curve of the plasma concentration versus time curve of 0 to 8 hours, is 1.59 in healthy subjects (Baerbel Wittke, unpublished data, 2006). Despite this, although there was a persistent hypotensive effect of sildenafil for 16 days, the clinic BP data show that there was some attenuation of the acute effect. This may be the result of neurohormonal counterregulatory mechanisms, such as stimulation of the renin–angiotensin system, after the initial vasodilatation-mediated reduction in BP. On day 16 of treatment, clinic BP was higher before the administration of sildenafil than at 1 hour afterward. Although average nighttime BP was reduced, this suggests that the overnight dose interval was sufficiently

TABLE 3. Effects on Peripheral Clinic BP, Arterial Wave Reflection, Central BP, CF-PWV, and FMD

	Absolute Values		Changes From Baseline	
Measure	Sildenafil	Placebo	Sildenafil	Placebo
Systolic BP, mm Hg				
Baseline	150 (2.6)	154 (3.3)		
1 h	141 (2.8)*	155 (3.2)	−10 (2.2)	1 (1.9)*
Day 16, predose	146 (2.8)	152 (3.1)	−4 (2.4)	−2 (1.8)
Day 16, postdose	145 (2.8)†	158 (3.9)	−5 (2.3)	4 (2.1)*
Diastolic BP, mm Hg				
Baseline	88 (1.5)	89 (1.6)		
1 h	82 (1.9)*	90 (1.7)	−6 (1.2)	2 (1.2)*
Day 16, predose	85 (1.5)*	88 (1.8)	−3 (1.0)	0 (1.1)
Day 16, postdose	83 (1.6)*	90 (2.0)	−5 (1.0)	2 (1.5)*
MAP (mm Hg)				
Baseline	111 (1.7)	113 (2.2)		
1 h	102 (2.3)*	113 (2.2)	−8 (1.5)	1 (1.5)*
Day 16, predose	107 (1.9)*	112 (2.2)	−4 (1.3)	−1 (1.3)
Day 16, postdose	105 (2.0)*	115 (2.6)	−5 (1.4)	2 (1.7)*
CAIx, %				
Baseline	32 (1.9)	32 (2.0)		
1 h	28 (2.1)*	33 (1.9)	−4 (0.9)	1 (0.9)*
Day 16, predose	30 (2.0)	31 (2.3)	−2 (1.1)	−1 (1.1)
Day 16, postdose	30 (2.0)†	31 (2.3)	−2 (0.8)	−1 (1.1)
CAIx@75, %				
Baseline	25 (1.7)	25 (1.9)		
1 h	22 (1.8)*	24 (1.8)	−3 (0.8)	−1 (0.6)†
Day 16, predose	23 (1.8)	24 (2.2)	−2 (0.9)	−1 (0.9)
Day 16, postdose	23 (1.8)*	23 (2.0)†	−1 (0.7)	−2 (0.8)
RAIx, %				
Baseline	88 (2.7)	89 (3.0)		
1 h	82 (2.9)*	90 (2.7)	−6 (1.2)	1 (1.4)*
Day 16, predose	85 (2.8)	87 (3.2)	−3 (1.6)	−2 (1.6)
Day 16, postdose	84 (2.8)*	88 (3.0)	−4 (1.2)	−1 (1.9)
Central systolic BP, mm Hg				
Baseline	142 (3.1)	145 (3.7)		
1 h	130 (3.2)*	148 (3.6)	−12 (2.2)	2 (1.9)*
Day 16, predose	137 (3.1)†	144 (3.6)	−5 (2.2)	−2 (1.8)
Day 16, postdose	136 (3.4)†	149 (4.4)	−6 (2.1)	4 (2.3)*
Central diastolic BP, mm Hg				
Baseline	89 (1.5)	90 (1.6)		
1 h	82 (2.0)*	90 (2.1)	−6 (1.2)	0 (1.7)*
Day 16, predose	86 (1.5)*	89 (1.8)	−3 (1.0)	0 (1.1)
Day 16, postdose	84 (1.6)*	91 (2.0)	−5 (1.1)	1 (1.5)*
Central pulse pressure, mm Hg				
Baseline	53 (2.9)	55 (3.0)		
1 hour	48 (2.3)*	57 (3.4)	−5 (1.7)	2 (1.7)*
Day 16, predose	51 (2.6)	54 (3.1)	−2 (1.8)	−1 (1.3)
Day 16, postdose	53 (2.8)	58 (3.5)	−1 (1.6)	3 (1.6)
CF-PWV, m/s				
Baseline	10.4 (0.49)	10.2 (0.63)		
1 h	10.2 (0.47)	10.4 (0.55)	−0.2 (0.20)	0.2 (0.18)
Day 16, predose	9.9 (0.55)	10.7 (0.62)†	−0.5 (0.28)	0.5 (0.20)†
Day 16, postdose	9.6 (0.56)‡	10.3 (0.53)	−0.8 (0.38)	0.1 (0.18)
FMD (peak dilatation, %)				
Baseline	2.6 (0.38)	2.1 (0.39)		
1 h	2.1 (0.44)	2.8 (0.47)	−0.4 (0.30)	0.7 (0.39)
Day 16, predose	2.7 (0.38)	2.2 (0.36)	0.1 (0.32)	0.1 (0.41)
Day 16, postdose	2.6 (0.63)	3.2 (0.48)†	0.0 (0.62)	1.1 (0.46)
FMD (AUC, AU)				
Baseline	7.1 (1.91)	5.6 (2.00)		
1 h	7.4 (2.35)	4 (3.14)	0.3 (2.91)	−0.3 (2.96)
Day 16, predose	7.3 (2.22)	6.9 (1.91)	0.3 (2.85)	0.5 (2.97)
Day 16, postdose	7.9 (2.53)	6.4 (2.28)	0.8 (3.40)	−0.3 (2.64)

For absolute values, comparisons are against baseline, and for changes from baseline, comparisons are between sildenafil and placebo.

*$P<0.01$; †$P<0.05$; ‡$P=0.063$.

long for the antihypertensive effect to begin to wane, which may be of clinical relevance given that the early morning surge in BP may be an important trigger of cardiovascular events.[30] The use of a modified-release preparation of sildenafil (not currently available) or a longer-acting PDE5 inhibitor might afford better protection at this time. A more stable steady-state plasma concentration that would occur with either a modified-release preparation or a longer acting agent might also result in a greater cumulative reduction in BP. Despite reducing BP, HR was not affected by sildenafil. This has been reported previously and is consistent with an influence on baroreflex regulation.[6]

Sildenafil reduced arterial wave reflection, whether measured as CAIx, CAIx@75, or RAIx and did so in a manner that was similar to the effect on clinic BP, with a greater reduction acutely than was evident after 16 days. However, this effect was relatively small and was no more than would be expected as a simple consequence of the reduction in systemic BP,[31] suggesting that sildenafil did not act specifically to reduce large artery stiffness. This conclusion may also be supported by the lack of effect of sildenafil on CF-PWV, a more direct measure of large artery stiffness. However, although the effects were not statistically significant, there was a trend to a progressive reduction in CF-PWV (−0.2 m/s at 1 hour, −0.5 m/s on day 16 before sildenafil, and −0.8 m/s on day 16 after sildenafil), and the possibility of a real effect on CF-PWV should not be dismissed; a larger sample size or a longer treatment period may have demonstrated a significant effect. With chronic treatment, sildenafil reduced central BP to an extent similar to its effect on peripheral BP. Measurement of central BP did not, therefore, provide any additional information on the antihypertensive action of sildenafil over and above the measurement of peripheral BP.

Sildenafil had no effect on FMD, indicating that it did not modulate endothelium-dependent vasomotion, either acutely or after chronic treatment. There are conflicting data on the acute effects of sildenafil on endothelium-dependent vasomotor responses, with some reports of an improvement and other reports showing no effect. Although these previous studies have recruited different patients and used different methodologies, there are no apparent consistent differences to explain the different effects observed. For example, there are positive and negative studies that used forearm plethysmography[20,22] or FMD[9,11,21] and that recruited smokers[9,21] or patients with CAD.[11,22] It should be noted that the use of a clamp to hold the ultrasound probe in place, as well as computerized analysis of arterial images, represent best practice in FMD studies, maximizing our confidence that the finding is truly negative.

Given that FMD is largely NO mediated, it would seem logical that sildenafil, by preserving NO-stimulated cGMP, would improve FMD. However, this might only be expected in subjects with impaired endothelium-dependent vasomotor function at baseline. Although we did not include a normotensive control group in the present study, in a further (unpublished) study in our department, mean baseline FMD was 5.6% (±0.4%) in 44 healthy men and women with mean age 42 years. Thus, endothelium-dependent vasomotion was most likely impaired in the hypertensive subjects, even if this was partly because they were, on average, older. A possible explanation for the lack of effect of sildenafil on FMD in the present study is that, compared with healthy subjects,[32] NO contributes relatively little to shear stress-induced vasodilatation at the brachial artery in hypertensive subjects. In support of this hypothesis, it has been shown previously that although vasodilatation to bradykinin in the forearm is NO mediated in healthy subjects, in hypertensive subjects it is not only reduced but is also mediated by a different pathway, possibly involving endothelium-dependent hyperpolarization.[33] Although not investigated previously, if similar changes occur in the brachial artery, sildenafil may be expected to have little or no effect on FMD in hypertension.

The relevance of an improvement in FMD to cardiovascular prognosis in hypertension is not clear. Although there is evidence that prognosis is better in patients that demonstrate improvements in FMD after treatment,[34] FMD is not consistently improved by antihypertensive drugs of different classes although, with the possible exception of β-blockers,[35] these drugs are substantially equivalent in reducing cardiovascular events.[36] Therefore, the lack of effect of sildenafil on FMD should not, in itself, detract from its potential as an antihypertensive in clinical practice.

Adverse effects from regular sildenafil treatment were relatively common but generally transient and of mild-to-moderate severity. Headache was experienced with a similar frequency to that seen in other studies, but dyspepsia was slightly more frequent.[5] Myalgia (reported as an aching sensation of the low back, buttocks, or legs) has generally been reported to occur in <5% of men taking single doses[37] but seems to occur more frequently with repeated administration, for example, in 28% of subjects in the present study and in 14% of subjects with pulmonary arterial hypertension.[5] The lack of any rise in plasma creatine kinase in 4 of our subjects with myalgia suggests that these symptoms were not because of an underlying myositis.

As a consequence of the range of outcome measures and assessment of these at multiple time points, a large number of statistical comparisons have been made, presenting the possibility of type 1 statistical errors. However, this possibility is of least concern for the data on the effect on ambulatory BP, the primary outcome measure, because ambulatory BP was only assessed at baseline and at the end of each treatment period. Moreover, the effect of sildenafil on both 24-hour and daytime BPs was highly statistically significant ($P<0.01$).

Perspectives

This is the first controlled clinical trial to demonstrate the potential of regular PDE5 inhibition in the chronic treatment of hypertension. Although sildenafil effectively reduced BP, its use in clinical practice is limited by its relatively short duration of action, requiring it to be administered 3 times daily. A modified-release preparation of sildenafil may overcome this problem but is not currently available. Alternatively, a longer acting PDE5 inhibitor, such as tadalafil, may be more suitable for further studies on PDE5 inhibition in hypertension. Assuming that such an agent is also shown to reduce BP when administered chronically, characterization of its adverse effect profile and an appropriate dosing strategy for chronic use would be research priorities. Clarification of the effects of chronic PDE5 inhibition on CF-PWV would also be of interest. Studies comparing both antihypertensive efficacy and tolerability of PDE5 inhibitors with established antihypertensives would help to determine their place in clinical practice. A potentially valuable indication for the use of PDE5 inhibitors as antihypertensives is in men with erectile dysfunction. However, it would be necessary to demonstrate that the effect of PDE5 inhibition on erectile function is maintained in the long term with the regular dosing pattern that would be required for the treatment of hypertension.

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Disclosures

None.

References

1. Stone JR, Marletta MA. Heme stoichiometry of heterodimeric soluble guanylate cyclase. *Biochemistry.* 1995;34:14668–14674.
2. Hofmann F, Ammendola A, Schlossmann J. Rising behind NO: cGMP-dependent protein kinases. *J Cell Sci.* 2000;113:1671–1676.
3. Rybalkin SD, Yan C, Bornfeldt KE, Beavo JA. Cyclic GMP phosphodiesterases and regulation of smooth muscle function. *Circ Res.* 2003;93:280–291.
4. Carson CC, Lue TF. Phosphodiesterase type 5 inhibitors for erectile dysfunction. *BJU Int.* 2005;96:257–280.
5. Galiè N, Ghofrani HA, Torbicki A, Barst RJ, Rubin LJ, Badesch D, Fleming T, Parpia T, Burgess G, Branzi A, Grimminger F, Kurzyna M, Simonneau G. Sildenafil citrate therapy for pulmonary arterial hypertension. *N Engl J Med.* 2005;353:2148–2157.

6. Jackson G, Benjamin N, Jackson N, Allen MJ. Effects of sildenafil citrate on human hemodynamics. *Am J Cardiol*. 1999;83:13C–20C.
7. Zusman RM, Morales A, Glasser DB, Osterloh IH. Overall cardiovascular profile of sildenafil citrate. *Am J Cardiol*. 1999;83:35C–44C.
8. Schalcher C, Schad K, Brunner-La Rocca HP, Schindler R, Oechslin E, Scharf C, Suetsch G, Bertel O, Kiowski W. Interaction of sildenafil with cAMP-mediated vasodilation in vivo. *Hypertension*. 2002;40:763–767.
9. Dishy V, Harris PA, Pierce R, Prasad HC, Sofowora G, Bonar HL, Wood AJJ, Stein CM. Sildenafil does not improve nitric oxide-mediated endothelium-dependent vascular responses in smokers. *Br J Clin Pharmacol*. 2004;57:209–212.
10. Dishy V, Sofowora G, Harris PA, Kandcer M, Zhan F, Wood AJ, Stein CM. The effect of sildenafil on nitric oxide-mediated vasodilation in healthy men. *Clin Pharmacol Ther*. 2001;70:270–279.
11. Halcox J, Nour K, Zalos G, Mincemoyer R, Waclawiw M, Rivera C, Willie G, Ellahham S, Quyyumi A. The effect of sildenafil on human vascular function, platelet activation, and myocardial ischemia. *J Am Coll Cardiol*. 2002;40:1232–1240.
12. Herrmann HC, Chang G, Klugherz BD, Mahoney PD. Hemodynamic effects of sildenafil in men with severe coronary artery disease. *N Engl J Med*. 2000;342:1622–1626.
13. Manfroi WC, Caramori PR, Zago AJ, Melchior R, Zen V, Accordi M, Gutierres D, Noer C. Hemodynamic effects of sildenafil in patients with stable ischemic heart disease. *Int J Cardiol*. 2003;90:153–157.
14. Mahmud A, Hennessy M, Feely J. Effect of sildenafil on blood pressure and arterial wave reflection in treated hypertensive men. *J Hum Hypertens*. 2001;15:707–713.
15. Vardi Y, Klein L, Nassar S, Sprecher E, Gruenwald I. Effects of sildenafil citrate (viagra) on blood pressure in normotensive and hypertensive men. *Urology*. 2002;59:747–752.
16. Webb DJ, Freestone S, Allen MJ, Muirhead GJ. Sildenafil citrate and blood-pressure-lowering drugs: results of drug interaction studies with an organic nitrate and a calcium antagonist. *Am J Cardiol*. 1999;83:21C–28C.
17. McDonald LJ, Murad F. Nitric oxide and cyclic GMP signaling. *Proc Soc Exp Biol Med*. 1996;211:1–6.
18. Hryniewicz K, Dimayuga C, Hudaihed A, Androne AS, Zheng H, Jankowski K, Katz SD. Inhibition of angiotensin-converting enzyme and phosphodiesterase type 5 improves endothelial function in heart failure. *Clin Sci (Lond)*. 2005;108:331–338.
19. Schofield RS, Edwards DG, Schuler BT, Estrada J, Aranda J, Juan M. Pauly DF, Hill JA, Aggarwal R, Nichols WW. Vascular effects of sildenafil in hypertensive cardiac transplant recipients. *Am J Hypertens*. 2003;16:874–877.
20. Kimura M, Higashi Y, Hara K, Noma K, Sasaki S, Nakagawa K, Goto C, Oshima T, Yoshizumi M, Chayama K. PDE5 inhibitor sildenafil citrate augments endothelium-dependent vasodilation in smokers. *Hypertension*. 2003;41:1106–1110.
21. Vlachopoulos C, Tsekoura D, Alexopoulos N, Panagiotakos D, Aznaouridis K, Stefanadis C. Type 5 phosphodiesterase inhibition by sildenafil abrogates acute smoking-induced endothelial dysfunction. *Am J Hypertens*. 2004;17:1040–1044.
22. Robinson SD, Ludlam CA, Boon NA, Newby DE. Phosphodiesterase type 5 inhibition does not reverse endothelial dysfunction in patients with coronary heart disease. *Heart*. 2006;92:170–176.
23. Vlachopoulos C, Hirata K, O'Rourke MF. Effect of sildenafil on arterial stiffness and wave reflection. *Vasc Med*. 2003;8:243–248.
24. Hirata K, Adji A, Vlachopoulos C, O'Rourke MF. Effect of sildenafil on cardiac performance in patients with heart failure. *Am J Cardiol*. 2005;96:1436–1440.
25. O'Brien E, Mee F, Atkins N, Thomas M. Evaluation of three devices for self-measurement of blood pressure according to the revised British Hypertension Society Protocol: the Omron HEM-705CP, Philips HP5332, and Nissei DS-175. *Blood Press Monit*. 1996;1:55–61.
26. Baumgart P, Kamp J. Accuracy of the Labs Medical 90217 ambulatory blood pressure monitor. *Blood Press Monit*. 1998;3:303–307.
27. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol*. 2002;39:257–265.
28. Webb DJ, Muirhead GJ, Wulff M, Sutton JA, Levi R, Dinsmore WW. Sildenafil citrate potentiates the hypotensive effects of nitric oxide donor drugs in male patients with stable angina. *J Am Coll Cardiol*. 2000;36:25–31.
29. Materson BJ, Reda DJ, Cushman WC, Massie BM, Freis ED, Kochar MS, Hamburger RJ, Fye C, Lakshman R, Gottdiener J, Ramirez EA, Henderson WG. Single-drug therapy for hypertension in men. A comparison of six antihypertensive agents with placebo. *N Engl J Med*. 1993;328:914–921.
30. Giles T. Relevance of blood pressure variation in the circadian onset of cardiovascular events. *J Hypertens Suppl*. 2005;23(suppl):S35–S39.
31. Wilkinson IB, MacCallum H, Hupperetz PC, van Thoor CJ, Cockcroft JR, Webb DJ. Changes in the derived central pressure waveform and pulse pressure in response to angiotensin II and noradrenaline in man. *J Physiol*. 2001;530:541–550.
32. Joannides R, Haefeli WE, Linder L, Richard V, Bakkali EH, Thuillez C, Luscher TF. Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo. *Circulation*. 1995;91:1314–1319.
33. Taddei S, Ghiadoni L, Virdis A, Buralli S, Salvetti A. Vasodilation to bradykinin is mediated by an ouabain-sensitive pathway as a compensatory mechanism for impaired nitric oxide availability in essential hypertensive patients. *Circulation*. 1999;100:1400–1405.
34. Modena MG, Bonetti L, Coppi F, Bursi F, Rossi R. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol*. 2002;40:505–510.
35. Lindholm LH, Carlberg B, Samuelsson O. Should beta blockers remain first choice in the treatment of primary hypertension? A meta-analysis. *Lancet*. 2005;366:1545–1553.
36. Blood Pressure Lowering Trialists Collaboration. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. *Lancet*. 2003;362:1527–1535.
37. Eardley I, Mirone V, Montorsi F, Ralph D, Kell P, Warner MR, Zhao Y, Beardsworth A. An open-label, multicentre, randomized, crossover study comparing sildenafil citrate and tadalafil for treating erectile dysfunction in men naive to phosphodiesterase 5 inhibitor therapy. *BJU Int*. 2005;96:1323–1332.