# INHIBITION OF PHOSPHODIESTERASE TYPE 5 AND EXERCISE IN ARTERIAL HYPERTENSION

**DR TERESA M ATTINÀ**

**PRESENTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

**UNIVERSITY OF EDINBURGH**

**JULY 2009**

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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.

**ATTINA, T.M.**, MALATINO, L.S., MAXWELL S.R., WEBB, D.J. Phosphodiesterase type 5 inhibition improves arterial stiffness after exercise but not exercise capacity in hypertensive patients. Submitted to *Journal of Hypertension*.

**ATTINA, T.M.**, MALATINO, L.S., MAXWELL S.R., PADFIELD, P.L., WEBB, D.J. (2008). Phosphodiesterase type 5 inhibition reverses impaired forearm exercise-induced vasodilatation in hypertensive patients. *J Hypertens*, 26, 501-7. Included in the appendix.

## PRESENTATIONS

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

**ATTINA T.M.** & WEBB, D.J. Phosphodiesterase type 5 inhibition reverses impaired forearm exercise-induced vasodilatation in hypertensive patients. Oral presentation at the 21st Scientific Meeting of the International Society of Hypertension 2006, Japan.

**ATTINA T.M.** & WEBB, D.J. Phosphodiesterase type 5 inhibition reverses impaired forearm exercise-induced vasodilatation in hypertensive patients. Oral presentation at the British Pharmacological Society 2005 winter meeting, London.

## 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.

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Dr Teresa Maria Attinà

## ACKNOWLEDGEMENTS

I am very much indebted to Professor David Webb, my principal supervisor, for his advice and guidance. I have learnt an enormous amount from Professor Webb, not only in relation to the specific research performed as part of this PhD, but for all aspects related to clinical research in an academic environment. Even though my career direction is now in Public Health, I will continue to value the experience that I have gained under his supervision. Professor Simon Maxwell, my assistant supervisor, has been a valuable source of friendly and sensible advice. I would also like to express my gratitude to Professor Lorenzo Malatino, my supervisor during my postgraduate specialty training in Italy, for his constant support throughout these years.

I am very much indebted to Vanessa Melville, Debbie Kerr, Susan Inch and the nurses at the Wellcome Trust Clinical Research Facility. The contributions of Vanessa and Debbie, both research nurses, to the forearm plethysmography studies were invaluable, as well as the contribution of Susan to the recruitment of my research participants. I am grateful to the nurses at the Wellcome Trust for their assistance with cardiopulmonary exercise testing. My thanks also go to Neil Johnston, the Head of the Clinical Pharmacology Laboratory, for his assistance and advice.

Although not directly involved in the research, the other people working within Clinical Pharmacology have helped to make the last few years enjoyable and stimulating. These are (in order of appearance) James Oliver, Rupert Payne, Nick Bateman, David Newby, Katsuaki Okubo, Bushra Ilyas, Neeraj Dhaun (Bean), Pajaree Lilitkarntakul (Bua), Takae Asai, James Dear, Iain McIntyre.

My sincerest thanks to all.

# ABBREVIATIONS

| | |
|---|---|
| ACE | Angiotensin converting enzyme |
| ACh | Acetylcholine |
| AIx | Augmentation index |
| ALLHAT | The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial |
| AP | Augmentation pressure |
| ARB | Angiotensin II receptor (type $AT_1$) blocker |
| AT | Anaerobic threshold |
| ß-blocker | ß-adrenoreceptor antagonist |
| BMI | Body mass index |
| BP | Blood pressure |
| bpm | Beats per minute |
| CAIx | Central augmentation index |
| CAIx@75 | Central augmentation index normalised to a heart rate of 75 beats per minute |
| CCB | Calcium channel blocker |
| CHD | Coronary heart disease |
| CF-PWV | Carotid-femoral pulse wave velocity |
| cGMP | Guanosine 3', 5'-cyclic monophosphate |
| CI | Confidence interval |
| CPET | Cardiopulmonary exercise testing |
| CRC | Clinical Research Centre |
| CVD | Cardiovascular disease |
| ECG | Electrocardiogram |
| EDRF | Endothelium-derived relaxing factor |
| ET-1 | Endothelin 1 |
| FBF | Forearm blood flow |
| FMD | Flow-mediated dilatation |
| HR | Heart rate |
| ISMN | Isosorbide mononitrate |
| ISDN | Isosorbide dinitrate |
| L-NMMA | $N^G$ monomethyl-L-arginine |
| LVH | Left ventricular hypertrophy |
| MAP | Mean arterial blood pressure |
| mmHg | Millimeters of mercury |
| MVC | Maximum voluntary contraction |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| PAH | Pulmonary arterial hypertension |
| PAP | Pulmonary arterial pressure |
| PDE | Phosphodiesterase |
| PP | Pulse pressure |
| PAH | Pulmonary arterial hypertension |
| PWA | Pulse wave analysis |
| PWV | Pulse wave velocity |

| | |
|---|---|
| RAIx | Radial augmentation index |
| RER | Respiratory exchange ratio |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| sGC | Soluble guanylate cyclase |
| SNP | Sodium nitroprusside |
| SVR | Systemic vascular resistance |
| SWG | Standard wire gauge |
| $VCO_2$ | Carbon dioxide output |
| $VO_2$ | Oxygen uptake |
| WGH | Western General Hospital |
| WR | Work rate |
| WTCRF | Wellcome Trust Clinical Research Facility |

## ABSTRACT

Hypertensive patients exhibit impaired exercise capacity, a strong independent risk factor for cardiovascular disease, and the mechanisms responsible for this are not fully determined. Potential candidates may include endothelial vasomotor dysfunction and arterial stiffness, both of which are associated with hypertension. Impairment of the nitric oxide-cyclic guanosine monophosphate (NO-cGMP) pathway plays a major role in the development of these abnormalities, suggesting that enhancement of NO-cGMP signalling through phosphodiesterase type 5 (PDE5) inhibition may offer therapeutic potential in arterial hypertension. This thesis investigated the effects of the PDE5 inhibitor sildenafil citrate on exercise-induced vasodilatation, maximal exercise capacity and arterial stiffness in hypertensive patients, using different studies involving local limb and whole body exercise.

Preliminary dose-ranging studies were initially performed to investigate the intra-arterial (brachial) effects of sildenafil on forearm blood flow (FBF), and to select an appropriate, cGMP-independent, vasodilator to use as a control. On the basis on these studies, it was established that sildenafil, infused at 50μg/min, and verapamil, infused at 5μg/min, had similar vasodilator effect on FBF. Ten untreated hypertensive patients and ten matched normotensive subjects were then studied in a three-way, randomised, single-blind and placebo-controlled FBF study. The aim was to investigate the effects of sildenafil on handgrip exercise-induced vasodilatation, and to compare this response with verapamil and saline (placebo). Preinfusion exercise-induced vasodilatation was significantly reduced in hypertensive compared with normotensive subjects ($P<0.001$). However, after the infusions, while verapamil did not affect the vasodilator response to exercise in either group, sildenafil substantially enhanced this response in hypertensive patients, but not in normotensive subjects ($P<0.05$). These results suggested that sildenafil, through an increase in cGMP levels in the vasculature, substantially and selectively improves the vasodilator response to handgrip exercise in hypertensive patients.

The effects of oral sildenafil on maximal exercise capacity and arterial stiffness were then investigated in a three-way, randomised, double-blind and placebo-controlled study. Fifteen untreated hypertensive and fifteen matched normotensive subjects received 50mg sildenafil, 25mg hydralazine (a control vasodilator) or placebo, 3 times daily for 1 week, and the effects on maximal exercise capacity, measured as peak oxygen consumption (peak $VO_2$), were evaluated. The effects of sildenafil on pulse wave velocity (PWV), a measure of arterial stiffness, were also investigated before and after maximal exercise. Peak $VO_2$ was significantly lower and PWV significantly higher in hypertensive than normotensive subjects ($P<0.0001$). Treatment with sildenafil did not affect peak $VO_2$ in either group. However, while PWV increased after exercise in hypertensive patients following placebo, sildenafil reversed these changes, significantly reducing PWV compared with placebo and hydralazine ($P=0.0001$).

In conclusion, although PDE5 inhibition did not affect maximal exercise capacity, sildenafil, by improving arterial distensibility in the recovery period after exercise, may, as well as blood pressure lowering, offer an additional beneficial effect in active hypertensive individuals.

# CHAPTER 1

## INTRODUCTION

## 1.1 THE ENDOTHELIUM AND THE NITRIC OXIDE SYSTEM

### 1.1.1 The endothelium

The endothelium, the inner layer of blood vessels, is a complex and dynamic organ that responds to environmental stimuli and generates vasoactive substances. With a surface area of around 4000-7000$m^2$, and a mass of more than 1 kg, it acts as the major communicating interface between the circulating blood and the vessel wall (Aird, 2007). A healthy endothelium plays a major role in modulating vascular tone through synthesis and release of vasoactive mediators, which determine both vascular structure and function and also provide protection from thrombosis and the development of atherosclerosis. Vasoactive mediators produced by the endothelium include relaxing (prostacyclin, nitric oxide, endothelium-derived hyperpolarising factor) and contracting (endothelin-1 and metabolites of arachidonic acid) factors (Vanhoutte, 1988). The discovery of the role of the endothelium in the modulation of vascular tone followed the major breakthrough by Furchgott and Zawadzki in 1980, who demonstrated that acetylcholine requires the presence of the endothelial cells to relax the underlying vascular smooth muscle cells (Furchgott & Zawadzki, 1980). The substance released by endothelial cells responsible for this effect was initially termed "endothelium-derived relaxing factor" (EDRF) and later identified as nitric oxide (NO) (Palmer *et al*., 1987). Since then, research in the field has been extensive, and NO is now widely recognised as a major determinant of vascular structure and function. Additional, NO-independent, pathways also cause vasodilatation, mainly involving activation of potassium channels and hyperpolarisation of vascular smooth muscle cells leading to vasorelaxation (Busse *et al*., 2002).

### 1.1.2 The NO-cGMP pathway

NO is synthesized by the enzyme nitric oxide synthase (NOS) from L-arginine and oxygen, consuming nicotinamide adenine dinucleotide phosphate (NADPH) in the process. NOS exists in 3 isoforms: endothelial (eNOS) and neuronal (nNOS) isoforms are constitutively expressed in the endothelium, platelets and some part of the nervous system, whereas the inducible (iNOS) isoform is controlled at the

transcriptional level (Stuehr, 1999; Toda & Okamura, 2003; Gorren & Mayer, 2007). The critical role played by the NOS system was demonstrated in a study showing, for the first time, that mice lacking all 3 NOS develop spontaneous myocardial infarction and exhibit markedly reduced survival (Nakata *et al*., 2008). NO synthesis is tightly controlled and linked to changes in ionized calcium concentration. Both eNOS and nNOS are activated via calcium/calmodulin and their activity is modulated by phosphorylation of serine, threonine and tyrosine residues (Fleming & Busse, 2003; Stuehr *et al*., 2004). Several agonists, including acetylcholine, bradykinin and substance P, act on specific membrane receptors that induce cytosolic calcium release and eNOS activation. Increased shear stress, the frictional force exerted by flowing blood, also serves as an important stimulus for NO production from eNOS. By contrast, iNOS is calcium independent and inducible by immunological mechanisms (Moncada *et al*., 1991).

NO plays a major role in the regulation of vascular tone and exerts many of its biological effects through the formation of cyclic guanosine monophosphate (cGMP) (McDonald & Murad, 1996; Schlossmann *et al*., 2003), although there are some effects that appear to occur independently of cGMP signalling (Cui *et al*., 2005). Once released by endothelial cells, NO diffuses to the vascular smooth muscle cells and stimulates the soluble guanylyl cyclase (sGC) enzyme. This results in cGMP formation and activation of cGMP-dependent protein kinase (PKG), which initiates a protein phosphorylation cascade with reduction in intracellular calcium concentration, ultimately leading to vasodilatation (Rybalkin *et al*., 2003).

The central role played by NO in the regulation of vascular tone has been evidenced by a number of studies. It was initially shown in animal studies (Rees *et al*., 1989), and Vallance and coworkers were the first to show it in humans. They demonstrated that the intra-arterial (brachial) infusion of the NOS inhibitor $N^G$-monomethyl-L-arginine (L-NMMA) reduces resting forearm blood flow and increases vascular resistance, clearly indicating that tonic generation of NO regulates basal vasomotor tone (Vallance *et al*., 1989). Subsequent studies, prompted by these findings, aimed at clarifying the role of NO in the regulation of blood pressure (BP), and showed that

systemic (intravenous) infusion of L-NMMA increases BP in healthy individuals (Haynes *et al*., 1993a, b).

### 1.1.3 Cyclic GMP and Phosphodiesterase type 5

Cyclic GMP (cGMP) has emerged recently as a principal focus in signal transduction and a possible pharmacological target, and much of the attention has derived from the fact that it mediates most of the effects of NO (Schlossmann *et al*., 2003; Bian *et al*., 2006; Murad, 2006). Following the discovery of cGMP and its importance to NO signalling, researchers focused on the regulation of this pathway, in particular the enzymes responsible for cGMP degradation, the cyclic nucleotide phosphodiesterases (PDEs) (Hardman *et al*., 1971). Currently, 11 different families of PDEs have been identified, and PDE type 5 (PDE5) is responsible for the hydrolysis of cGMP in smooth muscle cells (Bender & Beavo, 2006; Omori & Kotera, 2007). PDE5 was originally identified more than 25 years ago (Coquil *et al*., 1980; Francis *et al*., 1980) but it was only when this enzyme became a target for the PDE5 inhibitor sildenafil citrate that its important role in the regulation of vascular smooth muscle contraction emerged fully (Boolell *et al*., 1996).

PDE5 is present in smooth muscle cells and platelets and plays a major role under basal conditions, characterized by low intracellular calcium. However, under condition of increased calcium levels (associated with vasoconstriction), PDE1 may also be involved in cGMP breakdown (Rybalkin *et al*., 2003). The structure of PDE5 consists of 2 subunits, each containing a single catalytic domain and regulatory domain. The catalytic domain is highly specific for cGMP, whereas the regulatory domain contains two allosteric cGMP-binding sites (GAF domain, an acronym derived from the first three domains identified: mammalian c***G***MP binding PDEs, *Anabaena* ***a***denylyl cyclases, and plant ***F***hlA transcription factors) (Conti & Beavo, 2007). The regulatory domain is phosphorylated by cGMP-dependent protein kinase PKG: occupation of a GAF domain by cGMP is required for this phosphorylation, which, in turn, causes stimulation of both catalytic activity and cGMP binding to the GAF domain. Therefore, elevation of cGMP causes increased PDE5 activity,

representing a negative feedback mechanism in the cGMP pathway (Corbin *et al*., 2000; Francis *et al*., 2002; Bender & Beavo, 2006).

### 1.1.4 PDE5 inhibition and erectile dysfunction

On sexual arousal, NO is generated in the penile vasculature, diffuses in smooth muscle cells and binds with sGC, leading to cGMP formation. This results in relaxation of vascular and sinusoidal smooth muscle in the corpora cavernosa and increased penile blood flow, leading to expansion of erectile tissues and penile erection. Sildenafil, by preventing cGMP breakdown, prolongs vascular relaxation and promotes penile erection. This compound was initially developed for the treatment of angina pectoris, and it was only during early trials that its serendipitous effect on erectile function emerged, leading to the approval for the treatment of erectile dysfunction in 1998. Since then sildenafil has been very successfully used for the treatment of male erectile dysfunction (Carson & Lue, 2005).

### 1.1.5 The PDE5 inhibitors

The class of PDE5 inhibitors currently comprises 3 selective and orally active compounds, sildenafil (Viagra™), vardenafil (Levitra™) and tadalafil (Cialis™). Sildenafil was the first PDE5 inhibitor to become available for clinical use to treat penile erectile dysfunction and, to date, is the most extensively studied compound. The catalytic domain on PDE5, but not the GAF domain, binds sildenafil and blocks cGMP hydrolysis, resulting in increased cGMP levels (Boolell *et al*., 1996). Of note, the increased cGMP levels not only determine a greater degree of activation of PKG and PKG-dependent phosphorylation, but also potentiate sildenafil binding affinity to PDE5, which further contributes to elevating cGMP (Blount *et al*., 2004). This unique capacity for sildenafil to elevate cGMP levels and, at the same time, to further increase its own inhibitory capacity, represents a novel mechanism for the sustained generation of cGMP and explains the potent biological effects of this drug.

Sildenafil is a potent and reversible inhibitor of PDE5 ($IC_{50}$ of 3.9nM). It is highly selective (>1000-fold) for PDE5 when compared with PDE2, PDE3 and PDE4, and moderately selective over PDE1 (>80 fold). It is however only approximately 10-fold

as potent for PDE5 as for PDE6, found in the photoreceptors of the retina. Sildenafil is rapidly absorbed after oral administration and maximum plasma concentrations are reached within 30 to 120 minutes (median 60 minutes) of dosing in the fasted state, with a mean oral absolute bioavailability ranging from 38 to 41% and a plasma half-life of ~4 hours (Walker *et al*., 1999; Muirhead *et al*., 2002). For doses up to 200mg, systemic exposure of sildenafil is dose proportional, with an approximately linear exposure-response relationship.

The major metabolic pathway of sildenafil is hepatic, by the cytochrome P450, isoenzyme CYP3A4, and the principal metabolite is N-desmethyl sildenafil, which accounts for ≈20% of the overall pharmacological activity. Owing to its extensive metabolism, sildenafil is not detected unchanged in urine or faeces. Its metabolites are mainly excreted in faeces and, to a lesser extent, in urine. Inhibitors of the CYP3A4, such as erythromycin and the protease inhibitors ritonavir and saquinavir can interfere with the metabolism of sildenafil, increasing the area under the concentration-time curve (AUC) and maximum plasma concentration ($C_{max}$) (Muirhead *et al*., 2000; Muirhead *et al*., 2002). Grapefruit juice, an inhibitor of intestinal CYP3A4, also increases sildenafil bioavailability, although the effect seems to be variable (Jetter *et al*., 2002). Sildenafil clearance is reduced in healthy elderly patients (≥65 years) and in those with severe renal insufficiency (creatinine clearance <30mL/min) or hepatic cirrhosis (Muirhead *et al*., 2002).

The other PDE5 inhibitors available, vardenafil and tadalafil, differ in their selectivity for PDE5 (vardenafil>tadalafil>sildenafil), with $IC_{50}$ of 0.1-0.7 nM and 0.94 nM, respectively. They also differ with respect to other PDEs: selectivity of vardenafil for PDE5 is >1000-fold relative to PDE2-4 and 7-10; >300-fold relative to PDE11; >130-fold relative to PDE1; >15-fold relative to PDE6. Selectivity of tadalafil is >10 000-fold relative to PDE1-4 and 7-10; >700-fold relative to PDE6; >5-fold relative to PDE11 (Conti & Beavo, 2007).

The clinical efficacy of the PDE5 inhibitors is similar, the main difference being onset and duration of activity, especially for tadalafil, which has the longest duration

(Table 1.1). Most of the adverse events associated with PDE5 inhibitors are related to vasodilatation (headache, flushing, and nasal congestion) and gastrointestinal events (dyspepsia). Back pain and myalgia are more often reported with tadalafil (Setter *et al*., 2005). Visual disturbances (abnormal colour vision) seem to be more common with sildenafil, reflecting its limited selectivity against PDE6, localized in photoreceptors of the retina (Boolell *et al*., 1996).

When sildenafil was marketed in 1998, it was an immediate success and nowadays it can be found almost anywhere. Even in the Gaza Strip, during one of the recent humanitarian crisis, blackmarket sildenafil was available in large quantities (McGirk, 2008). The successful use of sildenafil in the treatment of male erectile dysfunction (Goldstein *et al*., 1998) has also created increasing interest in the therapeutic potential of PDE5 inhibition in cardiovascular diseases associated with dysfunction of the NO-cGMP signalling pathway.

| | Sildenafil | Vardenafil | Tadalafil |
|---|---|---|---|
| **$IC_{50}$ for PDE5 (nM)** | 3.9 | 0.1-0.7 | 0.94 |
| **Dose** | 50mg increased to 100mg | 10mg increased to 20mg | 10mg increased to 20mg |
| **$T_{max}$** | Median, 60 min | Median, 60 min | Median, 2 hours |
| **Mean $T_{1/2}$** | 4 hours; high-fat meal ↓ $C_{max}$ by 29% | 4-5 hours; high-fat meal ↓ $C_{max}$ by 18% | 17.5 hours; not affected by food |
| **Active metabolite** | N-desmethyl sildenafil 20% contribution to activity | N-desmethyl vardenafil 27% contribution to activity | None |
| **Renal excretion** | <1% | 1% | <0.3% |
| **Side effects** | Headache, dyspepsia, nasal congestion, flushing, abnormal vision | Headache, dyspepsia, flushing, rhinitis, dizziness, nausea, sinusitis | Headache, dyspepsia, myalgia, back pain, flushing, nasal congestion |

**Table 1.1. Comparisons of the commercially available PDE5 inhibitors: sildenafil, vardenafil and tadalafil.**

## 1.2 FITNESS AND CARDIOVASCULAR HEALTH

### 1.2.1 Physical fitness

Physical fitness can be defined as 'the ability to perform moderate-to-vigorous levels of physical activity without undue fatigue and the capability of maintaining this capacity throughout life' (Pollock *et al*., 1998). Two major elements contribute to physical fitness: performance-related physical fitness, linked to athletic skills and ability, and health-related physical fitness. The latter refers to the components of physical fitness related to health status (Caspersen *et al*., 1985; Vanhees *et al*., 2005).

| ***Component*** | ***Factor*** |
|---|---|
| Morphological | Body mass for height<br>Body composition<br>Flexibility |
| Muscular | Muscle strength<br>Muscle endurance<br>Power |
| Motor | Balance<br>Speed |
| Cardiovascular | Submaximal exercise capacity<br>Maximal aerobic capacity |

**Table 1.2. Major components and factors of health-related fitness**

The studies presented on this thesis will focus on the cardiovascular component of health-related fitness (cardiovascular fitness) (Table 1.2) and its value in relation to cardiovascular health.

### 1.2.2 Cardiovascular fitness and exercise capacity

Cardiovascular fitness refers to the combined efficiency of the heart, lungs and vascular system to deliver oxygen to the working muscles, which translates into the ability to perform dynamic exercise for a prolonged period of time. This determines an individual's maximal exercise capacity (a trait that describes how well an individual can perform dynamic exercise), and requires the interaction of the

cardiovascular, respiratory and skeletal muscle systems, whose responses are linked to each other and to cell respiration (Wasserman *et al*., 1999).

Exercise capacity is usually assessed by means of symptom-limited exercise testing (maximal incremental exercise testing), which allows the study of the integrated response of the cardiovascular, respiratory and muscle components under controlled exercise conditions. To assess an individual's maximal exercise capacity, an exercise test should:

- Employ at least 50% of the total muscle mass. Activities that meet this requirement include running and cycling
- Be independent of strength, speed, body size, and skills
- Be of sufficient duration for cardiovascular responses to be maximized (ideally between 8 and 12 minutes) (Wasserman *et al*., 1999).

Cardiopulmonary exercise testing (CPET) with appropriate gas exchange measurements is a valuable tool for objective exercise performance assessment. Measures of gas exchange primarily include oxygen consumption or uptake ($VO_2$), which represents the amount of oxygen transported and used by the working muscles during exercise. When we measure oxygen consumption, we are indirectly measuring an individual's maximal capacity to perform work aerobically (maximal aerobic capacity) (Wasserman *et al*., 1999) and, indeed, higher $VO_2$ values are associated with greater physical exercise capacity (Fletcher *et al*., 2001). In this context, CPET associated with gas exchange measurements allows evaluation of maximal exercise capacity, and can detect the presence and the degree of functional impairment, as well as objectively evaluate the response to interventions that may affect exercise capacity (McConnell *et al*., 1995; Wasserman *et al*., 1999).

An exercise test may be performed using a treadmill or a cycle ergometer. Both have advantages and disadvantages, and which one is the best choice for exercise testing is a matter of debate. Most subjects have higher peak $VO_2$ on the treadmill than the cycle ergometer, but movement artefacts and noise might be a problem, and it is

difficult to accurately quantify the work rate. On the contrary, the cycle ergometer offers less movement artefact and an accurate quantification of the work rate, and is also considered safer for patients (Gibbons *et al*., 2002; Myers *et al*., 2009).

### 1.2.3 Oxygen consumption and the Fick equation

Oxygen consumption is defined by the Fick equation as the product of cardiac output and arterial venous oxygen difference (Acierno, 2000):

$$VO_2 = (SV \times HR) \times (C_{aO2} - C_{VO2})$$

where SV is the stroke volume, HR is the heart rate, and $C_{aO2}$ and $C_{VO2}$ are the oxygen concentration of arterial and mixed venous gas, respectively. At maximal exercise, the components of the equation change as follows:

$$VO_2max = (SVmax \times HRmax) \times (C_{aO2}max - C_{VO2}max)$$

This defines the maximal aerobic capacity, i.e. how well an individual can metabolize oxygen and generate energy.

### 1.2.4 Maximal oxygen consumption and maximum oxygen consumption

The measurement of gas exchange variables has been simplified with the development of rapid gas analysers for oxygen and carbon dioxide and computerized on-line analysis systems (Beaver *et al*., 1973). Directly measured $VO_2$ is expressed as a rate, either in absolute values (litres per minute, L/min), or relative to body weight (millilitres per kg bodyweight per minute, ml/kg/min), making inter-subject comparisons easier. The human body has an upper limit for $O_2$ utilization at a particular state of fitness, and $VO_2$max represents the *maximal* volume of oxygen that the body can consume during intense exercise, from a resting value of 3.5 ml/kg/min. More precisely, $VO_2$max is defined as the point at which no further increase in measured $VO_2$ occurs despite an increase in work rate (a plateau is reached) during graded exercise testing. Instead, *maximum* oxygen consumption or peak $VO_2$ is the highest $VO_2$ attained during graded exercise testing, but the term does not imply that a plateau in measured $VO_2$ is reached (Wasserman *et al*., 1999) (Figure 1.1A). This distinction is relevant when considering exercise studies in normal (untrained) subjects or patients because, after reaching their peak $VO_2$ at maximal effort, most of them cannot tolerate the discomfort long enough to achieve a

plateau in $VO_2$. However, peak $VO_2$ is usually close to the predicted $VO_2$max, and is often the first parameter measured in exercise studies involving patients, because it evaluates whether the patient's response to exercise allows normal maximal aerobic function (Pardaens *et al*., 1996; Wasserman *et al*., 1999).

### 1.2.5 Aerobic exercise, anaerobic exercise, and the anaerobic threshold

Muscle contraction is associated with the breakdown of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). Because the reserve of ATP in the muscle is very limited, it has to be continuously replenished through the mitochondrial oxidation of substrates in order to continue exercise. In exercise activity of short duration requiring a high power output, oxygen demands cannot be met quickly enough and ATP is generated mostly through anaerobic metabolism, in which $CO_2$ is produced with very little $O_2$ being consumed. In exercise activity of longer duration (endurance exercise), oxygen demands can be met and ATP is generated aerobically during the initial phase of exercise. During this phase $CO_2$ output ($VCO_2$) increases linearly with $VO_2$ and reflects the aerobic production of $CO_2$. However, at a certain exercise level, the oxygen supply is no longer enough to meet the oxygen demands of the working muscle, and the anaerobic metabolism increases to increase energy generation. Lactic acid is the by-product of anaerobic metabolism and is buffered in the cell by bicarbonate, leading to $CO_2$ formation. This is the point at which $VCO_2$ increases exponentially relative to $VO_2$ to eliminate the excess $CO_2$ produced, and it is called anaerobic threshold (AT, Figure 1.1B) (Wasserman *et al*., 1999). The AT usually occurs at 47 to 64% of $VO_2$max in untrained subjects, and is widely used as a submaximal index of aerobic capacity and as an index of the metabolic and circulatory changes that occur during incremental exercise (Davis *et al*., 1976).

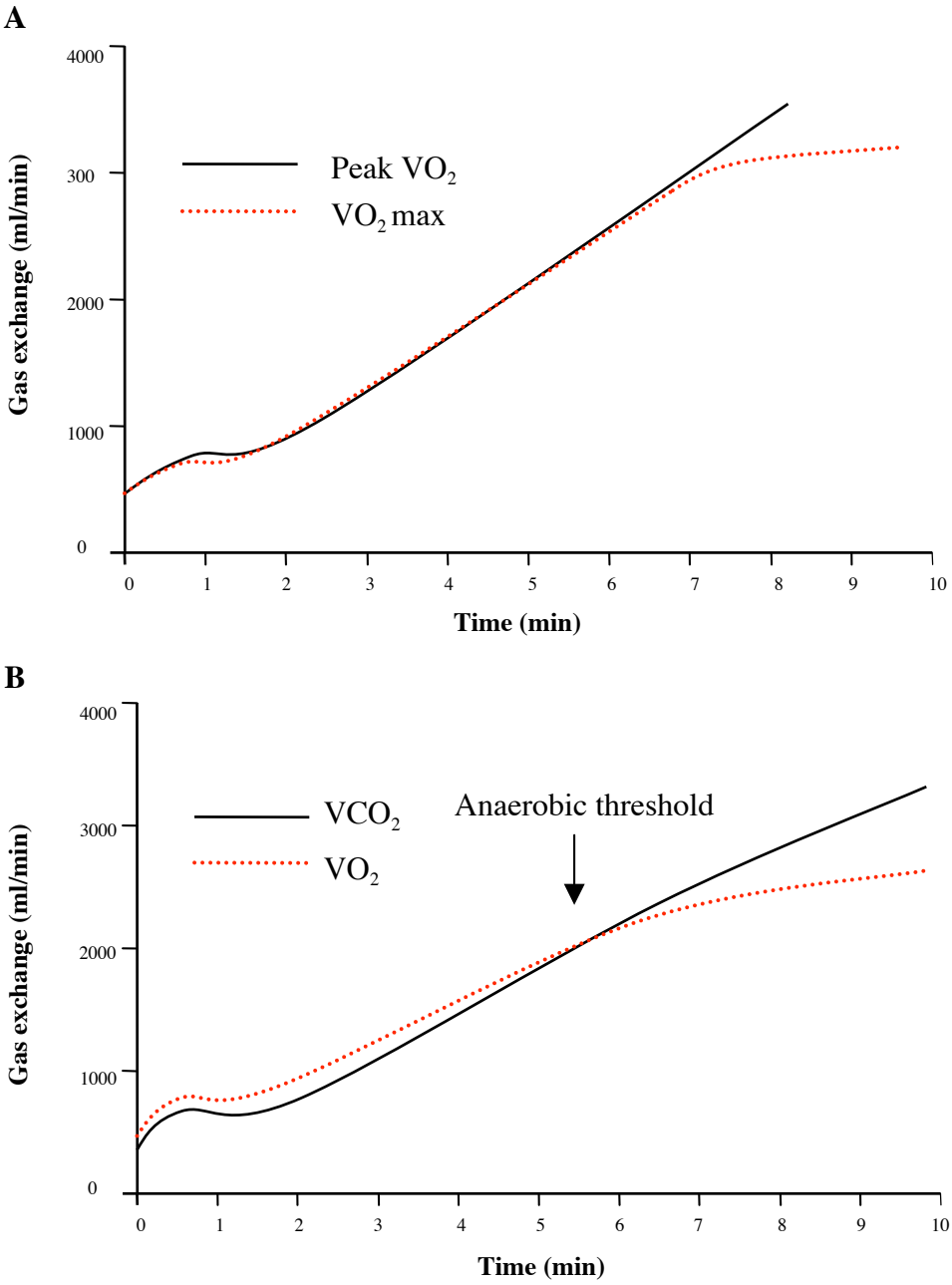


**Figure 1.1. Schematic representation of $VO_2$max and peak $VO_2$ (A) and anaerobic threshold (B).**
During incremental exercise testing, $VO_2$max is defined as the point at which no further increase in measured $VO_2$ occurs despite an increase in work rate (WR), represented as a plateau in the $VO_2$ curve. When the plateau is not reached, but the subject has reached the maximum tolerable WR, this is defined as peak $VO_2$ (A). The anaerobic threshold (AT) can be defined as the $VO_2$ just below the point at which a nonlinear increase in $VCO_2$ is observed, indicating the onset of anaerobic metabolism during incremental exercise (B).

### 1.2.6 The value of exercise capacity

Extensive evidence shows that aerobic exercise capacity is a strong independent predictor of cardiovascular risk and death in individuals with and without cardiovascular disease (CVD) (Paffenbarger *et al*., 1986; Ekelund *et al*., 1988; Blair *et al*., 1989; Sandvik *et al*., 1993; Blair *et al*., 1995; Myers *et al*., 2002; Evenson *et al*., 2004; Lee *et al*., 2005; Kokkinos *et al*., 2008; Peterson *et al*., 2008; Kodama *et al*., 2009). Greater exercise capacity has been shown to be protective against all-cause and cardiovascular mortality, regardless of whether clinic BP is controlled (Church *et al*., 2001), and its improvement is associated not only with reduced cardiovascular risk (Blair *et al*., 1996; Erikssen *et al*., 1998) but, also, with improved survival (Blair *et al*., 1995) and better quality of life (Brown *et al*., 2003; Martin *et al*., 2009). Furthermore, it seems to represent a more powerful predictor of mortality than traditional risk factors such as hypercholesterolaemia or diabetes (Mora *et al*., 2003; Balady *et al*., 2004). With age and with chronic cardiovascular conditions such as hypertension (Fleg *et al*., 2005), exercise capacity declines, and a concomitant decline in physical activity is observed, because it takes more effort to exercise, a person becomes more easily exhausted and breathless and, consequently, activities identified as requiring substantial effort tend to be avoided. This determines a vicious cycle leading to a further decrease of exercise capacity, with major implications not only for the risk of CVD, which has been recently demonstrated at the population level (Carnethon *et al*., 2005), but also on functional independence and long-term quality of life. For these reasons, the value of exercise capacity and its assessment is increasingly recognised, based on the understanding that resting pulmonary and cardiac function testing may not accurately predict exercise performance and functional capacity, and that overall health status correlates more closely with exercise capacity than with resting measurements.

## 1.3 HYPERTENSION AND EXERCISE CAPACITY

### 1.3.1 Hypertension

Cardiovascular diseases, which include coronary heart disease (CHD) and stroke, are a leading cause of morbidity and mortality in developed countries and are becoming increasingly common in less developed regions of the world (Murray & Lopez, 1997; Lopez *et al*., 2006). The final common pathway for the development of CVD is atherosclerosis of the vessel wall, and a number of traditional risk factors for atherosclerosis have been identified. Amongst them, arterial hypertension is one of the most important: recently published data show that worldwide about 54% of stroke and 47% of ischaemic heart disease can be attributed to hypertension (Lawes *et al*., 2008), and the risk further increases when hypertension is associated with other risk factors such as hypercholesterolaemia, obesity and smoking (Ezzati *et al*., 2003). Classifications of hypertension vary but British, European and American guidelines use a threshold of >140mmHg systolic or 90mmHg diastolic BP to diagnose hypertension (Chobanian *et al*., 2003; Williams *et al*., 2004; Mancia *et al*., 2007). However, it is acknowledged that this threshold is somewhat arbitrary and the risks of CHD and stroke begin to increase at a level of 115 mmHg.

Lowering of BP by non-pharmacological means should always come first in the management of hypertension. Weight loss, exercise, alcohol restriction, reduced salt intake should be encouraged, before and after commencing drug therapy, and they have a significant effect on BP reduction (Cutler *et al*., 1997; Xin *et al*., 2001; Whelton *et al*., 2002). More recently, the Dietary Approaches to Stop Hypertension (DASH) diet, already known to reduce BP (Appel *et al*., 1997), has also been shown to reduce the risk of CHD and stroke (Fung *et al*., 2008). However, for many individuals, pharmacological treatment will be required to control BP. Since the first evidence of the benefits of antihypertensive drug treatment (Mohler & Freis, 1960; Hamilton *et al*., 1964), a number of antihypertensive agents have been developed to control BP. Currently, they can be divided in 4 major classes: angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEIs/ARBs), beta-blockers, calcium channel blockers (CCBs), and thiazide diuretics. For all of them, the BP-

lowering effect seems to be the main determinant of cardiovascular risk reduction, but there has been much debate whether or not some of these agents offer additional benefits beyond BP control. Findings from the BP Lowering Treatment Trialists' Collaboration (Turnbull, 2003) showed that differences in outcomes between regimens were related to differences in BP reductions. Subsequent trials have compared active drugs regimens, such as the VALUE study (Julius *et al*., 2004) and ASCOT-BPLA (Dahlof *et al*., 2005). Again, because of unequal reductions in BP between treatment groups in these studies, the relative contribution of direct and BP mediated benefit is difficult to unravel. Meta-analyses, although useful, need to adjust for differences in BP and this makes their interpretation somewhat difficult. However, there is evidence suggesting that ACEIs and CCBs offer the best protection in the prevention of CHD (Verdecchia *et al*., 2005), and ACEIs, ARBs and diuretics seem to be the best choice in congestive heart failure (Wang *et al*., 2006). In addition, recent results from the ACCOMPLISH trial suggest that the combination of an ACE inhibitor and a CCB is superior to ACE inhibitor plus diuretic (hydrochlorothiazide) in reducing cardiovascular event in high-risk hypertensive patients (Jamerson *et al*., 2008). Data from meta-analyses also suggest that beta-blockers are not indicated as first-line treatment in patients with uncomplicated hypertension (Wiysonge *et al*., 2007), although the evidence reviewed was mainly based on the effect of atenolol, and it is always difficult to establish whether these findings can be extrapolated to the entire class of drug. The debate about the use of beta-blockers as first-line agents in hypertension is currently on going, and it is matter of controversy amongst different authors (Cutler & Davis, 2008; Messerli *et al*., 2008).

Despite clinically effective treatment, poor BP control in the UK is responsible for thousands of unnecessary deaths per year. It was calculated that if all hypertensive patients in the UK reduced their BP to target (<140/90mmHg) (Chobanian *et al*., 2003), approximately 41,400 ischaemic heart disease deaths and 21,400 stroke deaths could be prevented each year (He & MacGregor, 2003). Great benefit could be derived from adequate BP control and, because the adverse impact of hypertension on health usually occurs over a long period of time, it is important to consider BP control well in advance, before individuals exhibit any evidence of functional impairment or,

worse, disability. In fact, hypertension has been shown to be associated not only with an important reduction in the number of years lived without CVD (7.2 years for both sexes) and an increase in the time spent with CVD (Franco *et al*., 2005), but also with disability (Hajjar *et al*., 2007). Improving patient compliance and physician adherence with hypertension guidelines play a significant role in hypertension control (Wetzels *et al*., 2004; Okonofua *et al*., 2006). Nevertheless, treatment of hypertension to recommended targets can be a challenge in many patients. In an aging society and in a context of limited economic resources, this contributes to the overwhelming costs of care of advanced and chronic disease and highlights the public health burden of hypertension.

### 1.3.2 Exercise haemodynamics in hypertension

Established hypertension is characterized by increased peripheral vascular resistance, and is associated with reduced exercise capacity and exaggerated systolic BP response to exercise (Lim *et al*., 1996). Although in most epidemiological studies resting BP has been measured, and taken as an indicator of cardiac risk and the target for treatment, an increased cardiovascular mortality still persists in apparently well-controlled hypertensive patients treated accordingly to resting (office) BP values (Andersson *et al*., 1998; Almgren *et al*., 2005). One explanation is that resting BP may not accurately reflect the underlying cardiovascular changes occurring in patients with hypertension, particularly at an early stage. These changes are usually accentuated and better detected under stress conditions, such as during exercise. Physical exercise markedly increases blood flow to skeletal muscle to meet the metabolic demand of active muscle tissue, and a fundamental mechanism responsible for the increase in blood flow is vasodilatation. In normotensive subjects, this is evidenced by a progressive reduction of systemic vascular resistance (SVR) during exercise as a result of peripheral vasodilatation, which also limits the rise of systolic BP. By contrast, in hypertensive patients, exercise haemodynamics show an abnormal pattern characterized by lower stroke volume and, often, an exaggerated systolic BP response compared to normotensive subjects (Montain *et al*., 1988; Palatini, 1994). This reflects an impaired peripheral vasodilator capacity and consequent failure of SVR to fall (Lund-Johansen, 1991; Modesti *et al*., 1999), and

may contribute to the overall reduced exercise capacity and associated worse prognosis reported in hypertensive individuals (Amery *et al*., 1967; Fagard *et al*., 1988; Blair *et al*., 1991; Goodman *et al*., 1992; Missault *et al*., 1992; Lim *et al*., 1996). The clinical significance of these observations is supported by data showing that exercise systolic BP provides predictive information on cardiovascular death (Kjeldsen *et al*., 2001) and is also associated with coronary risk factors (Mundal *et al*., 1998). These findings support the value of exercise systolic BP and that of reduced exercise capacity as strong independent predictors of cardiovascular risk and death (Ren *et al*., 1985; Ekelund *et al*., 1988; Blair *et al*., 1989; Filipovsky *et al*., 1992; Miyai *et al*., 2000; Myers *et al*., 2002; Peterson *et al*., 2008). More recently, exercise capacity has been shown to be the strongest predictor of all-cause mortality in hypertensive men with and without additional cardiovascular risk factors. Furthermore, even small increases in exercise capacity seem to contribute substantially to mortality risk reduction (Kokkinos *et al*., 2009).

### 1.3.3 Vascular alterations in hypertension

The exact mechanisms underlying the impaired peripheral vasomotor response and reduced exercise capacity found in hypertension are not fully understood, but an important role may be played by the structural and functional alterations that develop in the hypertensive vessel wall. As previously mentioned, exercise capacity depends on an adequate oxygen supply to the heart and to the skeletal muscle, and may therefore be affected by the progressive functional and structural vascular changes occurring in hypertension. These changes occur at the level of small (resistance) arteries and large (conduit) arteries, leading to alteration of smooth muscle tone, reduced arterial distensibility (arterial stiffness) and vascular remodelling (Folkow *et al*., 1973; McVeigh *et al*., 1991; Heagerty *et al*., 1993; Park & Schiffrin, 2001). The final result is increased SVR, the hallmark of established hypertension (Lund-Johansen, 1980). Indeed, according to Poiseuille's law (Figure 1.2), vascular resistance varies inversely with the fourth power of the blood vessel radius, so that even a small decrease in the lumen markedly increases resistance (Folkow, 1982; Nichols, 1997). Conversely, reductions in the vasoconstrictor state of the peripheral

vasculature by greater local vasodilator influence would result in a significant change in blood flow.

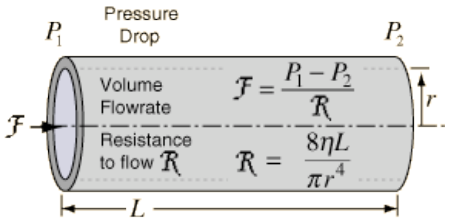


**Figure 1.2. Poiseuille's law**
The laminar flow rate of an incompressible fluid along a tube is directly proportional to the pressure difference between its ends and the fourth power of its internal radius, and inversely proportional to its length and the viscosity of the fluid. F, flow rate; P, pressure; R, resistance to flow; η, viscosity; L, length of the tube; r, radius.

In hypertension, cardiac adaptations also develop to confront the elevated systolic loads due to increased vascular resistance: these are represented by ventricular systolic stiffening and, later, hypertrophy (Kass, 2005; Zieman *et al*., 2005). The combination of ventricular-arterial stiffening affects the way in which elements of the cardiovascular system interact at rest and, particularly, under stress conditions, such as during exercise. This is further supported by evidence suggesting a relationship between increased vascular stiffness and reduced exercise capacity (Feske *et al*., 1988; Vaitkevicius *et al*., 1993; Cameron *et al*., 1999; Tanaka *et al*., 2000; Hundley *et al*., 2001; Ferreira *et al*., 2002; Boreham *et al*., 2004; Willens *et al*., 2005).

## 1.4 PDE5 INHIBITION AND EXERCISE

### 1.4.1 The NO-cGMP pathway and exercise

The main physiological stimulus to endothelial NO production is increased blood flow through the vessel lumen (Pohl *et al*., 1986; Rubanyi *et al*., 1986; Davies, 1995; Corson *et al*., 1996), resulting in increased vascular wall shear-stress that is sensed by the endothelium and translated into a vasodilator response (Hutcheson & Griffith, 1991; Koller & Kaley, 1991; Niebauer & Cooke, 1996). NO has been demonstrated to be essential for this flow-mediated vasodilator response in human peripheral conduit and resistance vessels (Joannides *et al*., 1995; Paniagua *et al*., 2001) raising the possibility that it may also contribute to exercise-induced vasodilatation. In fact, during exercise, the increase in cardiac output and tissue perfusion result in shear stress-induced enhancement of eNOS activity. This suggests that, with increasing blood flow and oxygen demand, the role of NO-mediated vasodilatation may become increasingly important (Dyke *et al*., 1995; Meredith *et al*., 1996; Maxwell *et al*., 1998). Furthermore, inhibition of NO synthesis has been shown to reduce exercise-induced vasodilatation in healthy subjects (Gilligan *et al*., 1994).

### 1.4.2 Systemic vascular effects of sildenafil

Given that PDE5 enzymes are widely represented throughout the vascular system, PDE5 inhibition might be expected to have effects on the general cardiovascular system. In particular, the enhancement of NO-cGMP-mediated relaxation on vascular smooth muscle may result in systemic BP reduction. In healthy subjects, sildenafil has been shown to reduce systolic and diastolic BP in most but not all studies. In a study by Jackson and coworkers, the mean maximum reduction in supine systolic/diastolic BP was 10/7 mmHg, and no changes in heart rate were observed (Jackson *et al*., 1999). A similar effect was observed in another study performed in healthy subjects (Zusman *et al*., 1999). However, a study by Schalcher and coworkers did not evidence any effect of sildenafil on BP in healthy subjects (Schalcher *et al*., 2002). In the same way, in individuals with coronary disease some investigators have reported BP reductions following sildenafil administration (Herrmann *et al*., 2000), but others have not (Manfroi *et al*., 2003). In hypertension,

most studies have been performed in individuals already on antihypertensive treatment (Webb *et al*., 1999; Mahmud *et al*., 2001; Vardi *et al*., 2002), and have shown a consistent BP lowering effect of sildenafil. In the only study investigating the effects of regular sildenafil treatment (for 16 days) in untreated hypertensive patients, both systolic and diastolic clinic BP were significantly reduced compared with placebo (Oliver *et al*., 2006). In summary, the effects of sildenafil in these studies are compatible with mild/moderate systemic vasodilatation, and no clear evidence of a positive chronotropic effect.

### 1.4.3 Effects of other PDE5 inhibitors on BP

Although less evidence is available on the effects of vardenafil and tadalafil, a study comparing sildenafil and vardenafil showed a greater BP decrease with the latter (8 mmHg systolic and 6 mmHg diastolic) than with sildenafil (5 mmHg systolic and 4 mmHg diastolic) (Pomara *et al*., 2004). With regard to tadalafil, a single oral dose of 10 or 20 mg did not seem to significantly change systolic BP, but diastolic BP was reduced by 5 mmHg compared with placebo (Kloner *et al*., 2003b). Most recently, Wolk and coworkers investigated the effect of different doses of a new, long-acting, PDE5 inhibitor, administered once daily for 28 days, on daytime systolic BP in patients with mild to moderate hypertension. The novel PDE5 inhibitor significantly decreased mean daytime systolic BP by approximately 7 mmHg compared with placebo, and the response, although greater at the beginning, was sustained until the end of the study period (Wolk *et al*., 2009).

### 1.4.4 PDE5 inhibitors and interactions with cardiovascular drugs

#### *1.4.4.1 Interaction between PDE5 inhibitors and organic nitrates*

The PDE5 inhibitor-nitrate interaction is well known and has been extensively studied. NO donors, such as nitroglycerine, isosorbide mononitrate (ISMN) and dinitrate (ISDN), work by stimulating the enzyme sGC and increasing the formation of cGMP, leading to vasodilatation and reduction of BP. These drugs are widely used to treat angina and heart failure and, when given in concomitance with PDE5 inhibitors, the increased cGMP levels together with the reduced breakdown can lead to marked vasodilatation and hypotension. This interaction has been shown in

healthy subjects with the concomitant administration of sildenafil and nitroglycerine (Webb *et al*., 1999), and has also been shown for tadalafil (Kloner *et al*., 2003a) and vardenafil (Reffelmann & Kloner, 2007). In patients with angina, this interaction has been demonstrated with nitroglycerine and ISMN (Webb *et al*., 1999; Webb *et al*., 2000). As a result, the combined use of organic nitrates and PDE5 inhibitors is an absolute contraindication because of the potential for harm from hypotension.

#### *1.4.4.2 Interaction between PDE5 inhibitors and alpha-adrenoceptor antagonists*

Alpha-adrenoceptor antagonists such as doxazosin and terazosin, used in both hypertension and benign prostatic hypertrophy, can interact with PDE5 inhibitors, but the synergistic hypotensive effect appears to be much less important than with NO donors. Therefore, the concomitant administration of these drugs is not an absolute contraindication, but some restrictions and close medical monitoring are advised (Reffelmann & Kloner, 2006).

### 1.4.5 Effects of PDE5 inhibition on exercise capacity

#### *1.4.5.1 Studies in primary pulmonary hypertension*

PDE5 is highly expressed in the pulmonary vasculature, and early studies investigating the haemodynamic effects of PDE5 inhibition in pulmonary arterial hypertension (PAH) showed a decrease in pulmonary arterial pressure (PAP) and vascular resistance (PVR), with little change in systemic BP, therefore suggesting pulmonary vascular selectivity (Wilkens *et al*., 2001; Zhao *et al*., 2001). Michelakis and coworkers investigated the effects of a 3-month treatment with sildenafil in 5 patients with PAH. This was a small, uncontrolled, study, but showed a significant increase in the 6-minute walking distance, which increased from 376±3 to 540±27 meters. These findings were also accompanied by a significant decrease in PAP and PVR (Michelakis *et al*., 2003). These results were confirmed in subsequent studies, such as the one by Galie and coworkers, who assessed safety and efficacy of sildenafil in 278 patients with PAH. This was a 12-week, placebo-controlled study, and showed a dose-dependent increase of the 6-minute walking distance (of 45, 46 and 80 meters) with sildenafil treatment (Galie *et al*., 2005). Most recently, the same

group demonstrated that tadalafil, at the dose of 40 mg, improves exercise capacity and quality of life measures in patients with PAH (Galie *et al*., 2009).

### *1.4.5.2 Studies in chronic heart failure*

The effects of PDE5 inhibition in patients with heart failure have been investigated by several groups. One of the first studies was performed by Bocchi and coworkers, who reported the effects of sildenafil on haemodynamics and exercise capacity in 24 patients with chronic heart failure (CHF). In this study sildenafil 50mg did not change peak exercise systolic or diastolic BP but increased oxygen uptake at peak $VO_2$ compared with placebo (17.7±3.4 *vs* 16.6±3.4 ml/kg/min, p=0.02) (Bocchi *et al*., 2002). Guazzi and coworkers investigated the acute effects of sildenafil on exercise performance in 16 patients with CHF, and showed that sildenafil increased peak exercise $VO_2$ from 16.2 to 19.4 ml/kg/min, whereas no difference was observed after placebo. No change in exercise capacity was reported in the control group after sildenafil. The same group also investigated the effects of chronic (6 months) treatment with sildenafil in 21 heart failure patients, showing a substantial, sustained effect of sildenafil on exercise capacity (peak $VO_2$ increment from 14.8 to 18.7 ml/kg/min) at 6 months, with no changes in heart rate (Guazzi *et al*., 2007). These findings are consistent with results obtained by Lewis and coworkers, who assessed the effects of sildenafil treatment in 34 patients with systolic heart failure and secondary pulmonary hypertension. After 12 weeks of treatment, peak $VO_2$, the primary endpoint, increased significantly from 12.2 to 13.9 ml/kg/min (Lewis *et al*., 2007a). The same group also reported improved exercise haemodynamics and oxygen uptake after the acute administration of sildenafil 50mg in 13 patients with heart failure (Lewis *et al*., 2007a).

### *1.4.5.3 Studies in healthy subjects*

Few studies have investigated the effects of PDE5 inhibition on exercise haemodynamics and oxygen uptake in healthy subjects. Ghofrani and coworkers investigated the effects of sildenafil on exercise capacity in healthy trained subjects under hypoxia, which determines a pulmonary hypertensive response. Sildenafil 50 mg reduced PAP at rest and during exercise, and increased maximum workload and

cardiac output (Ghofrani *et al*., 2004). Ghofrani and coworkers did not evaluate the effects of sildenafil under normoxia, which were investigated in a study by Hsu and coworkers. This study confirmed the findings by Ghofrani and also showed that sildenafil does not affect exercise performance under normoxic conditions (Hsu *et al*., 2006). With regard to other PDE5 inhibitors, there is only one published study in which the effects of tadalafil on exercise performance were investigated in young athletes. In this study, tadalafil did not influence exercise capacity and cardiopulmonary responses to maximal exercise under normoxia (Di Luigi *et al*., 2008).

### 1.4.6 Summary of data on the effects of PDE5 inhibition on exercise capacity

Current available evidence suggests that, in healthy subjects, PDE5 inhibition may increase exercise capacity under hypoxic but not normoxic conditions. In patients with PAH, data from published studies clearly show the beneficial effects of sildenafil on exercise capacity. In 2005, on the basis of this data, sildenafil was licensed for the treatment of patients with PAH classified as WHO functional class III to improve exercise capacity. Results from studies conducted in patients with CHF suggest that sildenafil could also improve exercise capacity in these patients, although these findings will need to be confirmed in larger studies. To date, the effects of PDE5 inhibition on exercise BP and exercise capacity in systemic arterial hypertension have never been investigated.

## 1.5 ENDOTHELIAL DYSFUNCTION

### 1.5.1 Assessment of endothelial function in humans

A balance between vasoconstrictor factors, such as endothelin-1 (ET-1) responsible for cell growth and pro-inflammatory effects, and vasodilator factors such as NO, which generally inhibit cell growth and inflammation, is a key point in the maintenance of normal vascular function and structure. When this balance is disrupted, the result is endothelial dysfunction, cell growth and inflammation, ultimately leading to vascular dysregulation and remodelling. An impaired activity of the NO system has been recognised as a major contributory factor underlying endothelial dysfunction, an early event in the atherogenic process and an important contributor to the clinical expression of atherosclerosis (Bonetti *et al*., 2003).

An accurate assessment of endothelial function is important in linking pathophysiology with clinical conditions. In humans, endothelial function can be assessed biochemically by dosing different markers (adhesion molecules, cytokines and prostanoids) or functionally (Deanfield *et al*., 2007). The most common method to evaluate the endothelial functional capacity is the use of flow studies to test vasomotor reactivity. It is performed by measuring the degree of endothelium-dependent dilatation with respect to the basal value, using stimuli that increase production of endothelium-derived NO. This can be evaluated *in vitro* (isolated arteries) and *in vivo,* either in response to receptor-dependent agonists or to changes in flow in the forearm, coronary or peripheral circulation. *In vivo* methods can be either non-invasive or invasive, and allow the study of blood vessels in their physiological environment.

### 1.5.2 *In vivo* non-invasive assessment

As already mentioned, the endothelium responds to shear stress by releasing NO, with consequent vasodilatation. One of the most commonly used tests to assess endothelial function non-invasively is based on this principle. It involves measurement of post-ischaemic endothelium-dependent vasodilatation modulated by flow (flow-mediated vasodilatation, FMD), and it is usually performed on

conductance arteries, such as the brachial artery. Post-ischaemic dilatation is caused by a 5-minute distal ischaemia obtained by cuff occlusion of the radial or brachial artery (reactive hyperaemia). The vasodilatation obtained with this technique is quantified by measuring the change in the arterial diameter in the post-ischaemic period with high-resolution ultrasonography. This endothelium-dependent vasodilatation is then compared with the vasodilatation produced by drugs that are NO donors, such as sublingual nitroglycerine, and for this reason termed 'endothelium-independent' vasodilatation (Corretti *et al*., 2002; Deanfield *et al*., 2007). This technique, although non-invasive, is technically demanding and requires appropriate training.

### 1.5.3 *In vivo* invasive assessment

These methods evaluate the endothelial function of arteries by measuring changes in their diameter (ultrasonography) or volume (plethysmography) after infusion in the coronary or peripheral circulation of endothelium receptor-dependent agonists (acetylcholine, substance P) or increased blood flow. One of the most commonly used receptor agonist is acetylcholine (ACh), which relaxes human vessels by stimulating the release of NO. Several studies of endothelial vasomotor function have used intra-arterial infusion of ACh into a local vascular bed to evoke NO-dependent vasodilatation, and an impaired blood flow response, compared with the response caused by an endothelium-independent NO donor such as sodium nitroprusside (SNP), has been taken as evidence of endothelial dysfunction. Studies performed by catheterization of coronary arteries and infusion of ACh measured the percentage of vasodilatation obtained (either by quantitative angiography or Doppler ultrasound), and found that ACh produced paradoxical vasoconstriction when infused in coronary arteries with atherosclerotic lesions (Ludmer *et al*., 1986; Vita *et al*., 1990). This response has been attributed to the direct vasoconstrictor action of ACh on smooth muscle cells, a response not present in a functional endothelium, and denotes endothelial dysfunction.

### *1.5.3.1 Venous occlusion plethysmography technique*

In the peripheral circulation, the forearm vascular bed has been extensively used to evaluate endothelial vasomotor function, because it is the only readily accessible vascular bed that allows the study of resistance vessels. These studies involve infusion into the brachial artery of different compounds, and the response obtained is then measured using the venous occlusion plethysmography technique. This approach is considered *minimally* invasive because is performed in a more accessible vascular bed, and the technique can be used to assess the effect of various endogenous ligands and drugs on blood vessels *in vivo*, to examine dose-response relationships and, when coupled with administration of endothelium receptor-dependent agonists, to assess endothelial function. This technique involves cannulation of the brachial artery, followed by the infusion of specific agonists or antagonists at doses that are systemically ineffective but result in changes in the local forearm circulation. The principle is simple: venous outflow, but not arterial inflow, is obstructed from the arm, resulting in a proportional change in the volume of the forearm, which is detected by strain gauges and translated into changes in forearm blood flow (Benjamin *et al*., 1995; Wilkinson & Webb, 2001).

The main advantage of the venous plethysmography technique is that drugs are infused locally, via the brachial artery, and are effective only within the circulation of the upper limb. This is because forearm blood flow is approximately 50ml/min, compared with a cardiac output of approximately 5000ml/min and, therefore, doses 100-1000 fold lower than those active systemically can be used. For the same reason, the amount of drug reaching the systemic circulation is insignificant and does not influence systemic haemodynamics, which allows the study of the direct vascular actions of the drugs infused without confounding factors. This technique has been extensively used and is considered one of the 'gold-standards' in the assessment of vascular function in resistance arteries (Joyner *et al*., 2001; Wilkinson & Webb, 2001).

### 1.5.4 Venous occlusion plethysmography and exercise

Exercise is the most common factor to determine a substantial increase in shear stress in conduit arteries *in vivo,* which results in increased arterial blood flow. Venous occlusion plethysmography has been widely used to elucidate the mechanisms responsible for the blood flow response to exercise in humans (Joyner *et al*., 2001). Several studies using this technique have shown that blood flow to exercising muscles can increase 10- to 20-fold and have investigated the role of substances such as adenosine, NO and prostaglandins on exercise-induced vasodilatation (Kilbom & Wennmalm, 1976; Dyke *et al*., 1995; Hellsten *et al*., 1998).

### 1.5.5 Endothelial dysfunction and hypertension

Endothelial dysfunction has been demonstrated in patients with several risk factors for CVD, such as hypercholesterolaemia (Chowienczyk *et al*., 1992), diabetes (Calver *et al*., 1992), and smoking (Celermajer *et al*., 1993). Current evidence suggests that an impaired endothelium-dependent vasomotor function is predictive of cardiovascular events, and its value is independent of other, well-established, risk factors. Although the forearm vascular bed is not a target for atherosclerosis, a number of studies have shown that impaired endothelial function in the forearm circulation is an independent predictor for cardiovascular events (Heitzer *et al*., 2001; Perticone *et al*., 2001; Fichtlscherer *et al*., 2004). In hypertension, a large number of studies (Panza *et al*., 1990; Endemann & Schiffrin, 2004; Brunner *et al*., 2005) with few exceptions (Cockcroft *et al*., 1994), have demonstrated the presence of endothelial dysfunction, as evidenced by reduced NO-mediated vasodilatation at the level of both conduit and resistance arteries (Panza *et al*., 1990; Park *et al*., 2001; Lind, 2006) and in the coronary circulation (Hasdai & Lerman, 1999). The reduced NO-mediated vasodilatation observed in hypertension not only affects the response to pharmacological agonists (such as ACh) but may also limit vascular responsiveness to shear stress, particularly during exercise. This contributes to the increase/maintenance of SVR and underperfusion of exercising muscles and may ultimately affect exercise capacity. In support of this hypothesis there is evidence

showing that hypertensive patients have a reduced vasodilator response to handgrip exercise (McEniery *et al*., 2002).

The concept of endothelial dysfunction as part of the causal pathway in the pathogenesis of atherosclerosis is increasingly accepted, and strategies to reverse impaired endothelial function are currently being investigated, targeting the early, preclinical phase of the disease. Table 1.3 presents a summary of the most important studies evaluating the predictive value of endothelial dysfunction.

| Author, Year | Vascular Bed | No. of subjects | Follow-up | Events, *N* |
|---|---|---|---|---|
| Suwaidi *et al,* 2000 | Coronary | 157 | 2.4 y | 6 |
| Schachinger *et al,* 2000 | Coronary | 147 | 7.7 y | 28 |
| Halcox *et al*, 2002 | Coronary | 308 | 4 y | 35 |
| Targoski *et al*, 2003 | Coronary | 503 | 1.4 y | 25 |
| Perticone *et al*, 2001 | FBF | 225 | 2.5 y | 29 |
| Heitzer *et al*, 2001 | FBF | 281 | 4.5 y | 91 |
| Fichtlscherer *et al*, 2004 | FBF | 198 | 4 y | 31 |
| Neunteufl *et al*, 2000 | FMD | 73 | 5 y | 27 |
| Gokce *et al*, 2002 | FMD | 187 | 30 d | 45 |
| Modena *et al*, 2002 | FMD | 400 | 5.7 y | 47 |
| Gokce *et al*, 2003 | FMD | 199 | 1.2 y | 35 |
| Katz *et al,* 2005 | FMD | 149 | 2.4 y | 17 |
| Patti *et al,* 2005 | FMD | 136 | 6 m | 20 |
| Jeboah *et al,* 2007 | FMD | 2792 | 5 y | 674 |

**Table 1.3. Summary of studies evaluating the predictive value of endothelial dysfunction.**
y, years; m, months; d, days.

### 1.5.6 Effects of PDE5 inhibition on endothelial function

Given the important role played by PDEs in the modulation of vascular smooth muscle cells relaxation through the NO-cGMP pathway, PDE5 inhibition may be expected to influence this response. In particular, when testing endothelium-dependent vasomotor function, prolongation of cGMP signalling through PDE5 inhibition might improve endothelium-dependent vasodilatation. This hypothesis has been tested in a number of studies, both in healthy subjects and patients with impaired endothelial function. In healthy subjects, PDE5 inhibition with sildenafil does not appear to influence endothelium-dependent vasodilatation (Dishy *et al*., 2001; Halcox *et al*., 2002; Guazzi *et al*., 2004b). In patients with heart failure, sildenafil has been shown to improve endothelial vasomotor function (Katz *et al*., 2000; Guazzi *et al*., 2004b; Hryniewicz *et al*., 2005), although this response was not observed in a study by Robinson and coworkers, performed in patients with coronary artery disease (Robinson *et al*., 2006). Previously, Halcox and coworkers had reported an improved coronary response to ACh with sildenafil, which was more pronounced in patients with coronary heart disease, while the response to verapamil was unaffected. They also studied the effect of sildenafil on brachial FMD and found that, while peak response was unchanged, sildenafil prolonged post-reactive hyperaemia vasodilatation (Halcox *et al*., 2002). The evidence is also conflicting in healthy smokers, in which two studies have reported an improved endothelial vasomotor function (Kimura *et al*., 2003; Vlachopoulos *et al*., 2004), but no improvement was reported in another study (Dishy *et al*., 2004). With regard to the other two PDE5 inhibitors, tadalafil and vardenafil, less evidence is available. Tadalafil has been shown to improve endothelial vasomotor function in patients with increased cardiovascular risk (Rosano *et al*., 2005). Acute administration of vardenafil seems also to improve brachial FMD in men with erectile dysfunction (Mazo *et al*., 2006).

## 1.6 ARTERIAL STIFFNESS AND PDE5 INHIBITION

### 1.6.1 Pulse pressure, arterial distensibility and wave reflection

In the past decade, a number of epidemiological studies have focused on the importance of systolic BP, and pulse pressure in particular, as more adequate markers of cardiovascular risk, whereas, in the past, the focus was on diastolic BP (Black, 1999). In fact, it was not until 1980 that data from the Framingham Heart Study evidenced the association between increased systolic pressure and high cardiovascular risk (Kannel *et al*., 1980). Both diastolic and systolic BP increase with age but, while this increase is continuous for systolic BP, diastolic BP tends to plateau or even decrease after the middle age (Vokonas *et al*., 1988). For this reason, isolated systolic hypertension (ISH) is the most common subtype of hypertension found in middle-age and in the elderly (Chobanian, 2007). ISH is associated with increased pulse pressure and both are associated with a marked increase in cardiovascular and total mortality (Alli *et al*., 1999). Furthermore, in the elderly in particular, pulse pressure has been found to be the best predictor of coronary heart disease (Franklin *et al*., 1999).

In hypertension, in addition to increased peripheral resistance, abnormalities of the large arteries also play an important role. Pulsatile BP consists of two components, mean arterial pressure (MAP), the steady component, and pulse pressure (PP), the pulsatile component. MAP is the product of cardiac output multiplied by vascular resistance, while PP is the difference between systolic and diastolic pressure and is determined by cardiac and vascular factors. The main vascular determinants of PP are arterial distensibility and timing and intensity of arterial wave reflection. In the vasculature, large arteries act not only as conduits for the blood but also play an important role as cushions, buffering the pressure wave generated with each ventricular contraction and smoothing the pulsatile blood flow as it travels towards the peripheral tissues (Nichols & O'Rourke, 1998). This is possible because of one important property of the arterial wall, vascular elasticity, whose study began as early as the 19$^{th}$ century (Roy, 1881), but was properly introduced to the scientific community in 1905, when Otto Frank published a model of the arterial tree based on

the concept of the *windkessel* (air chamber). This theory considers the vascular system as an elastic reservoir: during systole, the elastic components of the large arteries store part of the energy and volume which are then released during diastole (Frank, 1905). Despite its simplicity, this model is still very useful to understand the mechanical properties of the walls of the large arteries.

The elasticity of the arterial wall is the result of two different components: the elastin and collagen fibres, the latter much less distensible than the former. The wall of large proximal arteries has a high elastin to collagen ratio, which makes them distensible, but the situation changes in the peripheral vasculature, where the content of collagen progressively increases, making the arteries stiffer (or less distensible). The elastic properties of the arteries are also linked to the distending pressure within the vessel: at low pressure only the elastic fibres are stretched and therefore the arterial wall is more distensible, whereas at higher pressure the collagen fibres are also recruited and the wall is stiffer (Roach & Burton, 1957). With increasing age, the composition of the arterial wall of large arteries changes due to the progressive decrease in elastin content, and the arteries become stiffer (Learoyd & Taylor, 1966). The other important vascular determinant of PP is arterial wave reflection. The contraction of the left ventricle generates a pressure wave that travels along the arterial tree at a given velocity (pulse wave velocity, PWV). This wave is reflected at points of discontinuity, mainly represented by the primary and secondary branches of the aorta and by high-resistance arterioles, and travels back towards the aorta, generating a secondary wave that adds up to the incident wave (Figure 1.3). The timing of reflection is determined by the distance to the reflection site and is influenced by PWV and aortic length. Stiffness of the arteries and reflection sites are also major determinants of the final shape of this wave, which shows a characteristic pattern of amplification as it travels from the heart to the periphery, leading to increased systolic pressure and pulse pressure in the peripheral arteries (Nichols & O'Rourke, 1998). However, this amplification decreases with age, a phenomenon mainly related to stiffening of the large arteries. In fact, in adults younger than 50 years, in whom arteries are distensible and the PWV is low, the reflected wave is observed in diastole and results in increased diastolic pressure and coronary perfusion, whereas

systolic and pulse pressure are not affected (Kroeker & Wood, 1955). In individuals older than 50 years of age, stiffer arteries and increased PWV result in early return of the reflected wave, which is observed in systole, determining a rapid increase in systolic aortic pressure and loss of pulse pressure amplification. This affects central haemodynamics, leading to increased left ventricular load and reduced coronary perfusion pressure during diastole (O'Rourke, 1995).

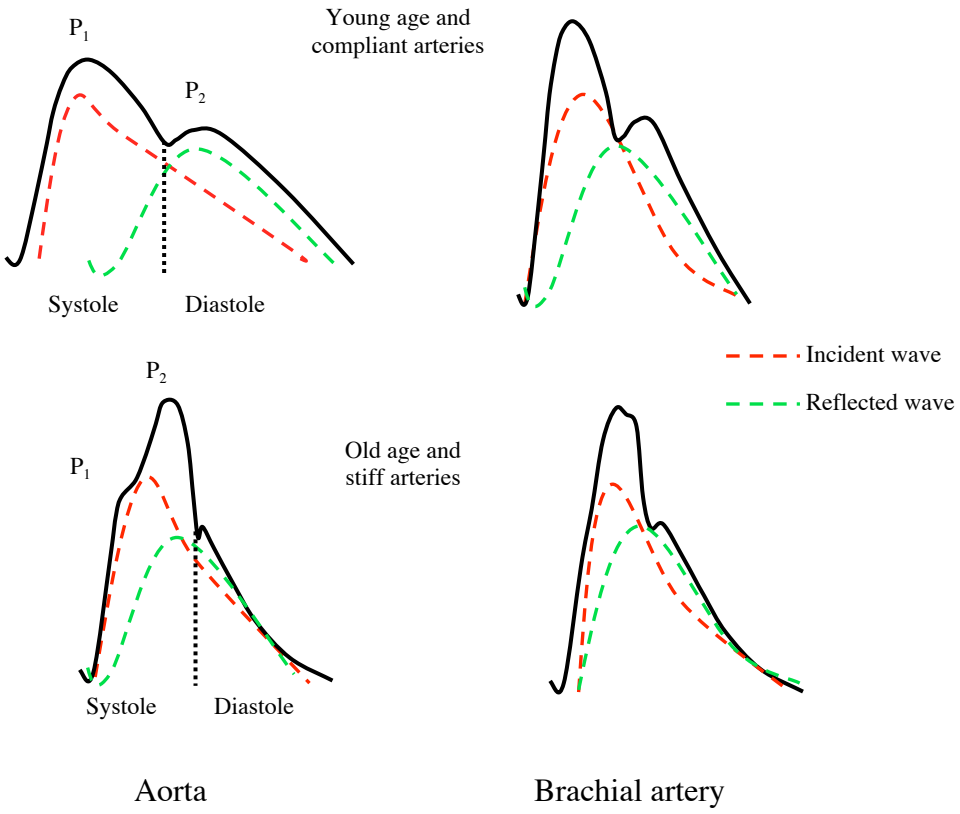


**Figure 1.3. Pressure waveforms at the aorta and brachial artery in young and old subjects.**
The pressure waveforms are composed of a forward travelling wave (incident wave, red dotted line) generated with each heartbeat, and a reflected wave (green dotted line), both of which determine the shape of the final measured wave (thick black line) in the aorta and in peripheral arteries such as the brachial artery. The incident wave is responsible for the initial systolic pressure wave $P_1$, whereas the reflected wave generates the reflected pressure wave $P_2$. In young subjects (top panel) with compliant arteries and low PWV, the reflection of the systolic wave takes place in diastole. In old subjects (lower panel) or subjects with hypertension, with stiff arteries and higher PWV, reflection occurs earlier and adds to the incident wave in systole, thereby increasing central systolic pressure and reducing diastolic pressure.

### 1.6.2 Arterial stiffness: methods of measurement

Vascular stiffening can affect cardiovascular function and, therefore, have clinical implications. For these reasons, a number of different methodologies have been introduced in an attempt to measure arterial stiffness and to assess its impact on cardiovascular prognosis. Evaluation of large artery stiffness can be done invasively with ultrasound or catheter tip manometers (Stefanadis *et al*., 1995), but non-invasive methods have been developed, and are more suitable for human studies. Of the three major non-invasive methodologies currently available, i.e. estimation of distending pressure and artery diameter change, measurement of PWV and analysis of the arterial pulse pressure waveform, the last two are now widely used in clinical research (Oliver & Webb, 2003).

#### *1.6.2.1 Measurement of pulse wave velocity*

It is well established that PWV is related to the elastic properties of the arterial wall and increases with stiffness of the arteries. It can be defined by the Moens-Korteweg equation:

$$PWV^2 = E \cdot h / 2\rho \cdot R$$

where h is the arterial wall thickness, R is the internal radius, ρ is the blood viscosity and E is the Young elastic modulus of the wall, which reflects the arterial wall properties (Nichols & O'Rourke, 1998). PWV can also be expressed by the Bramwell-Hill equation:

$$PWV^2 = \Delta P \cdot V / \Delta V \cdot \rho$$

where ΔP and ΔV represent changes in pressure and volume, V is the volume at baseline and ρ is the blood viscosity (Bramwell & Hill, 1922). In practice, PWV is measured as the distance travelled by the pulse between two recording sites divided by the time needed by the wave to travel from one site to the other (length/time), and determined by the delay between corresponding points, such as the foot of the 2 waves. The waves recorded between the two sites can be collected simultaneously or sequentially, using high-fidelity manometers. Commonly, estimation of the distance

covered by the incident wave is performed by superficial measurements between the carotid and the femoral artery, and measurement of PWV obtained between these two sites (CF-PWV) is now considered the "gold-standard" (Laurent *et al*., 2006). Validations studies have shown that automatic measurement of PWV are accurate and reproducible (Asmar *et al*., 1995).

Major determinants of large artery stiffness are age, systolic BP (Kelly *et al*., 1989a; Mitchell *et al*., 2004) and sex (London *et al*., 1995), and values of PWV in healthy adults generally range from 5m/s to 7m/s (Blacher & Safar, 2005; Kullo & Malik, 2007). Increased PWV is found in association with a number of cardiovascular risk factors (Lehmann *et al*., 1998), including hypercholesterolaemia (Lehmann *et al*., 1992b) and diabetes (Lehmann *et al*., 1992a), and PWV is now widely recognised as an important predictor of cardiovascular outcome. One of the landmark studies showing the predictive value of PWV was performed by Blacher and coworkers. In a cohort of patients with end-stage renal failure (ESRF), they showed that individuals with PWV values less than 9.4m/s were still alive at the end of the follow-up, but not those with PWV values greater than 12m/s (Blacher *et al*., 1999). These findings were then confirmed in three population-based studies, the Baltimore study (Sutton-Tyrrell *et al*., 2005), the Rotterdam study (Mattace-Raso *et al*., 2006) and the Copenhagen study (Willum-Hansen *et al*., 2006), demonstrating that PWV is an independent predictor of cardiovascular outcome in normal populations. Furthermore, the Copenhagen study showed that PWV, after adjusting for other factors, is a better predictor of outcome than 24-hour ambulatory BP monitoring.

In hypertension, PWV has been shown to be an independent predictor of all-cause and cardiovascular mortality (Laurent *et al*., 2001), and to predict primary coronary events (Boutouyrie *et al*., 2002) and stroke (Laurent *et al*., 2003). Increased PWV has also been found associated with manifestations of cerebral small-vessel disease in hypertensive patients (Henskens *et al*., 2008). Measurement of PWV has been proposed as part of the cardiovascular risk assessment and is present in the 2007 European Society of Hypertension guidelines (Mancia *et al*., 2007).

### *1.6.2.2 Measurement of pulse wave reflection*

As previously mentioned, when arteries stiffen, the return of the reflected wave is observed in systole, not in diastole, reaching the heart when the aortic valve is still open and thus resulting in a secondary rise in central BP. This increase is expressed as the augmentation index (AIx, %) or the augmentation pressure (AP, mmHg). The AIx is commonly used as a measure of wave reflection in arterial stiffness, and is calculated as the ratio between AP and PP (Figure 1.4). It is influenced by both the amplitude and the timing of wave reflection, increases linearly with mean arterial pressure (Wilkinson *et al*., 2001), and is inversely related to heart rate (Wilkinson *et al*., 2000; Gatzka *et al*., 2001). Central BP and AIx are important because they determine cardiac workload (Murgo *et al*., 1980; Nichols *et al*., 1985), and AIx determined invasively has been shown to be predictive of coronary disease (Hayashi *et al*., 2002). Invasive measurements are not applicable in routine practice, therefore methods to measure central haemodynamics non-invasively have been developed. Estimation of the AIx can be obtained at the carotid artery, as a surrogate of the aortic AIx (Chen *et al*., 1996), or at the radial artery, using applanation tonometry to obtain a radial waveform and radial AIx (RAIx) (Kelly *et al*., 1989b). From this, a generalised transfer function generates a central aortic waveform, central BP and central AIx (CAIx) (Chen *et al*., 1997). This technique is known as pulse wave analysis (PWA) and the principle of the transfer function is very simple. The waveform recorded at the radial artery is broken down into harmonics and then, using Fourier analysis, it is reconstituted to provide the central waveform (O'Rourke & Gallagher, 1996; Pauca *et al*., 2001). Pauca and coworkers validated the use of the transfer function in a prospective study, showing the close agreement between measured and estimated central pressure.

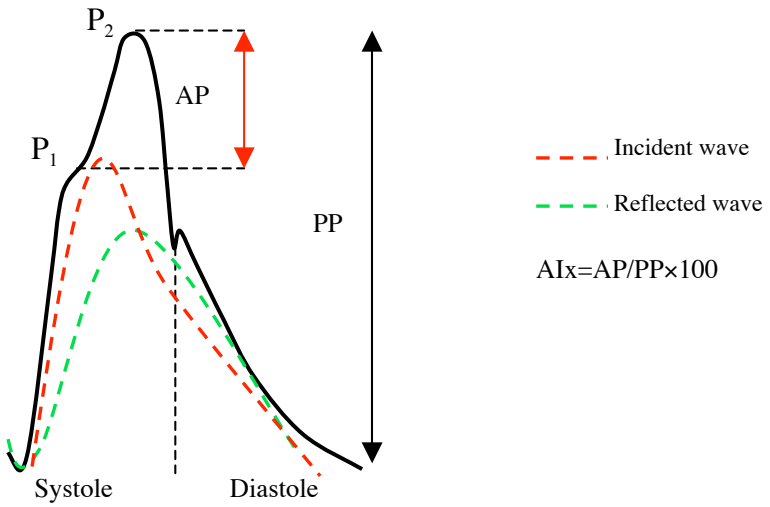


**Figure 1.4. Schematic representation of pulse pressure amplification**
The initial systolic pressure wave ($P_1$) generated by the incident wave is responsible for peak systolic BP. In stiff arteries, the reflected pressure waves ($P_2$), occurring in systole, augments systolic BP, (augmentation pressure, AP), and the magnitude of this increase can be quantified by the augmentation index (AIx), which represents the ratio between the AP and the pulse pressure (PP).

The validity and utility of the transfer function have been questioned, and studies have reported that its use underestimates aortic systolic pressure (Davies *et al*., 2003) and that the CAIx derived with this approach can differ substantially from the one obtained directly with invasive measurements (Hope *et al*., 2003). Others have also suggested that information about the central pressure can be obtained directly from the radial pressure, with no need for a transfer function (Millasseau *et al*., 2003). Studies investigating the relationship between AIx and cardiovascular risk have collected data with and without the transfer function. In a cohort of patients with ESRF, London and coworkers showed that increased AIx at the carotid artery, obtained without the transfer function, was an independent predictor of all-cause and cardiovascular mortality (London *et al*., 2001). In a subsequent, cross-sectional, study, Nurnberger and coworkers measured AIx, using the transfer function, in a cohort of 219 subjects with and without cardiovascular disease, and showed that increased AIx is a marker of cardiovascular risk (Nurnberger *et al*., 2002). In another cross-sectional study, Weber and coworkers found the AIx to be a strong, independent predictor of premature coronary disease (Weber *et al*., 2004). These

findings suggest that CAIx determined non-invasively, with or without the use of a transfer function, offers additional, prognostic information on cardiovascular risk.

The SphygmoCor® system (AtCor Medical Pty Ltd, Sydney, Australia) is a commercially available device for PWA and incorporates a generalised transfer function. Studies presented in this thesis used the SphygmoCor to derive central haemodynamics and CAIx.

### 1.6.3 Arterial stiffness and cardiovascular drugs

The independent predictive value of arterial stiffness for cardiovascular morbidity as well as cardiovascular and all-cause mortality has prompted investigation on the effects of different drug regimens on this parameter, but it still has to be demonstrated that reduction of arterial stiffness translates into reduction of cardiovascular risk. Several studies have evaluated the effect of antihypertensive treatment on PWV, but it is important to consider that any BP decrease is likely to result in decreased PWV, and that the length of the treatment is also likely to affect PWV in different ways. Available evidence suggests that ACEIs reduce PWV (Asmar *et al*., 1988; Lacourciere *et al*., 2004) and AIx (Jiang *et al*., 2007), and seem to be more effective than CCBs or ARBs, despite equal BP reduction (Rajzer *et al*., 2003). In turn, CCBs seem to be more effective than thiazide diuretics. In a cross-over study comparing the CCB felodipine with hydrochlorothiazide, the former significantly reduced PWV, while the latter did not have any detectable effect (Asmar *et al*., 1993). The effect of beta-blockers appears to be variable, depending on the agent used. Overall, they seem to reduce PWV but the effects are less consistent with regard to arterial wave reflection (Asmar *et al*., 2001; Dhakam *et al*., 2008). In this respect, results from two outcome trials have been recently published, the CAFE study (a substudy of ASCOT) and a substudy of the ANBP2. The ASCOT study compared two very different BP lowering regimens: one based on atenolol ± thiazide, the other based on amlodipine ± perindopril. The latter regimen was more effective at reducing stroke and all-cause mortality than the atenolol-based regimen, despite equal reduction of brachial BP (Dahlof *et al*., 2005). The CAFE study analysed the effects of the two different drug regimens on central BP, and showed

that the amlodipine-based regimen was more effective than the atenolol-based regimen at lowering central BP (Williams *et al*., 2006). However, these results conflict with those reported in a substudy of the ANBP2. This trial showed a better prognosis in hypertensive individuals treated with an ACEI than with a diuretic, despite same BP reduction at the brachial artery (Wing *et al*., 2003), but no difference was found in central BP between the two regimens (Dart *et al*., 2007). The techniques used to evaluate central haemodynamics in the two studies were different; in addition, patients in the ANBP2 were older (mean age 72 yrs *vs* 62 yrs in the CAFE study), and vascular aging is a major determinant of wave reflection. Furthermore, heart rate response to treatment was different in the CAFE study, mainly because of atenolol, which slows heart rate and results in earlier wave reflection. Clearly, more long-term studies are needed to evaluate the impact of arterial stiffness reduction on cardiovascular outcomes.

### 1.6.4 Arterial stiffness and PDE5 inhibition

The effects of PDE5 inhibition on arterial stiffness have been examined following acute and chronic administration. In patients with coronary heart disease sildenafil acutely reduced PWV (Vlachopoulos *et al*., 2003), and the same effect was observed in patients with heart failure (Hirata *et al*., 2005). In the former study, sildenafil reduced PWV by 0.65 m/s, CAIx by 4.4% and central BP by 6.7 mmHg. In the study by Hirata and coworkers, PWV was reduced by 0.8 m/s and AIx by 3.6%. In both studies these effects appeared to be independent of BP reductions. In a small study performed in subjects with erectile dysfunction, acute administration of sildenafil reduced PWV, but this effect was likely to be related to concomitant BP reduction (Shigemura *et al*., 2006). Acute and chronic effects of sildenafil on arterial stiffness were also investigated in a study by Oliver and coworkers. Sildenafil administration acutely reduced arterial wave reflection and central BP, an effect that might be related to the reduction in peripheral vascular resistance. A similar, smaller effect, was observed after chronic administration (16 days), but this was not different from placebo. Neither acute nor chronic effects of sildenafil on PWV and FMD were reported in the study, although there was a trend toward a progressive reduction (Oliver *et al*., 2006).

## 1.7 ENDOTHELIUM AND ARTERIAL STIFFNESS

Many studies have established a role for endothelial dysfunction in the development of the structural and functional alterations of the hypertensive vascular wall. In particular, available evidence suggests that the endothelium, in part through the release of NO, is an important regulator of arterial stiffness (Kinlay *et al*., 2001; Wilkinson *et al*., 2002a; Wilkinson *et al*., 2002c; Schmitt *et al*., 2005). More recently, endothelial function has been shown to be inversely related to PWV and AIx (McEniery *et al*., 2006). In addition, conditions associated with endothelial dysfunction are also associated with increased arterial stiffness (Cruickshank *et al*., 2002; Wilkinson *et al*., 2002b; Mahmud & Feely, 2003), including hypertension (Ceravolo *et al*., 2003), suggesting that impairment of the NO-cGMP system may be a common denominator. In particular, in hypertension, impaired endothelial vasomotor function may be a key element linking high exercise systolic BP and low exercise capacity with the high risk of future cardiovascular events exhibited by hypertensive patients.

## 1.8 RESEARCH HYPOTHESES AND AIMS

The favourable effects shown by PDE5 inhibition in the cardiovascular field justify a further and more complete exploration of the therapeutic potential related to enhanced cGMP signalling. In particular, in hypertension, a therapeutic strategy directly aimed at improving exercise capacity might offer additional benefits in terms of cardiovascular outcomes and contribute to preventing physical decline, resulting in improved quality of life. If impaired vascular function limits exercise capacity, then PDE5 inhibition, in view of its effects on the NO-cGMP pathway in the vasculature, might influence vascular responsiveness to exercise. Indeed, the potential improvement in vasodilator response to shear stress through enhanced cGMP signalling may set the stage for improved exercise capacity in hypertensive patients.

In this thesis, the following hypotheses will be addressed:

1. The reduced forearm blood flow response to exercise observed in hypertensive patients will be reversed by PDE5 inhibition with sildenafil but not by a control vasodilator.
2. The reduced exercise capacity and exaggerated exercise systolic BP response observed in hypertensive patients will be reversed by PDE5 inhibition with sildenafil but not by a control vasodilator.
3. Parameters of arterial stiffness in hypertensive patients will be improved by PDE5 inhibition with sildenafil but not by a control vasodilator.

These hypotheses will be investigated in two clinical studies performed in hypertensive patients with local limb and whole body exercise, aiming at the evaluation of the effects of PDE5 inhibition on vascular function and exercise capacity in arterial hypertension.

# CHAPTER 2

## METHODS

## 2.1 GENERAL REQUIREMENTS

### 2.1.1 Subjects and study environment

Participants were asked to abstain from alcohol for at least 24 hours and from tea, coffee or caffeine-containing beverages and food for 12 hours before study visits. Studies were conducted in rooms kept at temperature between 22ºC and 24ºC.

#### *2.1.1.1 Identification*

Suitable hypertensive patients were identified from the Western General Hospital (WGH) Cardiovascular Risk Clinic database, and healthy volunteers were identified from the existing Clinical Research Centre (CRC) community database.

##### *2.1.1.1.1 Inclusion criteria*

- Hypertensive subjects
    - Male
    - Aged between 20 and 70 years
    - At least 3 separate office measurements of systolic BP ≥160mmHg (maximum 180mmHg) and/or diastolic BP ≥90mmHg
    - Not on treatment

- Normotensive subjects
    - Male
    - Healthy
    - Aged between 20 and 70 years
    - Systolic BP ≤140mmHg and diastolic BP ≤80mmHg

##### *2.1.1.1.2 Exclusion criteria (all subjects)*

- Female
- History of coronary artery, cerebrovascular or peripheral vascular disease
- Total cholesterol >6.5 mmol/L
- Body mass index (BMI) ≥30 $kg/m^2$
- Current alcohol abuse

- Diabetes mellitus
- Asthma
- Smoking
- Taking any vasoactive or endothelial function modifying drugs which cannot be withdrawn for the purpose of the study
- Previous serious drug allergy
- ECG evidence of clinically significant arrhythmia, cardiac ischaemia or left ventricular hypertrophy (LVH)
- Evidence of secondary hypertension
- Clinically significant abnormality on screening blood test
- Contraindication to strenuous exercise
- Presence of other clinically relevant conditions.

Hypertensive subjects and healthy normotensive controls were matched for age and cholesterol values.

### 2.1.2 Research governance and ethics

All studies were approved by the Lothian Research Ethics Committee and performed in accordance with the Declaration of Helsinki of the World Medical Association. Each participant provided signed evidence of informed consent before entry to the study.

## 2.2 METHODOLOGIES

### 2.2.1 Blood pressure and heart rate

Resting systolic and diastolic BP and heart rate (HR) were recorded, with an appropriate sized cuff, using a validated oscillometric sphygmomanometer, the Omron HEM-705CP (Omron Healthcare Ltd, Milton Keynes, UK) (O'Brien *et al*., 1996).

Exercise BP was measured using the Tango+ exercise BP monitor (SunTech Medical Instruments, NC, USA), which has been previously validated and provides reliable

automatic BP measurements during exercise (Cameron *et al*., 2004; Myers *et al*., 2009). This device is synchronized with an ECG and integrates the signals from the blood pressure cuff, which contains a microphone able to distinguish the Korotkoff sounds from artefact noise, with the subject's ECG R-wave. Mean arterial pressure values were obtained during PWA analysis recordings using the SphygmoCor apparatus (AtCor Medical Pty Ltd, West Ride, Australia).

### 2.2.2 PWA and CF-PWV

#### *2.2.2.1 PWA*

Peripheral pressure waveforms were obtained at the radial artery of the dominant arm using a pencil-shaped probe connected with the SphygmoCor apparatus (Figure 2.1). The probe incorporates a high fidelity micromanometer (SPC-301; Millar Instruments, Houston, Texas, US), based on the principle of applanation tonometry, and interfaced with a laptop computer running the SpygmoCor software version 7.1. The SphygmoCor apparatus allows continuous on-line recordings of the radial artery waveform, and the last 10 seconds of each recording were averaged and used to calculate the radial AIx (RAIx) and to generate the corresponding central (ascending aorta) waveforms. The RAIx is calculated as:

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

Once the central waveform is generated, central parameters such as central systolic and diastolic BP, central PP, CAIx and CAIx@75 (CAIx adjusted to a standard HR of 75bpm) are also calculated.

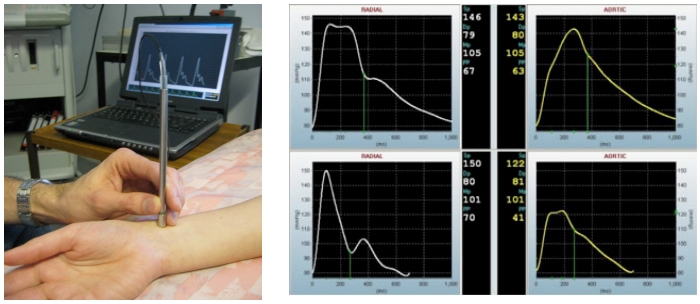

**Figure 2.1. PWA measurement at the radial artery.**
The radial artery waveform is recorded at the wrist (left panel) and, with the use of a validated transfer function, the corresponding central aortic waveform is generated (right panel).

#### *2.2.2.2 CF-PWV*

The SpygmoCor was also used to measure CF-PWV using the foot-to-foot method (the foot of the wave is identified by the software as the beginning of the sharp systolic upstroke). Path length was estimated from the distance between the sternal notch and the carotid and femoral artery, measured over the skin surface with a tape. During continuous ECG monitoring, pressure waveforms were recorded sequentially at the carotid and femoral artery, and the time delay between the feet of the pulse waves recorded at the two different sites is measured by subtraction of the two time intervals $\Delta T = T2 - T1$. T1 is the time interval measured between the ECG signal (R wave) and the foot of the proximal (carotid) wave, and T2 is the time interval measured between the ECG signal and the foot of the distal (femoral) wave. The distance travelled by the pulse wave is the CF-PWV and is calculated as:

$$\text{CF - PWV} = \frac{\text{Distance}}{\text{Transit time}}$$

and expressed as meters per second (m/s).

### 2.2.3 Forearm blood flow studies

These studies were performed combining the technique of venous occlusion plethysmography with intra-arterial (brachial) administration of vasoactive drugs (sildenafil and verapamil). Handgrip exercise was used to evoke forearm active hyperaemia and arterial vasodilatation in the forearm was measured as change in forearm blood flow (FBF).

#### *2.2.3.1 Venous occlusion plethysmography*

Upon arrival, subjects rested supine in a quiet, temperature-controlled room (22-24ºC), with both arms elevated above the heart level by resting the elbows on foam pads and supporting the hands with pillows. Blood pressure cuffs were placed around the upper arms and the wrists. The upper cuffs were intermittently inflated to 40mmHg for 10 seconds in every 15 seconds to temporarily prevent forearm venous outflow and obtain plethysmographic recordings. The hand was excluded from the blood flow determination through inflation of the wrist cuff above arterial pressure (220mmHg). This is because hand blood flow is predominantly through skin blood

vessels rather than skeletal muscle and has different control mechanisms than FBF. Rapid cuff inflation was obtained using an air source coupled with two cuff inflators (Hokanson E20 Inc., Bellevue, USA). An additional blood pressure cuff was placed over the noninfused arm to obtain BP recordings at the end of each set of measurements. After selecting the appropriate size, strain gauges (Hokanson Inc., Bellevue, USA) were securely placed around the widest part of the forearm and calibrated to the chart recorder software program (PowerLab Chart 5, version 5.1, ADInstruments Ltd, Chalgrove, UK). Blood flow was measured simultaneously in both arms by use of a dual-channel strain gauge plethysmograph (EC4 plethysmograph, Hokanson Inc., Bellevue, USA). Briefly, rapid proximal cuff inflation above venous pressure, but below arterial diastolic pressure, stops venous outflow abruptly, causing an increase in limb volume due to arterial inflow. As the volume of the limb changes, and thus arm circumference, the strain-gauge is "stretched" and the electrical resistance increases: this information is recorded by the plethysmograph and then processed and displayed as a waveform (Figure 2.2). FBF recordings were made every 10 minutes over a 3-minute period, and the mean of the final 5 measurements was used for analysis. Plethysmographic data were first extracted from Chart data files and then FBF values were calculated using a template spreadsheet (Excel 5.0; Microsoft). Blood flow responses to exercise and vasodilators were expressed as changes in absolute blood flow per unit volume of forearm (ml/min/100ml of forearm volume) in the infused arm. FBF measurements obtained immediately before handgrip exercise and drug infusion were used as baseline.

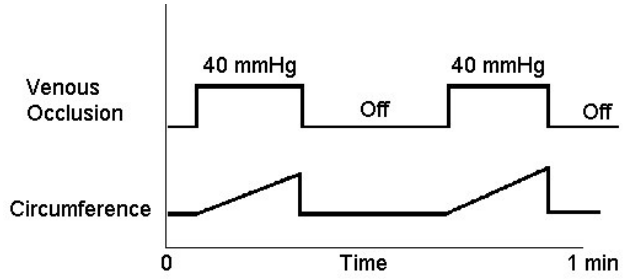


**Figure 2.2. Schematic representation of changes in limb circumference following venous occlusion.**

### *2.2.3.2 Intra-arterial drug administration*

The brachial artery of the non-dominant arm was cannulated under local anaesthesia (1% lidocaine, Hameln pharmaceuticals gmbh, Hameln, Germany) with a 27-SWG needle (Coopers Needle Works, Birmingham, UK) connected to a 16G epidural catheter for drug infusion (Portex Ltd, Hythe, Kent, UK) (Figure 2.3). Patency was maintained by normal saline (Baxter Healthcare Ltd. Thetford, UK), and the infusion rate kept constant at 1ml/min by means of constant rate infusion pumps (Asena, Alaris Medical UK Ltd, Basingstoke, UK). The intra-arterial needle was well tolerated and none of the subjects reported bleeding or haematoma formation following the cannulation. The noninfused arm was used as a control during the studies, to exclude a systemic drug effect and to take into account minor changes in blood flow occurring as result of stress or changes in temperature that affect both arms.

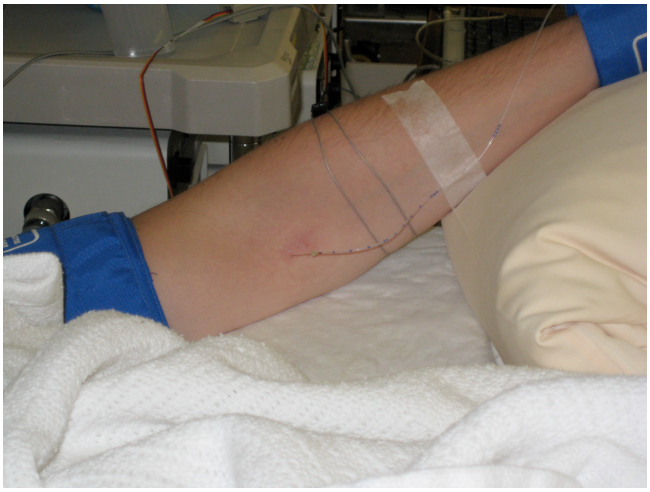

**Figure 2.3. Arterial needle and cannula for drug infusion**

### *2.2.3.3 Drugs*

All intra-arterial drugs were dissolved in 0.9% physiological saline on the day of the study, and different concentrations were prepared by serial dilution and infused at a constant rate of 1 ml/min. Drug infusion was always preceded by a 30-minute saline infusion, to allow stabilisation of FBF after the insertion of the intra-arterial needle and baseline FBF recordings. Sildenafil citrate (Pfizer Ltd. Sandwich, Kent, UK) was infused at 50 μg/min; the cGMP-independent control vasodilator verapamil (Abbott

Laboratories Ltd, Queenborough, Kent, UK) was infused at 5 μg/min; normal saline was infused during control, non-vasodilating studies. Drugs were infused for 6 minutes at 1ml/min; the doses of sildenafil and verapamil used in the study were selected on the basis of previously published literature (Robinson *et al*., 1982; Millgard & Lind, 1998; Jackson *et al*., 1999) and confirmed in preliminary dose-ranging studies performed in healthy subjects (Chapter 3).

## 2.3 EXERCISE TESTS

### 2.3.1 Handgrip exercise

The handgrip task was performed with a calibrated handgrip dynamometer (MLT 003 Hand Dynamometer ADInstruments Pty Ltd), following a previously validated method to evoke active forearm hyperaemia (Longhurst *et al*., 1974). Subjects rhythmically squeezed the device using the non-dominant arm in 15-second cycles, consisting of 5 seconds of steady handgrip pressure alternating with 10 seconds of rest, during which FBF measurements were taken.

### 2.3.2 Dundee Step test

The Dundee step test is a single stage, light, exercise test, consisting of each subject stepping up and down (step height 17.5 cm) for 3 minutes, at a stepping rate of 92 per minute, set using a metronome. Exercise BP was measured with the Tango+ immediately before and after 3 minutes of step testing. This test is simple and reproducible, and its low exercise intensity is similar to the activities of daily living (Lim *et al*., 1998).

### 2.3.3 Cardiopulmonary exercise testing

These studies were performed at the Wellcome Trust Clinical Research Facility (WTCRF) at the WGH. During the preliminary screening visit all subjects had the opportunity to familarise themselves with the exercise equipment and performed a practice run. Incremental cardiopulmonary exercise testing (CPET) with assessment of respiratory gas exchange was performed using an upright bicycle ergometer (Lode, Rehcor, Groningen, The Netherlands). Continuous 12-lead ECG monitoring

(CardioDirect 12, Reynolds Medical Ltd, Hertford, UK) was performed during each test for assessment of heart rate, arrhythmias and myocardial ischaemia. BP was measured with the Tango+ immediately before exercise and during the last 30 seconds of each exercise stage. After 2 minutes of unloaded pedalling, workload was increased by 20 watts per minute (added at the start of each minute) to maximal exercise tolerance. Subjects were asked to look at the rpm meter and to maintain a constant pedalling rate of 60rpm throughout the exercise test. In the absence of chest pain, ECG abnormalities, arrhythmias or critical BP changes, all tests were continued until exhaustion.

| **Indication for exercise termination** |
|---|
| • Ischaemic ECG changes |
| • Chest pain |
| • Arrhythmias |
| • Fall in systolic BP > 20mmHg |
| • Systolic BP > 250mmHg and/or diastolic BP > 120mmHg |
| • Dizziness or faintness |
| • Unable to maintain a cycling rate above 40rpm |

#### *2.3.3.1 Gas exchange variables*

Respiratory gas exchange variables were measured continuously during the unloaded cycling period and the exercise test using the Pulmolab EX670 mass spectrometer gas analyser (Morgan Medical Ltd, Kent, UK). This breath-by-breath system measures airflow continuously and calculates oxygen uptake ($VO_2$) and carbon dioxide output ($VCO_2$) during each breath. Subjects wore a nose clip and breathed through a low resistance mouthpiece assembly that monitors the composition and flow rate of the breath (Figure 2.4 and Figure 2.5).

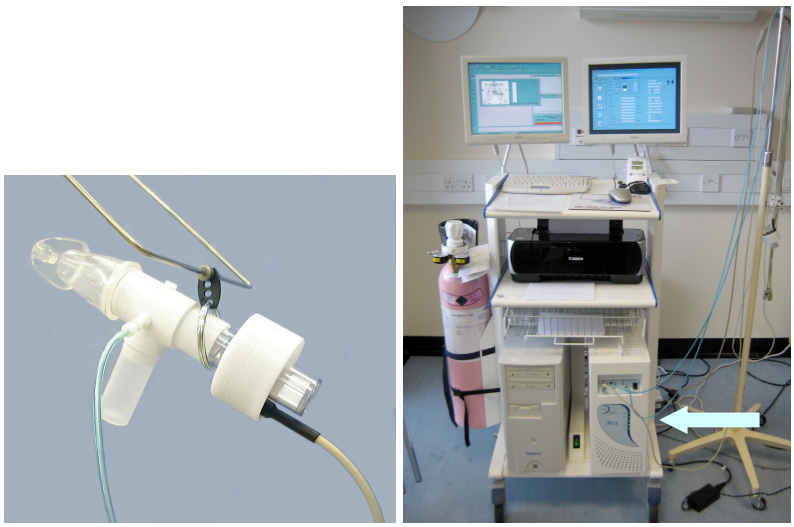

**Figure 2.4. Gas analyser and mouthpiece assembly.**
The respiratory flow rate is measured with a turbine device connected to the end of the mouthpiece (left), and a capillary tube transports the sample from the mouthpiece to the analyser (right).

The sample is drawn from the mouth into the analyser and subjected to ionisation by an electron beam. The ions formed, representing the gases, are projected into the influence of a magnetic field and form currents with different directions, which are then collected by detectors and measured. The system was calibrated prior to each test for barometric pressure, temperature and gas concentration using gases of known concentration ($O_2$ 14.99%, $CO_2$ 5%, Linde Gas UK Ltd, Aberdeen, Scotland). A 3L calibration syringe was used for calibration of flow rate. Calibration stability was monitored over the duration of the studies. $VO_2$ and $VCO_2$ acquired on a breath-by-breath basis were interpolated second-by-second and then averaged over 30-second intervals to reduce the variability of breath-by-breath measurements (Sue *et al.*, 1980). Peak $VO_2$ was defined as the highest 30-second average of oxygen uptake in the last minute of exercise. The anaerobic threshold (AT) during incremental exercise was determined by the V-slope method. When the slopes of $VCO_2$ and $VO_2$ are plotted together this method allows the determination of the break-point, i.e. the point at which $VCO_2$ increases faster than $VO_2$ and departs from a line with a slope equal to 1.00 (Beaver *et al.*, 1986).

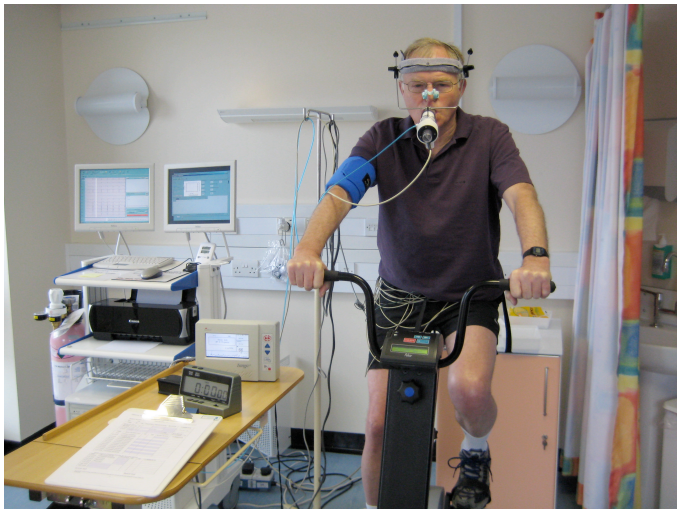

**Figure 2.5. Cardiopulmonary exercise testing**

Repeatability of exercise data was confirmed in 6 healthy subjects in whom the study protocol was repeated 14 days apart. These data are presented under "Methodology development" (Chapter 3).

## 2.4 SCREENING BLOOD SAMPLES

Blood samples were taken from subjects for full blood count, serum urea, creatinine, sodium, potassium, lipid profile and plasma glucose. Samples were analysed by the WGH Haematology and Biochemistry laboratories.

## 2.5 ASSESSMENT OF LEFT VENTRICULAR HYPERTROPHY

To evaluate the presence of left ventricular hypertrophy (LVH) during the preliminary visit, the Cornell voltage criteria were used (LVH present if the sum of the R-wave in lead aVL and the S wave in lead V3 was greater than 25mm) (Casale *et al*., 1987).

## 2.6 DRUGS

Sildenafil citrate 50 mg and matched placebo tablets were obtained from Pfizer Ltd. Sandwich, Kent, UK, and administered three times daily. Hydralazine 25 mg (Alpharma, Barnstaple, Devon, UK) was administered as a control, cGMP-independent vasodilator (Mulvihill-Wilson *et al*., 1985) and obtained through the

WGH pharmacy. Hydralazine has been previously used in studies involving healthy subjects and hypertensive patients (Mulvihill-Wilson *et al*., 1985; Tomlinson *et al*., 1988) and, after oral administration, peak plasma concentration is reached within 30 to 90 minutes (median 60 minutes) (Shepherd *et al*., 1980), showing a time to peak effect similar to sildenafil (Muirhead *et al*., 2002). On the basis of previously published literature, the doses of hydralazine and sildenafil selected for these studies were expected to result in similar BP reductions (Fagan *et al*., 1984; Oliver *et al*., 2006).

## 2.7 DATA ANALYSIS

Methods used to analyse data differed between studies and 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.

Test-retest repeatability of two measurements was assessed according to the method described by Bland and Altman (Bland & Altman, 1986). Agreement between variables obtained during the two tests was examined by plotting the differences between the individual measurements against their mean value (Bland-Altman plots); bias was calculated as the mean difference between measurements during the two tests. The limits of agreement for variables between the first and second test were calculated as bias ± 1.96 SDs, and approximately 95% of the differences are expected to fall within this range. 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.

# CHAPTER 3

## METHODOLOGY DEVELOPMENT

## 3.1 CHAPTER STRUCTURE

Two methodology development studies were performed and are presented in this chapter. The first consists of a series of venous occlusion plethysmography studies aimed at the investigation of the time course effect of intrabrachial sildenafil and selection of an appropriate control vasodilator; the repeatability of the forearm vasodilator response to local exercise was also investigated. The second study was designed to investigate the repeatability of peak $VO_2$ at maximal exercise and of arterial stiffness parameters before and after maximal exercise testing.

## 3.2 TIME COURSE EFFECTS OF INTRA-ARTERIAL SILDENAFIL ON FOREARM BLOOD FLOW AND COMPARISON WITH A CONTROL VASODILATOR

### 3.2.1 Background

There is a paucity of published data on the effects of intra-arterial sildenafil on forearm blood flow (FBF). In the only available study, Jackson and coworkers investigated the effects of increasing doses of intrabrachial sildenafil on FBF (from 3μg/min up to 300μg/min), and reported a dose-dependent vasodilator effect on resistance vessels. The highest dose used in this study (300μg/min) resulted in local FBF concentrations of approximately 10μg/ml, which is >10 times the plasma concentration present after the highest recommended oral dose (100mg) (Jackson *et al*., 1999). Because PDE1 and PDE5 are both present in vascular smooth muscle, and sildenafil is only moderately selective for PDE5 over PDE1 (>80 fold) (Wallis *et al*., 1999), it is possible that concentrations this high might result in loss of selectivity for PDE5. For this reason, it was decided to investigate the vasodilator effects of a lower dose of sildenafil (50 μg/min), to avoid loss of selectivity for PDE5, and to evaluate the duration of such effect on FBF. It was also important to identify an appropriate vasodilator that could be used as control for sildenafil in subsequent plethysmography studies. As discussed in the introduction, the vasodilator actions of sildenafil are mediated by the NO-cGMP pathway (see section 1.1.3), and it was therefore essential to choose a control dilator whose effect was cGMP-independent. Verapamil hydrochloride is a calcium ion influx inhibitor (slow-channel blocker) that exerts its pharmacologic effects by modulating the influx of ionic calcium across the cell membrane of arterial smooth muscle and myocardial contractile cells. In particular, verapamil targets the L-type calcium channel, which is the dominant type in cardiac and smooth muscle and is known to contain several drug receptors. Verapamil acts from the inner side of the membrane and binding of the drug reduces the frequency of opening in response to depolarization. The result is a marked decrease in transmembrane calcium current, resulting in long-lasting relaxation of smooth muscle cells (Antman *et al*., 1980). Previously published data indicate that the vasodilator effect of verapamil is cGMP-independent (Millgard & Lind, 1998)

and it was decided to use it as control vasodilator for sildenafil in the studies presented.

### 3.2.2 Aims

The aims of this study were to:

1. Characterize, in healthy subjects, the time course of the intrabrachial effects of sildenafil on FBF
2. Compare the forearm effects of sildenafil with different doses of verapamil, and select a dose of verapamil that produced a comparable vasodilator effect to that of sildenafil on FBF
3. Evaluate within day and between days test-retest repeatability of FBF data during handgrip exercise.

### 3.2.3 Methods

#### *3.2.3.1 Subjects*

*3.2.3.1.1 Identification*

Suitable subjects were identified from a database of healthy subjects at the Clinical Research Centre (CRC).

*3.2.3.1.2 Inclusion criteria*

- Healthy
- Male
- Aged 20 to 70 years

*3.2.3.1.3 Exclusion criteria*

- Female
- History of coronary artery, cerebrovascular or peripheral vascular disease
- Total cholesterol >6.5 mmol/L
- Current alcohol abuse
- Diabetes mellitus
- Asthma
- Smoking

- Body mass index (BMI) ≥30 kg/$m^2$
- Taking any vasoactive or endothelial function modifying drugs which cannot be withdrawn for the purpose of the study
- Previous serious drug allergy
- Clinically significant abnormality on screening blood test
- Presence of other clinically relevant conditions.

#### *3.2.3.2 Arterial cannulation and drug infusions*

The brachial artery of the non-dominant arm of each subject was cannulated under local anaesthesia (1% lidocaine, Hameln pharmaceuticals gmbh, Hameln, Germany) with a 27-SWG needle (Coopers Needle Works, Birmingham UK) attached to a 16G epidural catheter (Portex Ltd, Hythe, Kent, UK) for drug infusions. Sildenafil citrate (Pfizer Ltd. Sandwich, Kent, UK) was infused at 50 μg/min; the cGMP-independent control vasodilator verapamil (Abbott Laboratories Ltd. Queenborough, Kent, UK) was infused at different doses (1.25, 2.5, 5 and 10 μg/min); normal saline (Baxter Healthcare Ltd. Thetford, UK) was used a placebo. Each dose was infused for 6 minutes at 1ml/min.

#### *3.2.3.3 Measurements*

##### *3.2.3.3.1 Forearm venous plethysmography*

Details of the methodology are described in the method section (see 2.2.3.1). Briefly, arterial vasodilatation in the forearm was measured as change in FBF using venous occlusion plethysmography, with mercury-in-silastic strain gauges securely applied around the widest part of the forearm. The hand was excluded from the blood flow determination through inflation of a wrist cuff to 220mmHg. An upper arm cuff was intermittently inflated to 40mmHg for 10 seconds in every 15 seconds to temporarily prevent forearm venous outflow and obtain plethysmographic recordings. Forearm blood flow recordings were made over a 3-minute period. The mean of the final 5 measurements was used for analysis. Blood flow was measured simultaneously in both arms by use of a dual-channel strain gauge plethysmograph.

*3.2.3.3.2 Blood pressure*

Blood pressure was monitored, with an appropriate sized cuff, in the noninfused arm by use of a validated oscillometric sphygmomanometer (HEM-705CP, Omron Corporation) (O'Brien *et al*., 1996). Blood pressure measurements were taken after FBF recordings, to avoid any effect due to the venous congestion caused by inflation of the BP cuff.

### 3.2.4 Data analysis

Plethysmographic data were extracted from Chart data files and FBF calculated for each subject using a template spreadsheet (Excel 5.0; Microsoft). Recordings made in the first minute after wrist cuff inflation were excluded from the analysis because this results in transient vasoconstriction (Kerslake, 1949). Forearm blood flow data are presented as changes in absolute blood flow (ml/min/100ml of forearm volume) in the infused arm, as the intra-subject variability of the response to vasodilators is significantly reduced when presented as absolute values of FBF than when analysed as percentage change in the FBF ratio (Wilkinson & Webb, 2001). In part I, given the number of subjects (3 subjects receiving sildenafil and 3 subjects receiving verapamil), formal statistical analysis was not considered appropriate. In part II data were analysed with repeated measures ANOVA and 2-tailed Student's *t*-test as appropriate. Statistical analysis was performed with Graph-Pad Prism (GraphPad Software, Inc, San Diego, CA). Significance was accepted at the 5% level in all cases.

Test-retest repeatability of baseline and exercise FBF data were assessed according to the method described by Bland and Altman (Bland & Altman, 1986) (see section 2.7).

### 3.2.5 Protocol

All subjects attended a preliminary screening visit, when a fasting blood sample was taken for routine biochemistry. They then attended the CRC at 9am on 6 different occasions at least one week apart, and after an overnight fast (>12 hours). They were required to abstain from alcohol and caffeine-containing food and beverages from 24

hours before the study. All visits were performed in a quiet, draught-free, temperature-controlled room (22-24 ºC). On each study day, subjects rested quietly for 30 minutes and during this time FBF measurements were recorded. The measurement period immediately before the infusion of drugs or the handgrip exercise was always used as baseline.

### 3.2.6 Part I

This was a one-way, single-blind, randomised study, undertaken to investigate the FBF effect of a single dose of sildenafil and the FBF responses to incremental doses of verapamil. Six healthy subjects were recruited for this study and randomised to sildenafil (3 subjects) or verapamil (3 subjects). On the study day, after cannulation of the brachial artery and baseline FBF measurement, subjects randomised to sildenafil received an intrabrachial infusion of the drug (50μg/min) for 6 minutes, with FBF recordings during the last 3 minutes of infusion. The infusion was then stopped and FBF recorded every 10 minutes for the following 60 minutes. Subjects randomised to verapamil received an intrabrachial infusion of the drug at 4 consecutive and incremental doses (1.25, 2.5, 5 and 10 μg/min), each for 6 minutes. The infusion was then stopped and FBF recorded every 10 minutes for the following 60 minutes.

#### *3.2.6.1 Results*

The clinical characteristics of the study subjects are shown in Table 3.1. Blood flow responses to sildenafil 50μg/min for each of the 3 subjects are shown in Figure 3.1A. Sildenafil infusion for 6 minutes resulted in a significant increase in FBF, still evident at 10 minutes after the end of the infusion. Blood flow then gradually returned to baseline levels by 60 minutes after the end of the infusion. Blood flow responses to incremental doses of verapamil for each of the 3 subjects are shown in Figure 3.1B. Verapamil infused at 5 and 10μg/min resulted in a significant increase in FBF, which was still present at 10 minutes after the end of the infusions, and then blood flow gradually returned to baseline values by the end of the study. Sildenafil 50μg/min and verapamil 5μg/min showed a similar vasodilator effect on FBF, and it was decided to use this dose of verapamil in part II.

| Parameters | Subjects (n=6) |
|---|---|
| Age (years) | 35±5 (range 25-52) |
| BMI (kg/m$^2$) | 24.4±0.7 |
| Total cholesterol (mmol/l) | 4.3±0.3 |
| Systolic BP (mmHg) | 122±2.7 |
| Diastolic BP (mmHg) | 73±3.9 |

**Table 3.1. Clinical characteristics of the study subjects.**
BMI, body mass index; BP, blood pressure.

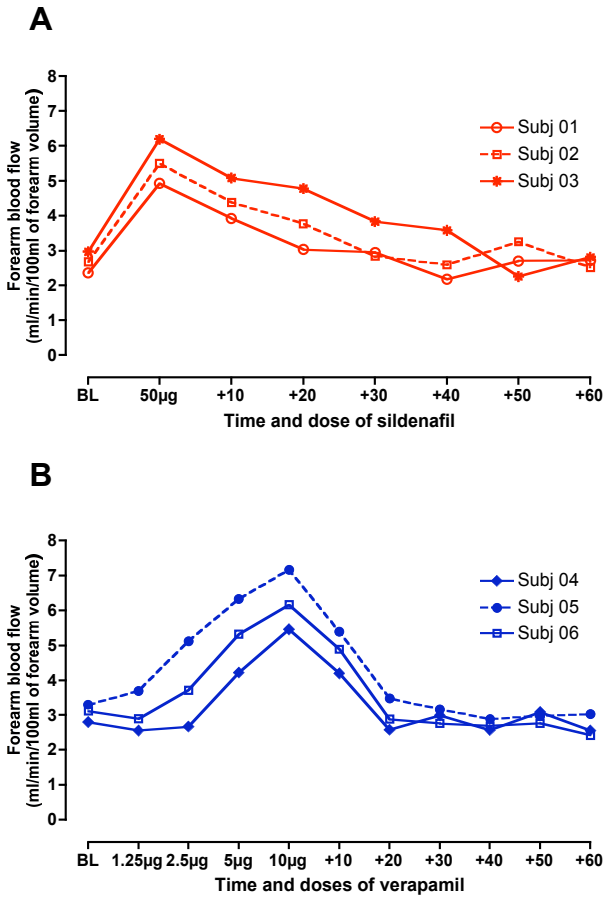


**Figure 3.1. Vasodilator responses to sildenafil and verapamil.**
Changes in absolute blood flow (ml/min/100 ml of forearm volume) in the infused arm of 3 subjects in response to the intrabrachial infusion of sildenafil 50µg/min (A) and in response to incremental doses of verapamil (1.25, 2.5, 5 and 10µg/min) (B). BL, baseline.

### 3.2.7 Part II

On the basis of the results obtained in part I, a three-way, randomised, single-blind and placebo-controlled study was undertaken to compare the vasodilator effects of sildenafil and verapamil. Normal saline was infused during a control, non-vasodilating study. The six subjects recruited for part I attended 3 further study visits, at least one week apart. On each occasion, after cannulation of the brachial artery and baseline FBF measurement, they received, in random order, a 6-minute infusion of sildenafil (50µg/min), verapamil (5µg/min) or placebo (saline), with FBF recordings made during the last 3 minutes of infusion.

#### *3.2.7.1 Results*

The effects of the 3 intra-arterial infusions on FBF are shown in Figure 3.2 and Table 3.2. Baseline FBF was not significantly different on each of the study visits, and the infusion of saline did not affect FBF. Both sildenafil and verapamil significantly increased FBF in the infused arm (P<0.001), and the extent of vasodilatation was similar between the two drugs. This effect was still significant at 10 minutes after the end of the infusion (P<0.05), and then gradually disappeared. None of the subjects reported adverse effects related to the local infusion of the study drugs. Blood pressure and heart rate did not change after any of the infusions (Table 3.3).

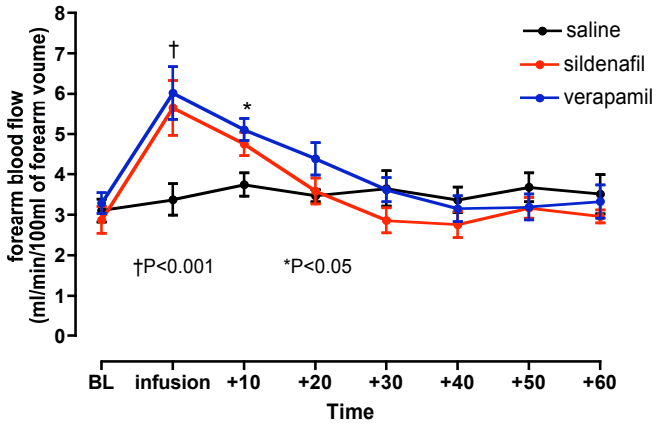


**Figure 3.2. Vasodilator responses to saline, sildenafil and verapamil.**
Changes in absolute blood flow (ml/min/100 ml of forearm volume) in the infused arm in response to the intrabrachial infusion of saline, sildenafil 50µg/min and verapamil 5µg/min. Data are mean ± SEM. BL, baseline.

| Subjects (N=6) | Infused arm | Noninfused arm | Ratio |
|---|---|---|---|
| ***Before infusion*** | | | |
| Saline | 3.27±0.63 | 3.73±0.67 | 0.89±0.07 |
| Sildenafil | 3.37±0.77 | 3.63±0.56 | 0.90±0.04 |
| Verapamil | 3.45±0.47 | 4.05±0.77 | 0.85±0.10 |
| ***During infusion*** | | | |
| Saline | 3.47±0.61 | 3.75±0.60 | 0.90±0.07 |
| Sildenafil | 5.64±0.68* | 3.33±0.57 | 1.79±0.21 |
| Verapamil | 6.18±0.78* | 4.17±0.66 | 1.52±0.13 |

**Table 3.2. Forearm blood flow values before and during infusions.**
Data are mean±SEM absolute FBF (ml/min/100ml of forearm volume) in the infused and noninfused arm, and FBF ratio (infused/noninfused) before and during the infusion of saline, sildenafil and verapamil. *P<0.001 infused *vs* noninfused arm.

| Subjects (n=6) | SBP | DBP | MAP | HR |
|---|---|---|---|---|
| ***Before infusion*** | | | | |
| Saline | 124±3 | 65±2 | 84±3 | 63±4 |
| Sildenafil | 125±5 | 66±2 | 86±4 | 65±2 |
| Verapamil | 123±5 | 69±3 | 87±3 | 66±3 |
| ***After infusion*** | | | | |
| Saline | 126±5 | 68±3 | 87±4 | 67±3 |
| Sildenafil | 128±3 | 62±5 | 86±2 | 62±2 |
| Verapamil | 125±5 | 71±2 | 91±3 | 65±3 |

**Table 3.3. Blood pressure and heart rate before and after the infusion of saline, sildenafil and verapamil.**
SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

### 3.2.8 Part III

The purpose of this part of the study was to evaluate, for each subject, within-day repeatability of baseline FBF data and repeatability of the forearm vasodilator response to handgrip exercise within and between days. Subjects (n=6) attended the CRC on 2 different occasions: during the first visit they performed the handgrip exercise twice on the same day (with a 30-minute rest in between), and then returned after 1 week to repeat the task (between days repeatability). The handgrip exercise was performed with a calibrated handgrip dynamometer (MLT 003 Hand Dynamometer ADInstruments Pty Ltd). Subjects rhythmically squeezed the device using the nondominant arm in 15-second cycles, consisting of 5 seconds of steady handgrip pressure alternating with 10 seconds of rest, and they were instructed to avoid Valsalva-like manoeuvres during the task. The exercise was performed for 5 minutes at 45% of maximum voluntary contraction (MVC), which was determined for each subject during the initial screening visit. Forearm blood flow was recorded in the last 3 minutes of exercise, during the 10-second period of relaxation between contractions, immediately before the next handgrip contraction.

#### *3.2.8.1 Results*

There was good repeatability for baseline and handgrip exercise FBF data measured at two different time points during the same study day. In the same way, no significant difference was observed between the mean FBF responses to handgrip exercise performed on two different study days (Figure 3.3).

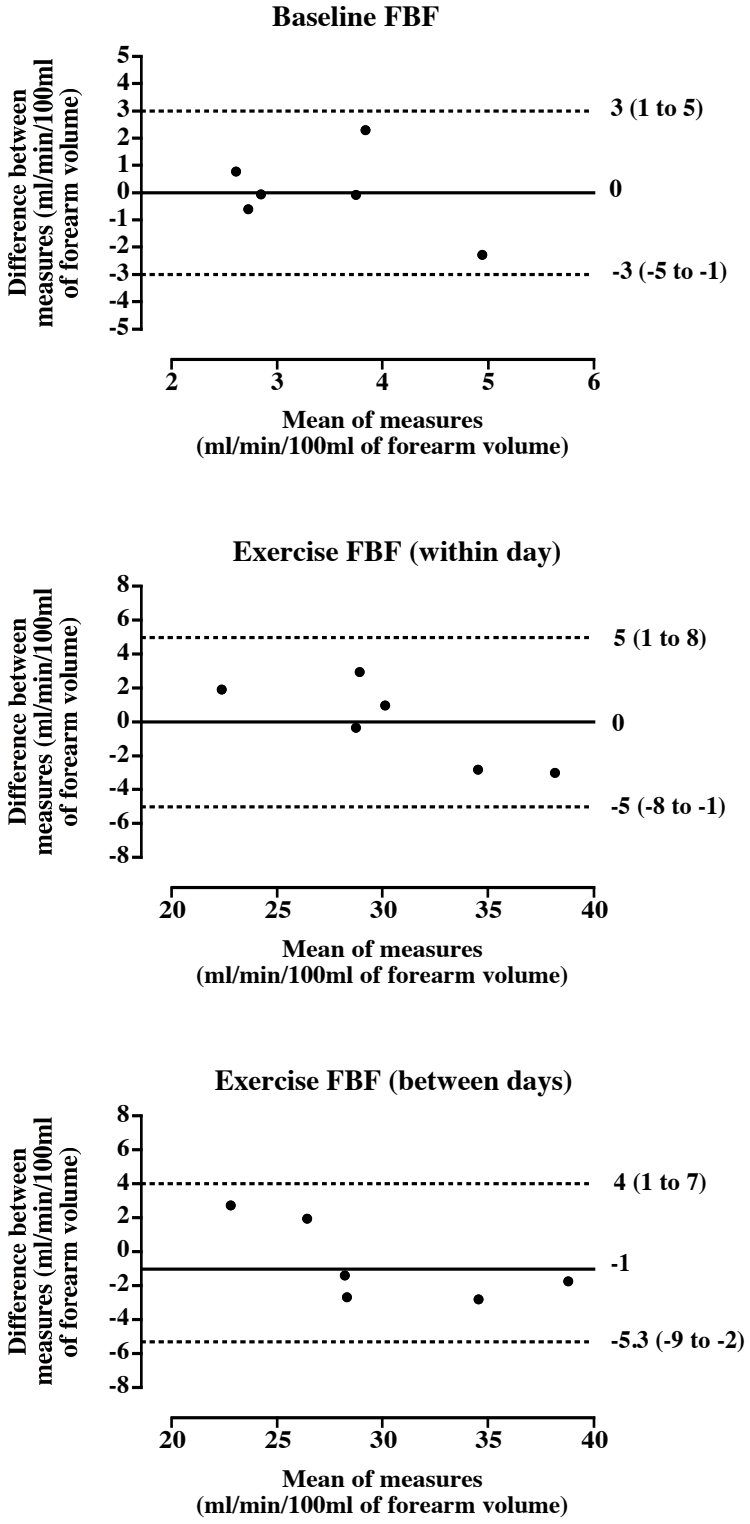


**Figure 3.3. Bland-Altman plots of baseline and exercise FBF data.**
Solid lines are mean differences and dotted lines are upper and lower 95% limits of agreement (for which 95% CIs are given in brackets).

### 3.2.9 Discussion

#### *3.2.9.1 Part I and II*

The present studies showed that a 6-minute intra-arterial infusion of sildenafil at 50μg/min results in a significant vasodilator effect on FBF. A comparable vasodilator effect was evoked by the calcium channel blocker verapamil infused at 5μg/min. On the basis of these results, this dose of verapamil was selected and used as a control for sildenafil in the subsequent plethysmography studies presented in this thesis.

The vasodilator effects of sildenafil have been investigated in vitro and in vivo. In vitro, sildenafil has been shown to potentiate NO-cGMP signalling leading to smooth muscle relaxation in human corpus cavernosum tissue (Ballard *et al*., 1998; Moreland *et al*., 1998), and to enhance the vasorelaxant effect of a NO donor, glyceryl trinitrate, in isolated aortic rings (Wallis *et al*., 1999). In the only available study in which the intrabrachial effects of sildenafil were investigated, Jackson and coworkers reported a dose-dependent vasodilator effect of the drug on FBF in healthy subjects (Jackson *et al*., 1999), in agreement with findings presented here. The present studies also showed that the effect of sildenafil is still significant 10 minutes after the end of the infusion, after which time FBF gradually returns to baseline values.

With respect to verapamil, available evidence indicates that its vasodilator effects are cGMP-independent (Millgard & Lind, 1998; Xu *et al*., 2002), and it has been extensively used in studies involving the use of a control, endothelium-independent vasodilator (Dawes *et al*., 1997; Mills *et al*., 2005; Mills *et al*., 2007). For these reasons, verapamil was chosen as a control vasodilator for sildenafil in these preliminary studies. It was also necessary to select a dose of verapamil that produced a similar effect to that of sildenafil on FBF. This is because the response to any intervention that follows drug infusions is affected by the pre-existing values of FBF (Wilkinson & Webb, 2001) and, if the two vasodilators have different effects on FBF, comparisons would not be valid. Results obtained in part I showed that verapamil, infused at 5μg/min, had a vasodilator effect on FBF similar to that of

sildenafil. It was therefore decided to directly compare verapamil 5μg/min to sildenafil 50μg/min in part II of the study, whose results confirmed that the two drugs, at the doses selected, produced a similar vasodilator effect on FBF. Furthermore, the results presented show that, at the doses selected, sildenafil and verapamil produced significant changes in FBF without affecting systemic blood pressure.

#### *3.2.9.2 Part III*

As shown by the Bland-Altman plots presented, there was good repeatability of FBF baseline measurements, with no tendency for repeatability to vary with mean values. The Bland-Altman plots also show good within day and between days repeatability of the FBF response to handgrip exercise. The repeatability of baseline and exercise FBF data compare well with previously published data (Roberts *et al*., 1986).

### 3.2.10 Summary

The preliminary forearm plethysmography studies presented in this section show that sildenafil, at a dose of 50μg/min, produces a significant increase in FBF that is still significant at 10 minutes after the end of the infusion. Verapamil, infused at 5μg/min, produced a comparable vasodilator effect to that of sildenafil 50 μg/min on FBF.

## 3.3 REPEATABILITY OF PEAK OXYGEN UPTAKE DURING MAXIMAL EXERCISE AND OF PARAMETERS OF ARTERIAL STIFFNESS BEFORE AND AFTER EXERCISE

### 3.3.1 Background

Repeatability values of exercise variables are necessary for a valid interpretation of the results of exercise testing and to evaluate the effect of a treatment on these parameters. Biological and technological variation can be responsible for changes in these variables when the same subject performs a test on two or more occasions. Factors such as physiological circadian variations and environmental conditions, both examples of biological variation, can influence exercise performance (Kung *et al*., 1980; Atkinson & Reilly, 1996). In addition, variation can be the result of differences in equipments and laboratories (technological variation) (Jones & Kane, 1979; Bloch *et al*., 1995). With respect to arterial stiffness, the repeatability of measures obtained using pulse wave velocity and analysis techniques has been shown to be high (Wilkinson *et al*., 1998), but it was important to assess the repeatability of such measures before and after maximal exercise testing.

### 3.3.2 Aims

The aims of this study were to:

1. Investigate test-retest repeatability of peak oxygen uptake (peak $VO_2$) during maximal exercise testing
2. Investigate test-retest repeatability of arterial stiffness parameters before and after maximal exercise testing.

### 3.3.3 Methods

#### *3.3.3.1 Subjects*

##### *3.3.3.1.1 Identification*

Suitable subjects were identified from a database of healthy subjects who had previously taken part in research at the CRC.

*3.3.3.1.2 Inclusion criteria*

- Healthy
- Male
- Aged 20 to 70 years

*3.3.3.1.3 Exclusion criteria*

- Female
- History of coronary artery, cerebrovascular or peripheral vascular disease
- Total cholesterol >6.5 mmol/L
- Current alcohol abuse
- Diabetes mellitus
- Asthma
- Smoking
- Body mass index (BMI) ≥30 kg/$m^2$
- Regular exercise training
- Taking any vasoactive or endothelial function modifying drugs which cannot be withdrawn for the purpose of the study
- Previous serious drug allergy
- Clinically significant abnormality on screening blood test
- Presence of other clinically relevant conditions.

***3.3.3.2 Measurements***

Details of the methodology are described in the methods section (see 2.2.2 and 2.3.3). Briefly, exercise capacity was assessed through maximal incremental exercise testing on an electromagnetically braked cycle ergometer (Lode, Rehcor, Groningen, The Netherlands), and measured as peak $VO_2$. Breath-by-breath gas analysis was performed using the Pulmolab EX670 mass spectrometer gas analyser (Morgan Medical Ltd, Kent, UK), connected to a personal computer running analysis software. From this peak $VO_2$, defined as the highest 30-second average of oxygen uptake in the last minute of exercise, was derived. Exercise BP was measured immediately before exercise and every 2 minutes during exercise using an ECG-gated auscultatory device (Tango+ exercise BP monitor, SunTech Medical

Instruments, NC, USA). Continuous 12-lead ECG monitoring (CardioDirect 12, Reynolds Medical Ltd, Hertford, UK) was performed during each test for assessment of heart rate (HR), arrhythmias and myocardial ischemia.

The SphygmoCor apparatus (AtCor Medical Pty Ltd, West Ride, Australia) was used to measure CF-PWV and for PWA. During arterial stiffness measurements, clinic BP and HR were monitored, with an appropriate sized cuff, using a validated oscillometric sphygmomanometer (HEM-705CP, Omron Corporation) (O'Brien *et al*., 1996).

### 3.3.4 Protocol

Subjects attended the unit at 9am on 3 different occasions (1 preliminary visit and 2 study visits 14 days apart) and after an overnight fast (>12 hours). They were required to abstain from alcohol and caffeine-containing food and beverages from 24 hours before each study. All studies were performed in a quiet, draught-free, temperature-controlled room (22-24 ºC). During the initial preliminary visit all subjects had the opportunity to familiarise themselves with the exercise equipment and to perform a practice run. A blood sample was also taken during this visit. On each study day, after 30 minutes resting in the supine position, baseline measurement of BP, HR, PWV and PWA were recorded. Subjects were then asked to perform an upright bicycle exercise to their maximum tolerance, with the use of a work rate progressively increasing at 20 watts/minute, after the first 2 minutes of unloaded pedalling (Wasserman *et al*., 1999). Respiratory gas exchange variables were measured continuously during the unloaded cycling period and the exercise test and, in the absence of chest pain, ECG abnormalities, arrhythmias or critical blood pressure changes (systolic BP > 250mmHg and/or diastolic BP > 120mmHg) all tests were continued until exhaustion. The criteria used to establish maximal effort included a respiratory exchange ratio (RER, the ratio of $VCO_2$ and $VO_2$) ≥1.10, a failure to maintain a pedalling rate above 40rpm, and no change in HR with change in workload. At the end of the test, subjects rested supine for 1 hour and measurements of BP, HR, PWA and PWV were taken at 10, 40 and 60 minutes after exercise.

### 3.3.5 Statistical analysis

Test-retest repeatability of the parameters investigated were assessed according to the method described by Bland and Altman (Bland & Altman, 1986) (see section 2.7).

### 3.3.6 Results

Six healthy volunteers were recruited for this study, and their characteristics are shown in Table 3.4.

| Parameters | Subjects (n=6) |
|---|---|
| Age (years) | 47± 5 (range 28-66) |
| BMI ($kg/m^2$) | 25.6±1.1 |
| Total cholesterol (mmol/l) | 4.5±0.4 |
| Systolic BP (mmHg) | 121±2.3 |
| Diastolic BP (mmHg) | 72±3.3 |

**Table 3.4. Clinical characteristics of the study subjects.**
BMI, body mass index; BP, blood pressure.

All subjects completed both exercise tests until exhaustion, and none of the tests had to be discontinued because of chest pain, ECG abnormalities, arrhythmias or critical blood pressure changes. There was good repeatability between the two measures for the various clinical parameters recorded during the first and second exercise test. Figure 3.4 shows Bland-Altman plots for peripheral BP, central BP and peak exercise systolic BP; Figure 3.5 shows Bland-Altman plots for PWA parameters, CF-PWV and peak $VO_2$.

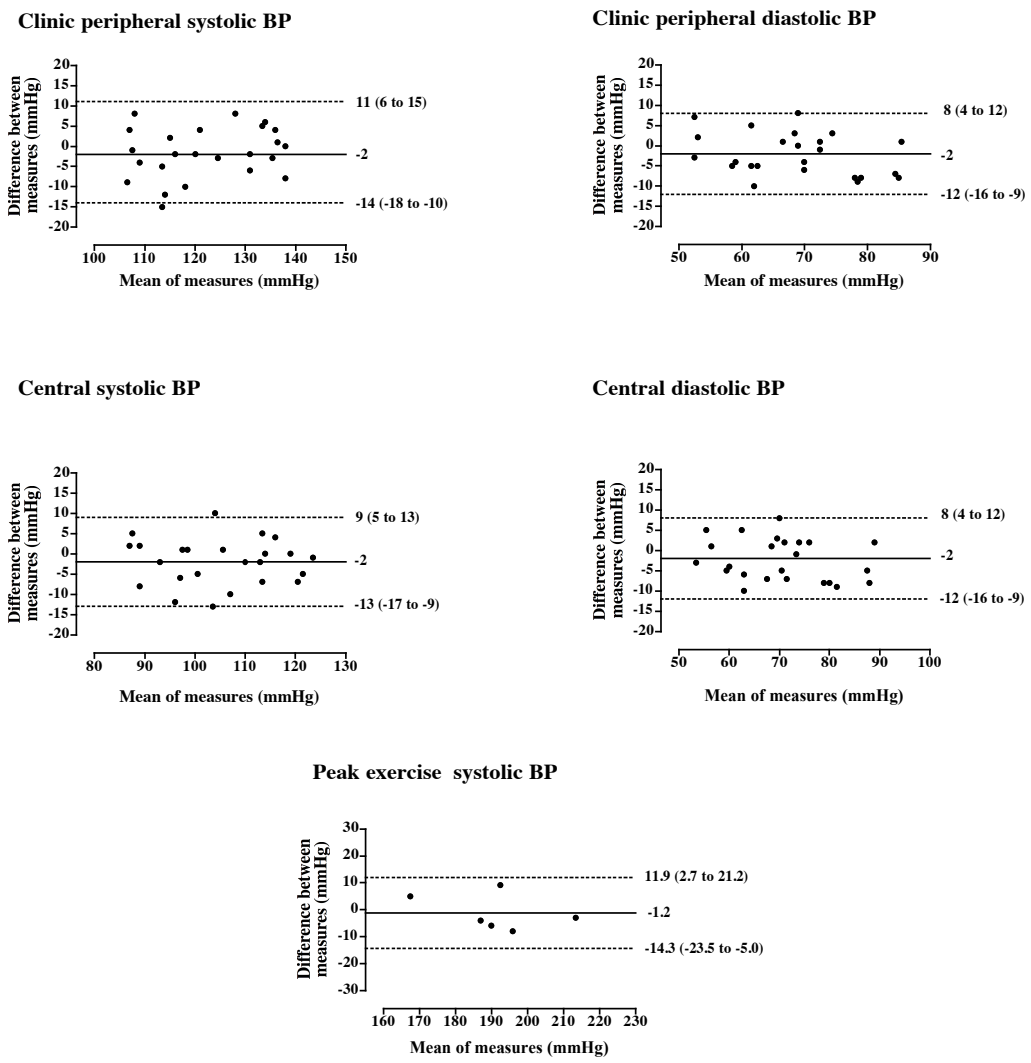


**Figure 3.4. Bland-Altman plots of peripheral BP, central BP and peak exercise systolic BP.**

Solid lines are mean differences and dotted lines are upper and lower 95% limits of agreement (for which 95% CIs are given in brackets).

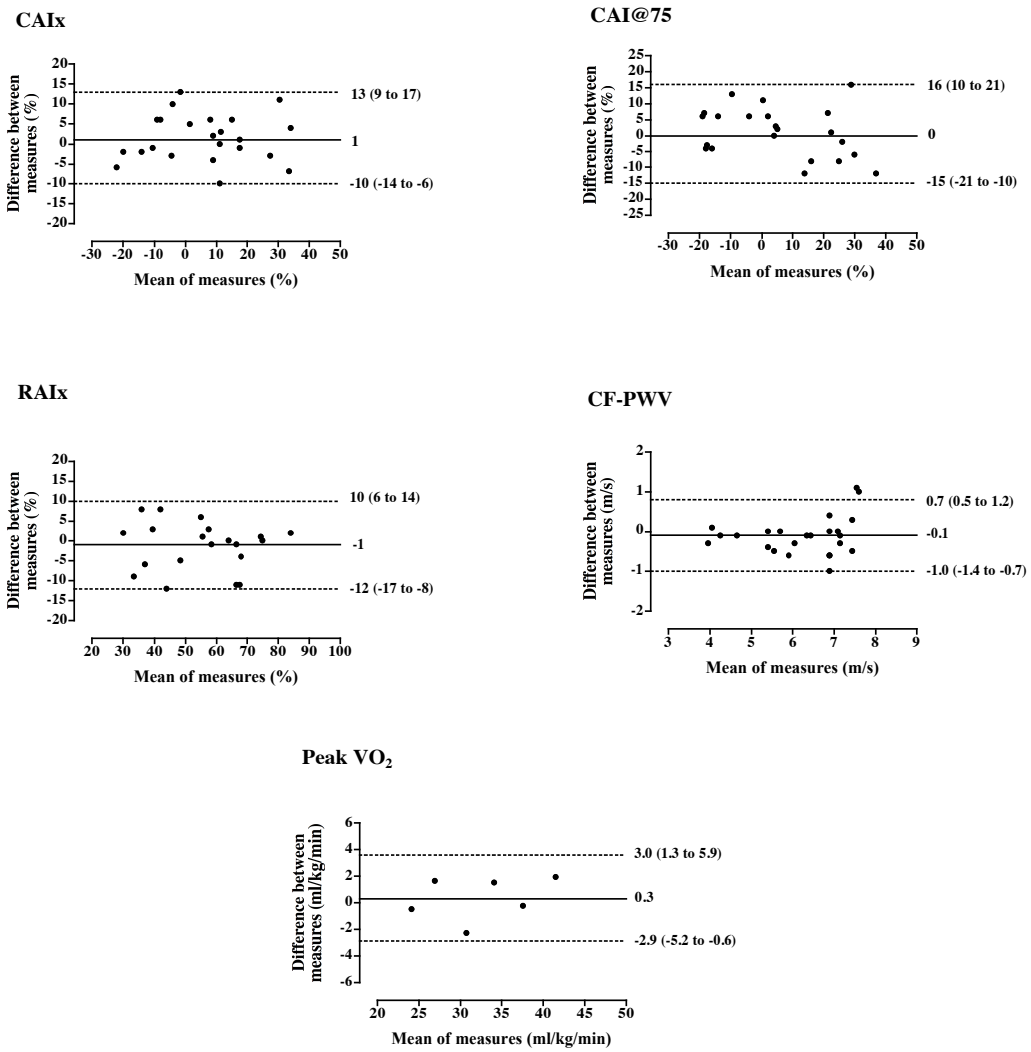


**Figure 3.5. Bland-Altman plots of CAIx, CAIx@75, RAIx, CF-PWV and peak $VO_2$.**
Solid lines are mean differences and dotted lines are upper and lower 95% limits of agreement (for which 95% CIs are given in brackets). CAIx, central augmentation index; CAIx@75, CAIx adjusted to a standard HR of 75bpm; RAIx, radial augmentation index; CF-PWV, carotid-femoral pulse wave velocity.

### 3.3.7 Discussion

The repeatability of variables studied during maximal exercise testing is essential to evaluate whether changes of such variables, measured in successive exercise tests, are the result of changes in physical condition of the subject or the result of variability of measurements. In particular, in order to assess changes in clinical status or the effects of therapeutic interventions on exercise capacity, repeatability studies are necessary to evaluate normal test-retest variation, which can be attributed to a number of factors such as normal circadian rhythm, subject motivation and prior pre-test activity. Repeatability data are also specific to the test equipment and the exercise protocol used, and it is recommended that laboratories develop repeatability coefficients for their own specific test conditions (Bingisser *et al*., 1997). In this study extra care was taken to ensure each test was performed under the same standard conditions, in agreement with current guidelines (Myers *et al*., 2009), and the data obtained show a good repeatability and no significant differences in the variables measured during the first and second exercise test.

### 3.3.8 Summary

The results presented attest the repeatability of peak $VO_2$ and arterial stiffness parameters obtained using a specific equipment and exercise protocol. This is important for the valid interpretation of repeated exercise tests performed to investigate the effects of PDE5 inhibition on exercise capacity and arterial stiffness.

# CHAPTER 4

## PHOSPHODIESTERASE TYPE 5 INHIBITION AND FOREARM EXERCISE-INDUCED VASODILATATION IN HYPERTENSIVE PATIENTS

## 4.1 INTRODUCTION

### 4.1.1 Background

The successful use of sildenafil in the treatment of male erectile dysfunction (Goldstein *et al*., 1998) has created increasing interest in the therapeutic potential of PDE5 inhibition in cardiovascular diseases associated with dysfunction of the NO-cGMP signalling pathway. Elevation of cGMP by PDE5 inhibition seems a logical approach for treating conditions ranging from hypertension to vasospasm, as demonstrated by the growing literature on the potential clinical uses for PDE5 inhibitors. Sildenafil has been shown to improve the vasomotor response of blood vessels in patients with heart failure (Katz et al., 2000), type 2 diabetes (Desouza et al., 2002) and Raynaud's phenomenon (Fries *et al*., 2005), and to significantly improve exercise capacity in chronic heart failure and in pulmonary arterial hypertension (Guazzi *et al*., 2004a; Galie *et al*., 2005; Lewis *et al*., 2007a). In hypertensive patients, in whom endothelial dysfunction and reduced exercise-induced vasodilatation are important features, the impaired vasomotor response to exercise might be improved by PDE5 inhibition with sildenafil. In turn, improvement in vasodilator responses to physiological stimuli (shear stress) through enhanced cGMP signalling may set the stage for improved exercise capacity in hypertensive patients. The effects of PDE5 inhibition on forearm exercise-induced vasodilatation in arterial hypertension have not been investigated previously.

### 4.1.2 Aims

The aims of this study were to:

1. Assess the forearm blood flow (FBF) response to a previously validated handgrip exercise task in hypertensive and normotensive subjects before and after the local (intrabrachial) administration of sildenafil
2. Compare this response with the one obtained after the infusion of the calcium channel blocker verapamil, a cGMP-independent vasodilator (control) and placebo (saline).

## 4.2 METHODS

### 4.2.1 Subjects

#### *4.2.1.1 Identification*

Suitable hypertensive patients were identified from the WGH Cardiovascular Risk Clinic database, and healthy volunteers were identified from the existing CRC community database.

##### *4.2.1.1.1 Inclusion criteria*

- Hypertensive subjects

- Male
- Aged between 20 and 70 years
- At least 3 separate office measurements of systolic BP ≥160mmHg (maximum 180mmHg) and/or diastolic BP ≥90mmHg
- Not on treatment

- Normotensive subjects

- Male
- Healthy
- Aged between 20 and 70 years
- Systolic BP ≤140mmHg and diastolic BP ≤80mmHg

##### *4.2.1.1.2 Exclusion criteria (all subjects)*

- Female
- History of coronary artery, cerebrovascular or peripheral vascular disease
- Total cholesterol >6.5 mmol/L
- Current alcohol abuse
- Diabetes mellitus
- Asthma
- Smoking
- Body mass index (BMI) ≥30 $kg/m^2$

- Taking any vasoactive or endothelial function modifying drugs which cannot be withdrawn for the purpose of the study
- Previous serious drug allergy
- Regular exercise training
- Clinically significant abnormality on screening blood test
- Presence of other clinically relevant conditions.

### 4.2.2 Screening visit

Potentially suitable subjects who agreed to be considered for the study attended a screening visit at the CRC. At the time of 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 fasting blood sample was also taken. During this visit subjects performed the handgrip exercise with the non-dominant arm to determine maximum voluntary contraction (MVC) and, from this, 45% of MVC was established for each subject and used for the subsequent visits.

### 4.2.3 Study design

Randomised, placebo-controlled, single-blind, 3-way crossover.

### 4.2.4 Study protocol

The study protocol is outlined in Figure 4.1. Subjects attended the CRC at 9am on 3 different occasions, at least one week apart, and after an overnight fast (>12 hours). They were required to abstain from alcohol and caffeine-containing food and beverages from 24 hours before each study. On each study day, subjects rested quietly for 30 minutes and during this time FBF measurements were recorded. Preinfusion handgrip exercise (at 45% of MVC) was then performed for 5 minutes, with FBF assessed in the final 3 minutes of the exercise, during each of the 10-second relaxation period between contractions. After a recovery period of 30 minutes, the brachial artery of the exercised arm was cannulated and, following a 30-minute saline infusion, subjects received a 6-minute infusion of sildenafil 50μg/min, verapamil 5μg/min or placebo (saline), in random order, with FBF recordings during the last 3 minutes of infusion. The cannula was then removed and handgrip exercise and FBF measurements repeated.

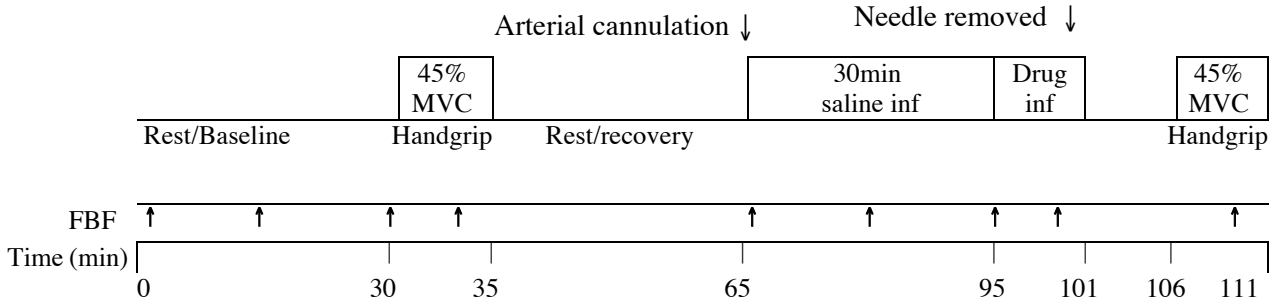


**Figure 4.1. Schematic representation of the experimental protocol.**
Drug infusion (inf) refers to 6-minute intrabrachial infusion of sildenafil (50 μg/min), verapamil (5 μg/min) or saline. MVC, maximum voluntary contraction.

### 4.2.5 Statistical analysis

Venous occlusion plethysmography is a very powerful technique, and it allows the detection of significant changes in forearm blood flow without the need for a large sample of subjects (Wilkinson & Webb, 2001). This is supported by previously published data in which a sample size of 8 (McEniery *et al*., 2002) or 12 subjects (Jackson *et al*., 1999) was enough to detect a statistically significant change in blood flow. On the basis of these studies, and also supported by the findings observed in the preliminary dose-ranging studies presented in Chapter 3, a sample size of 10 subjects was considered adequate for this study. Plethysmographic data were extracted from Chart data files and analysed by an independent observer blinded to the treatment. All values are expressed as mean±SEM. Forearm blood flow data are presented as changes in absolute blood flow (ml/min/100ml of forearm volume) in the infused (exercised) arm. Preinfusion and postinfusion FBF responses to handgrip exercise for the 3 treatments were then compared within each group. Data were analysed with repeated measures ANOVA with post-hoc Bonferroni corrections and 2-tailed Student's *t*-test as appropriate. Statistical analysis was performed with Graph-Pad Prism (GraphPad Software, Inc, San Diego, Calif). Significance was accepted at the 5% level in all cases.

## 4.3 RESULTS

### 4.3.1 Subjects

Ten normotensive and ten hypertensive subjects, none of whom taking regular antihypertensive treatment, were recruited, and all of them completed the study. The baseline clinical characteristics of the two groups are shown in Table 4.1, and they differed only by BP.

### 4.3.2 Resting FBF and preinfusion handgrip exercise

As shown in Table 4.2, resting FBF was similar in hypertensive and normotensive subjects on each study day. Preinfusion handgrip exercise produced an increase in FBF in both groups; however, the increase observed in hypertensive patients was significantly less than that in the control group ($P<0.001$) (Figure 4.2). Blood pressure tended to increase in hypertensive patients, but not in normotensive controls, on each of the 3 study visits during handgrip exercise, but this did not reach statistical significance (Table 4.3). Heart rate did not change significantly in either group (Table 4.3).

| Parameter | Hypertensive patients (n=10) | Normotensive subjects (n=10) | p* |
|---|---|---|---|
| Age, y (range) | 46±4 (31-67) | 43±3 (28-59) | NS |
| Body mass index, kg/m$^2$ | 27.4±0.9 | 25.7±0.8 | NS |
| Total cholesterol, mmol/L | 4.8±0.2 | 4.7±0.2 | NS |
| Creatinine, umol/L | 91.7±4.2 | 86.8±2 | NS |
| Urea, mmol/L | 5.1±0.3 | 5.2±0.4 | NS |
| Sodium, mmol/L | 142±0.7 | 141±0.6 | NS |
| Potassium, mmol/L | 4.2±0.09 | 4.4±0.08 | NS |
| Fasting glucose, mmol/L | 5.4±0.1 | 5.1±0.1 | NS |
| SBP, mmHg | 170±2 | 123±4 | |
| DBP, mmHg | 97±3 | 68±2 | |

**Table 4.1. Clinical characteristics of the hypertensive patients and normotensive subjects.**
Data are mean±SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure. *Differences between groups were evaluated by unpaired Student's *t*-test (normotensive subjects *vs* hypertensive patients).

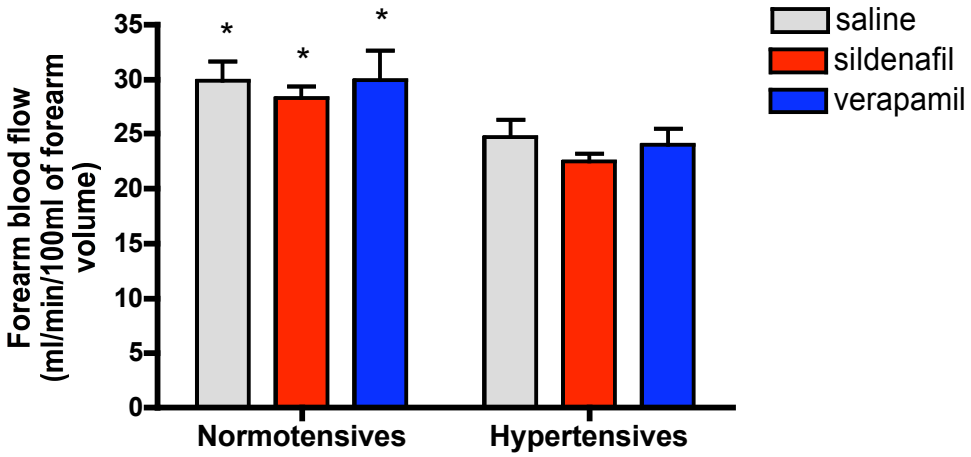


**Figure 4.2. Preinfusion exercise vasodilator responses.**
Changes in absolute blood flow (ml/min/100ml of forearm volume) in the infused (exercised) arm in response to handgrip exercise before saline, sildenafil and verapamil infusion. Data are mean± SEM. *P<0.001 in normotensive subjects *vs* hypertensive patients.

| | Hypertensive patients | | | Normotensive subjects | | |
|---|---|---|---|---|---|---|
| | Infused | Noninfused | Ratio | Infused | Noninfused | Ratio |
| *Before infusion* | | | | | | |
| Saline | 4.05±0.39 | 3.80±0.36 | 1.10±0.08 | 3.22±0.37 | 3.45±0.43 | 0.98±0.08 |
| Sildenafil | 3.34±0.25 | 3.10±0.30 | 1.14±0.09 | 3.35±0.26 | 4.04±0.62 | 0.91±0.07 |
| Verapamil | 3.93±0.59 | 3.71±0.35 | 1.05±0.07 | 3.96±0.54 | 4.06±0.59 | 0.99±0.06 |
| *During infusion*† | | | | | | |
| Saline | 4.07±0.33 | 3.71±0.32 | 1.14±0.08 | 3.35±0.36 | 3.53±0.38 | 0.98±0.08 |
| Sildenafil | 5.58±0.53* | 3.30±0.32 | 1.79±0.12 | 5.92±0.55* | 3.86±0.42 | 1.62±0.15 |
| Verapamil | 6.44±0.68* | 3.68±0.40 | 1.78±0.11 | 6.20±0.50* | 3.59±0.40 | 1.82±0.13 |

**Table 4.2. Forearm blood flow before and during the infusions.**
Data are mean ± SEM absolute FBF (ml/min/100ml of forearm volume) in the infused and noninfused arm, and FBF ratio (infused/noninfused) before and during the infusion of saline, sildenafil and verapamil. †FBF measurements during the last 3 minutes of drug infusion; *P<0.001 infused *vs* noninfused arm.

| | Normotensive subjects | | | | Hypertensive patients | | | |
|---|---|---|---|---|---|---|---|---|
| | **SBP** | **DBP** | **MAP** | **HR** | **SBP** | **DBP** | **MAP** | **HR** |
| *Pre-handgrip* | | | | | | | | |
| Saline | 125±5 | 67±3 | 85±3 | 66±2 | 154±3 | 75±3 | 99±3 | 62±3 |
| Sildenafil | 123±4 | 65±2 | 84±2 | 65±2 | 153±4 | 78±4 | 105±4 | 59±2 |
| Verapamil | 127±5 | 70±3 | 90±3 | 63±3 | 154±2 | 79±4 | 106±2 | 60±2 |
| *Post-handgrip* | | | | | | | | |
| Saline | 128±5 | 70±3 | 89±3 | 68±3 | 159±5 | 77±4 | 102±4 | 64±2 |
| Sildenafil | 125±4 | 66±2 | 87±2 | 66±3 | 157±4 | 82±4 | 109±3 | 60±3 |
| Verapamil | 131±5 | 71±3 | 92±3 | 65±2 | 160±3 | 81±3 | 110±4 | 62±2 |

**Table 4.3. Blood pressure and heart rate before and after exercise.**
Data are mean ± SEM. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate.

### 4.3.3 Effects of sildenafil and verapamil on resting FBF and handgrip exercise

The effects of the 3 intra-arterial infusions on resting FBF are presented in Table 4.2. Forearm blood flow did not change significantly in either group after the infusion of saline. The infusion of sildenafil and verapamil produced significant vasodilatation compared to saline, resulting in increased FBF in both groups ($P<0.001$). The extent of vasodilatation was similar between the two treatments and with no significant difference between hypertensive and normotensive subjects. Blood pressure and heart rate did not change after any of the infusions and none of the subjects reported adverse effects related to the local infusion of the study drugs. In the normotensive subjects, vasodilator response to handgrip exercise did not change significantly after the infusions when compared to preinfusion values, and no significant difference was detected amongst the 3 treatments ($P=0.35$) (Figure 4.3A). In the hypertensive group,

a significant difference in the response to handgrip exercise was observed amongst the 3 treatments (ANOVA, P=0.0167); indeed, while no significant changes were detected in the vasodilator response to exercise following the infusion of saline or verapamil, a significant improvement was observed after sildenafil, which significantly increased exercise-induced vasodilatation compared to both verapamil and saline (P<0.05) (Figure 4.3B).

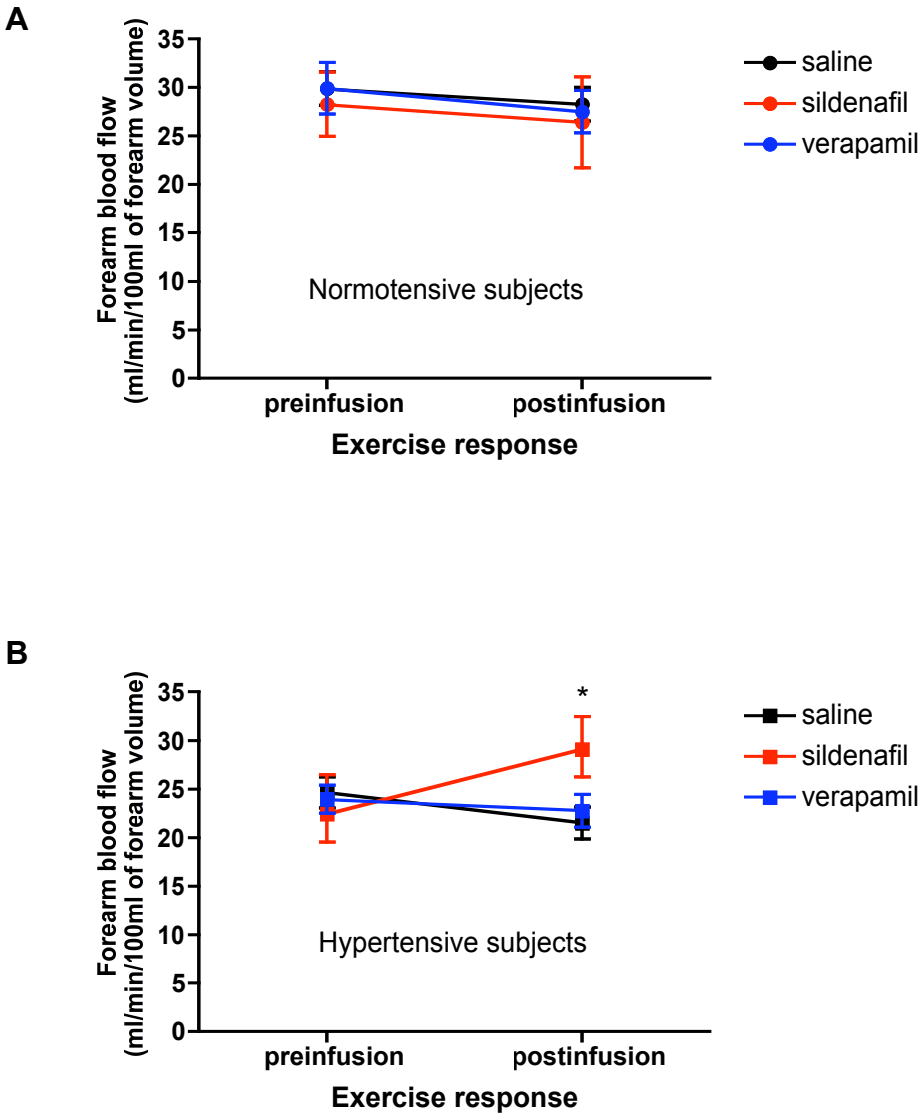


**Figure 4.3. Preinfusion and postinfusion exercise vasodilator responses**.
Changes in absolute blood flow (ml/min/100ml of forearm volume) in the infused (exercised) arm in response to exercise after 6-minute intra-arterial infusion of saline, sildenafil 50 µg/min and verapamil 5 µg/min in normotensive subjects (A) and hypertensive patients (B). Data are mean ± SEM. *P<0.05 *vs* saline and verapamil in the hypertensive patients.

## 4.4 DISCUSSION

The present study provides evidence in support of a beneficial effect of the PDE5 inhibitor sildenafil citrate on the vascular response to handgrip exercise in hypertensive patients. The results obtained confirmed previous work showing that the forearm vasodilator response to handgrip exercise is reduced in hypertensive patients compared with normotensive subjects (McEniery *et al*., 2002). Also, and the major novel observation from the current study, sildenafil substantially enhanced exercise-induced vasodilatation in hypertensive patients but not in normotensive subjects, an effect that is not seen with the control dilator verapamil. These findings suggest that cGMP signalling is critical in regulating this flow limitation, given that it can be selectively improved by PDE5 inhibition.

### 4.4.1 Vascular responses to intra-arterial infusions

The effects of local sildenafil infusion in this study are consistent with previous data, which demonstrate an increase in FBF after intra-arterial administration of sildenafil in healthy subjects (Jackson *et al*., 1999). The control vasodilator used in this study, verapamil, was chosen on the basis of the effects shown in preliminary plethysmography investigations (see Chapter 3), but it is important to acknowledge that, in studies comparing hypertensive and normotensive subjects, whose starting conditions differ, no perfect control exists. However, if anything, studies show an increased response to verapamil in hypertensive subjects (Robinson *et al*., 1982). As resting FBF was similar in both groups before the second bout of exercise, as it was for preinfusion exercise, potential influences of different resting FBF, and inherent vascular tone, on the subsequent vasodilator response to exercise in each group are eliminated.

### 4.4.2 Vasodilator response to handgrip exercise

On each study day, maximum preinfusion vasodilator response to handgrip exercise was significantly less in patients with hypertension than in healthy controls. Endothelial function was not directly addressed in the study subjects, but these findings are consistent with results obtained in a previous study (McEniery *et al*., 2002), and suggest that reduced endothelium-mediated vasodilatation may limit

vascular responsiveness to shear stress, contributing to increased vascular resistance during exercise. In healthy controls, when the effects of infusions on the vasodilator response to handgrip exercise were compared, no significant difference was observed amongst the 3 treatments. By contrast, in the hypertensive patients, a significant and substantial difference was detected amongst the 3 treatments in favour of sildenafil, which selectively improved the response, whereas this was not observed with the control, cGMP-independent, vasodilator verapamil. This is the most important finding of the study, supporting the involvement of the NO-cGMP pathway in the vasodilator response to exercise. It has previously been shown that inhibition of NO synthase abolishes the vasodilatation mediated by PDE5 antagonism (Wallace & Tom, 2000; Teixeira *et al*., 2006), thus findings derived from this study are likely to be explained by the increased activity of cGMP, which acts as a second messenger for NO and is ultimately responsible for smooth muscle cell relaxation. Further support to this explanation is provided by another study in which sildenafil, through an increased level of cGMP in the vasculature, reversed vascular alterations in an experimental model of chronic NO deprivation (Rossoni *et al*., 2007). Therefore, in situations associated with alterations of the NO-cGMP pathway, PDE5 inhibition could exert beneficial effects by increasing the intracellular level of cGMP and potentially contribute to restoring physiological responses.

Previous studies have shown conflicting results on the effects of sildenafil on brachial artery flow-mediated vasodilatation (FMD) (Halcox *et al*., 2002; Dishy *et al*., 2004; Vlachopoulos *et al*., 2004). In particular, in a previous study by Oliver and coworkers (Oliver *et al*., 2006), sildenafil had no effect on FMD in hypertensive patients. However, it should be noted that the stimulus for FMD is (passive) reactive hyperaemia, which occurs in response to a temporary occlusion of the vessel, whereas the present study investigated the effects of sildenafil on (active) exercise hyperaemia. This is a more complex phenomenon, in which the pattern of blood flow changes observed is the result of an integrated response also involving the skeletal muscle and the resistance arterioles. Furthermore, as blood flow progressively increases during sustained exercise, the contribution of the NO-cGMP pathway may become more prominent, and this might contribute to explaining the effect of

sildenafil on exercise-induced vasodilatation in the hypertensive subjects recruited for this study.

Local cGMP levels were not measured in this study, as venous cannulation would have interfered with handgrip exercise. However, measurement of cGMP levels after PDE5 inhibition has a high intersubject variability (Jackson *et al*., 1999) and seems not to correlate well with vasodilatation (Gardiner *et al*., 2004).

### 4.4.3 Summary

Results from this study suggest that sildenafil, through an increase in cGMP levels in the vasculature, substantially and selectively improves the vasodilator response to handgrip exercise in hypertensive patients. It was concluded that the impaired vasodilator response to exercise in hypertensive patients is, at least in part, related to reduced endothelium-dependent vasodilatation and can be substantially improved by PDE5 inhibition.

# CHAPTER 5

## EFFECTS OF PHOSPHODIESTERASE TYPE 5 INHIBITION ON EXERCISE CAPACITY AND ARTERIAL STIFFNESS IN HYPERTENSIVE PATIENTS

## 5.1 INTRODUCTION

### 5.1.1 Background

From the early phases of hypertension, a reduced peripheral vasodilator response during exercise is observed, which adversely affects exercise-induced vasodilatation and exercise capacity. Available evidence suggests that NO is a major contributing factor to exercise hyperaemia, and inhibition of NO synthesis has been shown to reduce exercise-induced vasodilatation in healthy subjects (see section 1.4.1). Other classes of drugs, such as ACEIs, can improve exercise capacity (Sumukadas *et al*., 2007) and may have indirect effects on NO (Henriksen & Jacob, 2003), but PDE5 inhibitors effects are specifically related to a direct action on the NO-cGMP pathway. In particular, there could be a unique therapeutic role for PDE5 inhibition in the improvement of vasomotor response to exercise and exercise capacity in hypertension. This is reinforced by the particular characteristic for PDE5 inhibitors to elevate cGMP levels and, at the same time, to further increase their inhibitory capacity, which represents a novel mechanism for the sustained generation of cGMP and explains the potent biological effects of these drugs (see section 1.1.5). To date, no studies have focused on the therapeutic potential of this class of drugs in the vascular responsiveness to exercise in arterial hypertension. This study will try to elucidate whether this therapeutic potential, already realised in pulmonary hypertension (Galie *et al*., 2005) and under investigation in heart failure (Lewis *et al*., 2007a), is also present in arterial hypertension.

### 5.1.2 Aims

The main aims of this study were to:

1. Investigate the effects of a 1-week treatment with oral sildenafil, hydralazine (a control, cGMP-independent vasodilator) and placebo on maximal exercise capacity in untreated hypertensive patients and matched normotensive subjects
2. Investigate the effects of these interventions on exercise systolic BP during a single-stage test of light exercise (Dundee step test) and during maximal exercise testing

3. Investigate the effects of these interventions on parameters of arterial stiffness before and after maximal exercise testing.

## 5.2 METHODS

### 5.2.1 Subjects

#### *5.2.1.1 Identification*

Suitable hypertensive patients were identified from the WGH Cardiovascular Risk Clinic database, and healthy volunteers were identified from the existing CRC community database.

#### *5.2.1.2 Inclusion criteria*

- Hypertensive subjects

- Male
- Aged between 20 and 70 years
- At least 3 separate office measurements of systolic BP ≥160mmHg (maximum 180mmHg) and/or diastolic BP ≥90mmHg
- Not on treatment

- Normotensive subjects

- Male
- Healthy
- Aged between 20 and 70 years
- Systolic BP ≤140mmHg and diastolic BP ≤80mmHg

#### *5.2.1.3 Exclusion criteria (all subjects)*

- Female
- History of coronary artery, cerebrovascular or peripheral vascular disease
- Total cholesterol >6.5 mmol/L
- Current alcohol abuse
- Diabetes mellitus
- Asthma

- Smoking
- ECG evidence of clinically significant arrhythmia, cardiac ischaemia or left ventricular hypertrophy (LVH)
- Body mass index (BMI) ≥30 kg/$m^2$
- Regular exercise training
- Taking any vasoactive or endothelial function modifying drugs which cannot be withdrawn for the purpose of the study
- Previous serious drug allergy
- Clinically significant abnormality on screening blood test
- Contraindication to strenuous exercise
- Presence of other clinically relevant conditions.

Hypertensive subjects and healthy normotensive controls were matched for age and cholesterol values.

### 5.2.2 Screening visit

Potentially suitable participants received an information sheet with details about the study and, those who expressed an interest, were invited to attend a preliminary screening visit at the CRC. During this visit the study procedure was fully explained and each participant signed a written consent. Medical history, physical examination and a 12-lead ECG were performed, and a fasting blood sample was taken. Subjects also underwent a practice run on the cycle ergometer to familiarise themselves with the study equipment and to detect any contraindication to exercise.

### 5.2.3 Study design

Double-blind, randomised, placebo-controlled, 3-way crossover.

### 5.2.4 Study protocol

After the preliminary visit, subjects were randomly assigned to oral sildenafil 50 mg, hydralazine 25mg or placebo for 1 week, and attended the research centre on day 7 and 8 of each treatment arm. On day 7, after a 30-minute rest in the sitting position, baseline measurements of BP and HR were made. Subjects were then asked to take their tablet and after 1 hour, time of the expected peak plasma concentrations and

biological effects (Shepherd *et al*., 1980; Muirhead *et al*., 2002), the same measurements were repeated, immediately followed by 3 minutes of step test, at the end of which BP was measured. On day 8 subjects underwent the protocol shown in Figure 5.1.

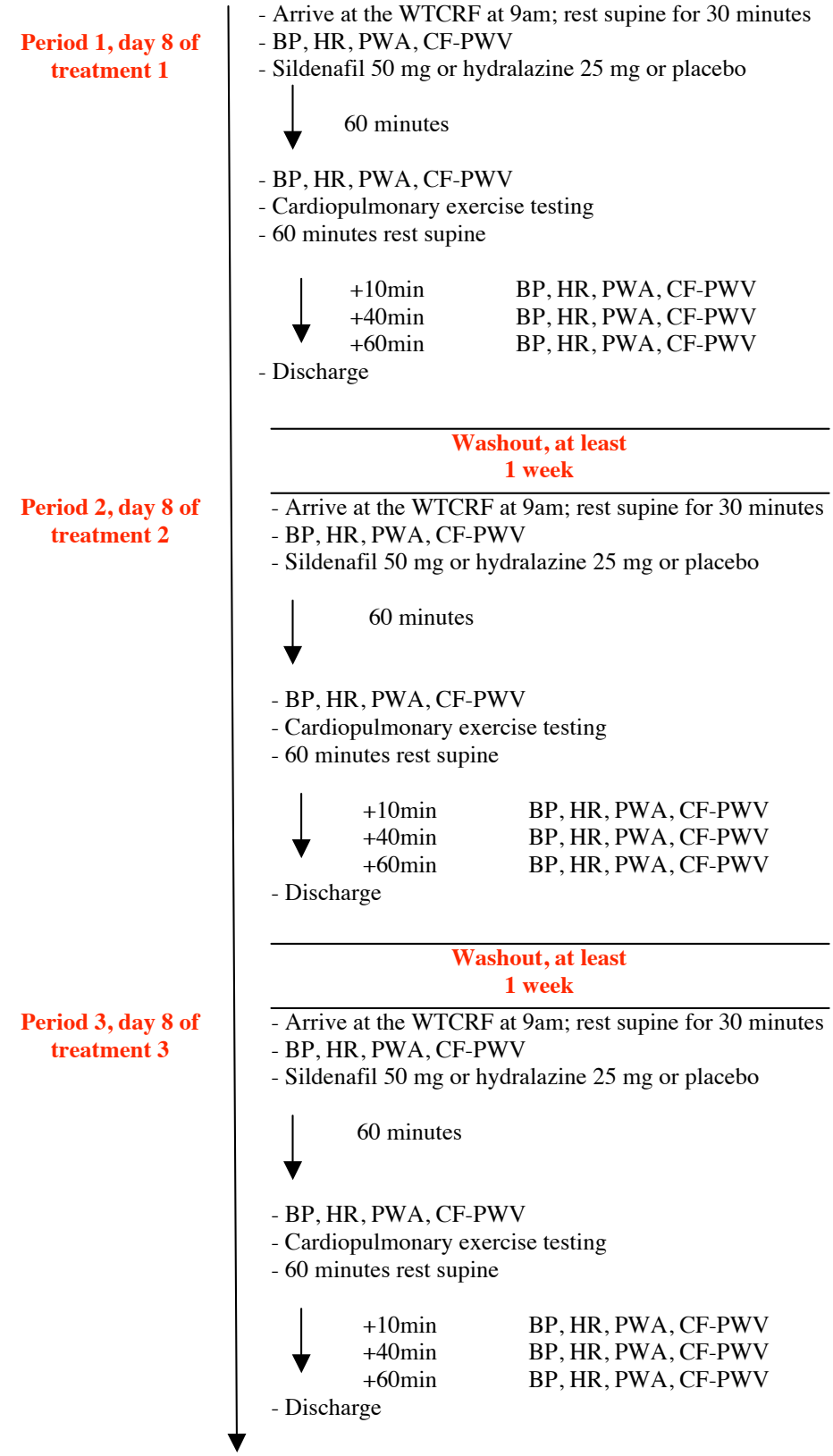


**Figure 5.1. Schematic representation of the study protocol**

### 5.2.5 Drugs

Subjects were instructed to take 1 tablet 3 times per day, at around 9am, 3pm and 10pm. They were given 25 tablets for each treatment arm (4 more than required), and asked to return all tablets left at the end of the treatment. Returned tablets were used to determine adherence to the treatment, which was calculated as $\frac{100 \times (25 - \text{number returned})}{21}$ and expressed as a percentage.

Hydralazine tablets were not matched, therefore this treatment arm was unblinded to the subjects but not to the investigator.

### 5.2.6 Adverse effects

During the screening visit, subjects received 3 cards, one for each arm of the study, and asked to report any symptoms experienced during the period of treatment. These cards were collected at the end of the study and details of symptoms experienced were clarified when subjects attended the research centre.

### 5.2.7 Statistical analysis

The primary end-point of the study was peak $VO_2$, and a standard deviation of ±4.8 ml/kg/min in hypertensive subjects was used for the power calculations (Goodman *et al*., 1992; Guazzi *et al*., 2004a). It was anticipated that a total of 30 subjects (i.e. 15 per group) would have 80% power to detect a 25% difference in peak $VO_2$ between treatment arms at the 5% level. Results are presented as mean±SEM. Repeated measures ANOVA with post-hoc Bonferroni corrections was used to assess the effect of time and intervention within each group. Differences between groups were analysed by 2-tailed unpaired Student's *t*-test.

## 5.3 RESULTS

### 5.3.1 Study subjects

The clinical characteristics of the study subjects are shown in Table 5.1. Subjects enrolled in the study were recreationally active but none of them was resistance- or endurance-trained. Thirty-two subjects were recruited, and a total of 30 (15 per

group) completed the study. During cardiopulmonary exercise testing, none of the subjects had to stop because of chest pain, ECG abnormalities, arrhythmias or critical blood pressure changes (systolic BP > 250mmHg and/or diastolic BP > 120mmHg), and the only reason for stopping exercise was leg fatigue in both groups. Mean maximum adherence was 97% during the placebo arm, 96% during the sildenafil arm, and 97% during the hydralazine arm.

| Parameter | Hypertensive patients (n=15) | Normotensive subjects (n=15) | p* |
|---|---|---|---|
| Age, y (range) | 48±4 (30-68) | 45±3 (27-66) | NS |
| Body mass index, kg/m$^2$ | 27.2±0.8 | 25.5±0.7 | NS |
| Total cholesterol, mmol/L | 4.7±0.2 | 4.5±0.2 | NS |
| Creatinine, umol/L | 90.3±4.0 | 90.8±3.1 | NS |
| Urea, mmol/L | 5.0±0.3 | 4.9±0.2 | NS |
| Sodium, mmol/L | 140±0.3 | 141±0.4 | NS |
| Potassium, mmol/L | 4.3±0.07 | 4.4±0.08 | NS |
| Fasting glucose, mmol/L | 5.1±0.1 | 4.9±0.1 | NS |
| Systolic BP, mmHg | 168±3 | 125±2 | |
| Diastolic BP, mmHg | 95±2 | 74±2 | |

**Table 5.1. Clinical characteristics of the hypertensive patients and normotensive subjects.**
Data are mean ± SEM. *Differences between groups were evaluated by unpaired Student's *t*-test. BP, blood pressure.

### 5.3.2 Blood pressure, arterial stiffness and exercise parameters after placebo

Following placebo, central and peripheral BP, parameters of arterial wave reflection and arterial stiffness were significantly higher in hypertensive patients than normotensive subjects at all time points (ANOVA P<0.0001) (Table 5.2). In addition, after maximal exercise, CF-PWV significantly increased compared with baseline values in hypertensive patients, whereas this increase was not observed in the normotensive group (Table 5.3). With respect to exercise systolic BP during the

step test (day 7) and at peak exercise (day 8), despite higher resting systolic BP in the hypertensive group, changes from baseline were not significantly different between groups (Table 5.4 and Table 5.5). Hypertensive patients also exhibited lower peak $VO_2$ (ANOVA P<0.0001), $VO_2$ at anaerobic threshold (ANOVA P=0.001), peak workload (ANOVA P=0.002), exercise time (ANOVA P=0.002) and lower $VO_2$/work rate relationship (ANOVA P<0.0001) than normotensive subjects (Table 5.5).

### 5.3.3 Effects of drug treatment before exercise

The effects of sildenafil and hydralazine on BP, PWA and CF-PWV are shown in Table 5.2 and Table 5.3. For a graphical representation of the data presented in the tables please see Figure 5.2, Figure 5.3 and Figure 5.4 (data shown as changes from baseline).

#### *5.3.3.1 Blood pressure and heart rate*

In the hypertensive group, brachial systolic BP was lower after 1 week of drug treatment than after placebo (ANOVA P=0.01), but this difference was significant only for hydralazine (P<0.05). Brachial diastolic BP was also lower after drug treatment than after placebo (ANOVA P=0.005), both for sildenafil (P<0.01) and hydralazine (P<0.05). Neither hydralazine nor sildenafil affected peripheral PP in hypertensive individuals. With regard to MAP, this was lower after drug treatment than after placebo (ANOVA P=0.01), both for sildenafil (P<0.05) and hydralazine (P<0.05). None of these parameters changed significantly 1 hour after drug administration compared with baseline. After 1 week of drug treatment with sildenafil and hydralazine, HR was not significantly different compared with placebo. On the study day, HR was significantly lower 1 hour after placebo and sildenafil compared with baseline, but this was not observed after hydralazine. However, differences in changes from baseline were not statistically significant when compared amongst the three interventions.

In the normotensive group, brachial systolic and diastolic BP, peripheral PP and MAP were not different from placebo at baseline after 1 week of drug treatment. On

the study day, brachial diastolic BP and MAP were significantly lower 1 hour after hydralazine compared with placebo; sildenafil did not affect these parameters. Heart rate was not significantly different at baseline after 1 week of drug treatment. On the study day, 1 hour after drug administration, no significant changes in HR were observed compared with baseline.

#### *5.3.3.2 Pulse wave analysis*

In the hypertensive group, CAIx and RAIx were lower after 1 week of drug treatment compared with placebo, and this almost reached statistical significance (P=0.07 and P=0.06, respectively); the magnitude of this effect was similar for sildenafil and hydralazine. On the study day, drug administration did not significantly affect these parameters compared with baseline. Central systolic and diastolic BP and central PP did not change significantly after 1 week of drug treatment compared with placebo, nor they changed 1 hour after drug administration on the study day.

In the normotensive group, after 1 week of drug treatment, CAIx and central PP were not significantly different compared with placebo, and they did not change on the study day 1 hour after drug administration. RAIx was not significantly different at baseline after 1 week of treatment, but was significantly lower one hour after hydralazine compared with baseline values and with placebo ($P<0.05$ for both).

#### *5.3.3.3 Pulse wave velocity*

In both groups, no significant differences were observed at baseline, after 1 week of drug treatment, compared with placebo. On the study day, drug administration did not affect PWV in either group.

### 5.3.4 Effects of sildenafil and hydralazine on exercise systolic BP and maximal exercise capacity

The effects of drug treatment on exercise systolic BP and parameters of exercise capacity are shown in Table 5.4 and Table 5.5.

#### *5.3.4.1 Exercise systolic BP*

During the step test (day 7) and at peak exercise during cardiopulmonary exercise testing (day 8), hypertensive subjects had a higher resting systolic BP than normotensive subjects, but changes from baseline were not significantly different between groups, and were not affected by drug treatment.

#### *5.3.4.2 Peak $VO_2$ and related parameters*

A mean peak RER ≥1.10 was achieved in both groups during each study day, consistent with maximum effort during exercise. Treatment with sildenafil and hydralazine did not affect peak $VO_2$, $VO_2$ at anaerobic threshold or any of the other related exercise parameters compared with placebo in either group.

### 5.3.5 Effects of drug treatment after exercise

The effects of sildenafil and hydralazine on BP, PWA and CF-PWV after exercise are shown in Table 5.2 and Table 5.3. For a graphical representation of the data presented in the tables please see Figure 5.2, Figure 5.3 and Figure 5.4 (data shown as changes from baseline).

#### *5.3.5.1 Blood pressure and heart rate*

In the hypertensive group, following hydralazine, brachial systolic BP was significantly reduced compared with baseline at 40 and 60 min after exercise ($P<0.01$), and brachial diastolic BP was also lower at all time points after exercise ($P<0.01$ and $P<0.05$), but these changes were not significantly different from placebo and sildenafil. MAP was also significantly decreased compared with baseline at 40 and 60 min after exercise following hydralazine ($P<0.01$); no significant changes in MAP were observed following placebo and sildenafil. After hydralazine, peripheral PP was significantly increased compared with baseline at 10 min after exercise and significantly increased 40 min after exercise ($P<0.05$), something that was not observed with sildenafil. Heart rate remained elevated compared with baseline at all time points after exercise following hydralazine treatment ($P<0.01$ and $P<0.05$); however, these changes were not significantly different when compared with placebo and sildenafil. In the normotensive group, at

10 min after exercise, brachial systolic BP was significantly increased compared with baseline following placebo and hydralazine but not sildenafil; however these changes from baseline were not significantly different amongst the 3 treatments. Brachial diastolic BP was significantly decreased compared with baseline at all time points after exercise, and these changes were also significant when compared with placebo. MAP was significantly decreased compared with baseline at 40 and 60 min after exercise following hydralazine. No significant changes were observed with either placebo or sildenafil. Peripheral PP was significantly increased at 10 min after exercise compared with baseline values, but these changes from baseline were not significantly different amongst the three interventions. HR remained significantly elevated compared with baseline at all time points after exercise.

#### *5.3.5.2 Pulse wave analysis*

In the hypertensive group, CAIx was significantly decreased compared with baseline at all time points after exercise following hydralazine ($P<0.01$ and $P<0.05$), and at 10 and 40 min after exercise following sildenafil ($P<0.01$ and $P<0.05$). CAIx@75 was reduced at 40 min after exercise compared with baseline following sildenafil ($P<0.05$) but not hydralazine. For both, CAIx and CAIx@75, changes were not significantly different between the two drug treatments. Central PP was significantly decreased compared with baseline at 10 min after exercise following sildenafil ($P<0.05$) and at 40 min after exercise for all treatments ($P<0.05$), but these changes were not significantly different amongst the three interventions. RAIx was significantly decreased compared with baseline at 10 and 40 min after exercise following sildenafil ($P<0.05$), and at all time points following hydralazine ($P<0.01$ and $P<0.05$). Central systolic BP was significantly lower than baseline at 40 and 60 min after exercise following placebo ($P<0.01$ and $P<0.05$) and hydralazine ($P<0.01$), and a similar trend, although not statistically significant, was observed with sildenafil; however, changes from baseline were not significantly different amongst the three interventions. Central diastolic BP was significantly decreased compared with baseline at 60 min after exercise following hydralazine ($P<0.01$), a change that was also significant when compared with placebo ($P<0.05$).

In the normotensive group, CAIx did not change significantly from baseline at all time points after exercise, except for a reduction at 60 min after exercise following treatment with hydralazine; CAIx@75 was significantly increased 10 min after exercise compared with baseline, with no differences detected amongst treatments. RAIx decreased at all time points after exercise compared with baseline following placebo or sildenafil, whereas, following hydralazine, the reduction became apparent only at 40 and 60 min after exercise. No difference was observed amongst the three interventions. At 40 and 60 min after exercise, central systolic BP was significantly lower than baseline following hydralazine, and this was also true for central diastolic BP. No significant changes were observed in central diastolic BP after exercise with placebo or sildenafil. Central PP was significantly increased compared with baseline at 10 min after exercise following hydralazine, but this change was not significant compared with placebo and sildenafil.

#### *5.3.5.3 Pulse wave velocity*

In the normotensive group, CF-PWV was not affected by either sildenafil or hydralazine treatment at any time point after exercise. By contrast, in hypertensive patients, CF-PWV was significantly lower after exercise following sildenafil treatment but not after placebo or hydralazine (ANOVA P=0.0001).

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| Brachial SBP (mmHg) | Baseline | 122(3.2) | | 122(3.0) | | 124(3.3) | | 152(3.4) | | 145(4.2) | | 143(3.1) | |
| | 1 hour | 123(3.0) | 1(1.1) | 121(3.2) | -2(1.1) | 123(3.3) | -2(1.4) | 150(4.2) | -2(2.1) | 142(4.6) | -2(2.5) | 144(3.0) | 0(1.7) |
| *Post exercise* | +10min | 129(3.4)† | 7(1.3) | 126(3.5) | 4(2) | 131(4.1)† | 6(1.4) | 152(3.1) | 0(2.2) | 141(3.8) | -3(2.9) | 144(2.9) | 1(2.7) |
| | +40min | 121(2.3) | -1(1.5) | 121(4.0) | -1(2.2) | 121(3.8) | -3(1.8) | 147(3.0) | -4(2) | 141(3.2) | -4(2.5) | 136(2.7)† | -7(2.1) |
| | +60min | 121(3.5) | -1(1.1) | 120(3.1) | -2(2.3) | 120(3.0)* | -3(1.9) | 148(4.4) | -4(2.2) | 141(2.9) | -4(2.6) | 137(3.2)* | -6(2.7) |
| Brachial DBP (mmHg) | Baseline | 71(3.4) | | 72(3.2) | | 73(3.5) | | 88(2.1) | | 81(3.2) | | 83(2.5) | |
| | 1 hour | 73(3.8)* | 2(1) | 70(2.8) | -2(1.6) | 67(3.2)* | -5(2.4)* vs P | 88(2.0) | 0(1.5) | 81(2.9) | 0(1.4) | 80(2.9) | -2(1.3) |
| *Post exercise* | +10min | 72(2.7) | 1(1.2) | 69(2.6) | -2(1.7) | 68(3.6)* | -6(2.4)* vs P | 88(2.7) | 0(1.4) | 79(1.7) | -3(1.4) | 78(2.3)* | -4(1.6) |
| | +40min | 70(3.0) | 0(1.1) | 69(3.1) | -3(1.5) | 66(3.2)† | -7(2.4)* vs P | 87(1.9) | 0(1.2) | 80(1.9) | -1(1.6) | 80(2.8) | -3(1.1) |
| | +60min | 71(3.2) | 0(1.2) | 70(3.0) | -2(2.7) | 66(3.2)† | -8(2.3)‡ vs P | 87(2.3) | -1(1.3) | 79(2.6) | -2(1.6) | 77(2.5)† | -5(1.2) |

**Table 5.2. Peripheral and central parameters at baseline, 1 hour after drug administration and after exercise.**
For absolute values comparisons are against baseline; for Δ (change) from baseline comparisons are among placebo, sildenafil and hydralazine; **P*<0.05, †*P*< 0.01, ‡*P*<0.001. P, placebo.

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| MAP (mmHg) | Baseline | 87(3.2) | | 88(3.0) | | 88(2.9) | | 109(3.1) | | 103(4.1) | | 103(3.4) | |
| | 1 hour | 89(3.1) | 2(1.2) | 86(3.5) | -2(1.4) | 84(3.0)† | -4(0.8)† vs P | 110(3.4) | 0(1.6) | 102(3.7) | -1(1.8) | 101(3.5) | -2(1.8) |
| *Post exercise* | +10min | 90(2.9) | 3(1.3) | 88(3.2) | 0(1.7) | 87(2.6) | -1(1.4)* vs P | 110(2.7) | 0(1.3) | 100(2.9) | -3(2.4) | 99(2.6) | -4(2) |
| | +40min | 86(3.4) | -1(1.1) | 85(3.1) | -3(1.8) | 83(3.3)† | -5(1.5) | 107(2.2) | -2(1.3) | 99(3.3) | -4(1.9) | 98(2.8)† | -5(1.5) |
| | +60min | 86(3.7) | -1(1.1) | 86(3.1) | -2(2.5) | 82(3.2)† | -6(1) | 107(3.4) | -3(1.7) | 100(2.5) | -3(2.1) | 96(2.3)† | -7(1.3) |
| Heart rate (bpm) | Baseline | 61(2) | | 60(3) | | 65(3) | | 64(2) | | 68(2) | | 69(2) | |
| | 1 hour | 56(2)† | -5(1.0) | 59(3) | -1(1.1) | 63(2) | -2(1.5) | 59(2)† | -5(1.3) | 63(2)† | -5(1.0) | 66(2) | -3(1.1) |
| *Post exercise* | +10min | 82(3)† | 22(2.8) | 83(3)† | 22(3.5) | 87(2)† | 22(3.3) | 78(3)† | 11(3.4) | 83(3)† | 15(2.0) | 86(3)† | 17(2.4) |
| | +40min | 70(3)† | 9(1.8) | 72(3)† | 12(2.6) | 77(2)† | 12(2.6) | 68(2) | 4(2.0) | 73(2)* | 4(1.7) | 76(3)† | 8(1.9) |
| | +60min | 69(3)† | 8(1.2) | 70(3)† | 10(1.3) | 73(2)† | 8(1.5) | 66(2) | 2(2.1) | 71(2) | 2(1.8) | 73(3)* | 5(1.9) |

**Table 5.2**
Continued from previous page

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| Central SBP (mmHg) | Baseline | 106(3.2) | | 107(3.3) | | 107(3.6) | | 132(4.1) | | 129(5.2) | | 128(4.1) | |
| | 1 hour | 108(3.1) | 1(1.4) | 105(3.4) | -2(1.2) | 104(3.2)* | -3(1.1) | 132(4.0) | 0(2.4) | 128(5.3) | -1(2.4) | 128(4.4) | 0(1.8) |
| *Post exercise* | +10min | 110(2.6)† | 4(1.3) | 107(3.1) | 0(1.8) | 110(3.2) | 3(1.3) | 129(3.6) | -3(2.3) | 122(3.1) | -6(3.1) | 123(3.8) | -4(2.3) |
| | +40min | 104(3.3) | -2(1.2) | 103(3.8) | -3(2.0) | 102(3.6)* | -5(1.7) | 127(3.2)† | -6(1.9) | 123(3.0) | -6(2.9) | 119(3.1)† | -8(2.1) |
| | +60min | 104(3.0) | -2(1.1) | 104(3.6) | -3(2.1) | 101(3.1)† | -6(1.4) | 127(4.2)* | -5(2.1) | 124(3.5) | -4(3) | 119(4.3)† | -5(2.6) |
| Central DBP (mmHg) | Baseline | 72(3.4) | | 73(3.1) | | 72(3.7) | | 84(2.2) | | 82(3.1) | | 84(2.3) | |
| | 1 hour | 74(3.2) | 2(1.0) | 71(2.3) | -2(1.6)* vs P | 69(3.3)† | -4(1.0)† vs P | 84(2.5) | 0(1.5) | 83(2.0) | 0(1.6) | 81(2.7) | -3(1.6) |
| *Post exercise* | +10min | 74(2.7) | 3(1.3) | 72(2.0) | -1(1.8) | 71(2.1) | -2(1.5)* vs P | 85(2.1) | 0(1.5) | 81(1.0) | -1(2.2) | 81(2.2) | -3(1.7) |
| | +40min | 72(3.0) | 0(1.1) | 70(2.7) | -2(1.6) | 69(3.4)* | -4(1.2)* vs P | 84(2.3) | 0(1.2) | 82(2.4) | 0(1.7) | 81(2.0) | -2(1.1) |
| | +60min | 72(3.2) | 0(1.0) | 71(3.2) | -2(2.8) | 67(3.2)† | -5(0.9) | 83(2.3) | -1(1.4) | 81(2.3) | -1(1.6) | 78(2.1)† | -6(1.2)* vs P |

**Table 5.2**
Continued from previous page

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| Peripheral PP (mmHg) | Baseline | 51(2.1) | | 50(2.6) | | 53(2.5) | | 64(3.4) | | 63(2.1) | | 61(2.1) | |
| | 1 hour | 51(2.7) | 0(1.4) | 51(2.0) | 1(1.5) | 55(2.4) | 2(1.4) | 62(3.0) | -2(1.5) | 61(3.2) | -2(2.3) | 63(3.1) | 3(2.0) |
| *Post exercise* | +10min | 57(3.0)† | 6(1.5) | 56(3.2)* | 6(2.5) | 63(3.2)† | 9(1.4) | 64(3.2) | 0(2.4) | 62(2.0) | -1(2.2) | 66(2.5)* | 5(2.3) |
| | +40min | 50(2.3) | -1(1.5) | 53(2.2) | 2(2.1) | 54(2.1) | 1(1.3) | 60(2.7) | -4(1.9) | 60(2.9) | -3(2.1) | 56(2.2)* | -4(2.1) |
| | +60min | 50(1.9) | -1(1.1) | 50(2.7) | 0(2.4) | 55(2.9) | 1(1.5) | 61(3.1) | -3(1.8) | 62(2.8) | -1(1.8) | 60(2.6) | -1(1.9) |
| Central PP (mmHg) | Baseline | 35(1.1) | | 34(1.3) | | 34(1.1) | | 48(3.2) | | 46(2.0) | | 44(3.3) | |
| | 1 hour | 34(1.6) | 0(1.2) | 34(1.2) | 0(1.2) | 35(1.2) | 1(1.1) | 48(3.5) | -1(1.2) | 45(3.3) | -1(1.9) | 46(3.4) | 2(1.6) |
| *Post exercise* | +10min | 36(1.9) | 1(1.1) | 35(1.7) | 1(1.7) | 39(2.3)* | 4(1.9) | 45(2.9) | -4(2.2) | 41(2.8)* | -4(1.7) | 43(2.5) | -1(1.9) |
| | +40min | 32(1.0)* | -2(1.0) | 33(1.9) | -1(1.7) | 33(1.2) | -1(1.0) | 43(2.1)* | -5(1.8) | 41(2.5)* | -4(2.0) | 38(2.7)† | -5(1.7) |
| | +60min | 32(1.1)† | -3(0.5) | 32(1.7) | -1(1.5) | 34(1.7) | 0(1.2) | 44(2.4)* | -4(1.6) | 44(2.2) | -2(1.8) | 41(2.1) | -3(1.7) |

**Table 5.2**
Continued from previous page

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| CAIx (%) | Baseline | 6(4.6) | | 6(4.8) | | 3(4.1) | | 20(4.1) | | 16(4.6) | | 15(4) | |
| | 1 hour | 8(3.8) | 2(1.7) | 6(4.2) | 0(2.7) | -1(4.1) | -4(2.3) | 22(3.9) | 2(1.0) | 16(4.7) | 0(2.1) | 15(5) | 0(1.3) |
| *Post exercise* | +10min | 8(4.0) | 2(2.9) | 9(3.5) | 3(2.5) | 4(4.0) | 0(3.6) | 18(3.1) | -2(2.3) | 10(3.7)† | -6(2.0) | 9(4)† | -6(1.4) |
| | +40min | 4(4.0) | -2(3.3) | 4(3.8) | -2(3.5) | -1(3.8) | -4(2.6) | 15(4.2) | -5(2.6) | 10(4.3)* | -7(1.4) | 10(4)* | -5(1.9) |
| | +60min | 4(4.1) | -2(2.3) | 1(4.3) | -5(2.2) | -2(3.6)* | -5(2.2) | 16(4.0) | -4(2.1) | 12(4.6) | -4(2.3) | 10(4)* | -5(1.7) |
| CAIx@75 (%) | Baseline | 2(7.4) | | -1(5.6) | | -2(4.5) | | 15(4.3) | | 14(4.6) | | 11(4.4) | |
| | 1 hour | 2(7.2) | 0(1.4) | -1(5.0) | 0(2.5) | -6(4.6) | -4(1.3) | 14(4.2) | -1(1.3) | 11(4.6) | -2(2.1) | 10(4.4) | -1(2.2) |
| *Post exercise* | +10min | 13(5.2)* | 11(4.1) | 12(3.4)† | 13(3.2) | 9(3.7)* | 11(4.3) | 17(3.6) | 3(1.9) | 14(3.7) | 0(1.9) | 12(3.8) | 1(1.6) |
| | +40min | 5(6.4) | 3(3.3) | 2(3.9) | 3(2.5) | -1(3.6) | 1(2.7) | 12(4.1) | -3(2.2) | 9(4.4)* | -5(1.9) | 10(4.1) | -1(1.4) |
| | +60min | 4(6.3) | 1(2.3) | -1(4.5) | 0(2.1) | -3(3.7) | -1(2.1) | 12(4.1) | -3(2.2) | 10(4.5) | -4(1.4) | 9(3.6) | -2(1.6) |

**Table 5.2**
Continued from previous page

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| RAIx (%) | Baseline | 55(5.7) | | 55(6.0) | | 50(5.6) | | 70(5.9) | | 66(6.6) | | 65(6.2) | |
| | 1 hour | 58(5.8) | 2(1.7) | 55(5.5) | 0(2.3) | 46(5.7)* | -4(1.6)* vs P | 73(5.4) | 2(1.6) | 67(6.4) | 2(2.7) | 66(6.1) | 0(1.7) |
| *Post exercise* | +10min | 48(4.7)† | -6(2.5) | 47(5.2)† | -8(2.4) | 48(6.1) | -2(4.2) | 67(5.0) | -3(4.1) | 58(4.6)* | -8(2.7) | 56(5.2)† | -9(2.2) |
| | +40min | 49(5.6)* | -6(2.4) | 46(5.1)† | -9(2.7) | 42(5.4)† | -8(2.7) | 67(5.2) | -3(1.6) | 59(5.4)* | -7(2.6) | 60(5.8)* | -5(2.2) |
| | +60min | 48(5.7)† | -7(1.8) | 48(5.2)† | -6(2.0) | 42(5.3)† | -8(2.3) | 69(5.5) | -1(1.6) | 62(5.8) | -4(2.4) | 60(6.0)* | -5(1.9) |

**Table 5.2**
Continued from previous page

| | | Normotensives | | | | | | Hypertensives | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | sildenafil | | hydralazine | | placebo | | sildenafil | | hydralazine | |
| | | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline | Mean (SEM) | Δ from baseline |
| PWV (m/s) | Baseline | 6.4(0.32) | | 6.3(0.28) | | 6.3(0.26) | | 8.7(0.55) | | 8.7(0.55) | | 8.4(0.40) | |
| | 1 hour | 6.5(0.31) | 0.1(0.11) | 6.4(0.38) | 0.1(0.18) | 6.3(0.26) | 0(0.12) | 8.8(0.56) | 0(0.14) | 8.6(0.57) | -0.1(0.15) | 8.5(0.47) | 0.1(0.19) |
| *Post exercise* | +10min | 6.5(0.22) | 0(0.15) | 6.5(0.27) | 0.2(0.13) | 6.8(0.29)‡ | 0.5(0.11)* vs P | 9.1(0.61) | 0.4(0.14) | 8.6(0.50) | -0.1(0.14) | 8.7(0.53) | 0.3(0.20) |
| | +40min | 6.6(0.32) | 0.1(0.10) | 6.2(0.25) | 0(0.20) | 6.4(0.26) | 0.1(0.11) | 9.3(0.65)* | 0.6(0.30) | 8.4(0.51) | -0.3(0.17)† vs P and H | 8.7(0.55) | 0.3(0.22) |
| | +60min | 6.5(0.29) | 0(0.14) | 6.2(0.31) | 0(0.14) | 6.3(0.25) | 0(0.09) | 9.4(0.65)* | 0.6(0.24) | 8.5(0.58) | -0.2(0.13)† vs P | 8.4(0.52) | 0(0.17) |

**Table 5.3. CF-PWV at baseline, 1 hour after drug administration and after exercise.**
For absolute values comparisons are against baseline; for Δ (change) from baseline comparisons are among placebo, sildenafil and hydralazine.
*P<0.05, †P< 0.01, ‡P<0.001. P, placebo; H, hydralazine.

| | | STEP TEST - SYSTOLIC BP | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | placebo | | | sildenafil | | | hydralazine | | |
| | | Mean (SEM) | | | Mean (SEM) | | | Mean (SEM) | | |
| | | Absolute value | Δ from baseline | %Δ from baseline | Absolute value | Δ from baseline | %Δ from baseline | Absolute value | Δ from baseline | %Δ from baseline |
| ***Normotensives*** | | | | | | | | | | |
| **Systolic BP (mmHg)** | **Baseline** | 122(2.1) | | | 122(2.2) | | | 121(2.1) | | |
| | **1 hour** | 123(2.6) | 2(1.6) | 1.2(1.3) | 119(2.0) | -2(1.5) | -1.5(1.3) | 121(2.0) | 1(1.3) | 0.8(1.1) |
| | **BP@3min** | 150(4.6)* | 28(3.1) | 22.7(2.3) | 144(2.7)* | 23(2.2) | 18.9(2.1) | 149(3.4)* | 28(2.2) | 23.5(1.8) |
| ***Hypertensives*** | | | | | | | | | | |
| **Systolic BP (mmHg)** | **Baseline** | 151(2.1) | | | 146(3.9) | | | 144(2.9) | | |
| | **1 hour** | 150(3.3) | -1(1.3) | -0.6(1.1) | 141(2.0) | -5(2.7) | -2.9(1.6) | 141(2.0) | -3(2.8) | -1.4(1.9) |
| | **BP@3min** | 181(3.6)*† | 30(3.4) | 20.1(2.3) | 170(4.2)*† | 24(4.4) | 17.2(3.0) | 175(4.0)*† | 31(4.1) | 22.2(3.1) |

**Table 5.4. Systolic BP response after 3 minutes of step test**.
For absolute values comparisons are against baseline; Δ, change. For Δ and %Δ from baseline comparisons are among placebo, sildenafil and hydralazine. *P<0.001 *vs* baseline; †P<0.001 *vs* normotensives.

| | Normotensives | | | Hypertensives | | |
|---|---|---|---|---|---|---|
| | **placebo** | **sildenafil** | **hydralazine** | **placebo** | **sildenafil** | **hydralazine** |
| | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) |
| Peak $VO_2$ (ml/kg/min) | 34.6(1.8) | 35.2(2.0) | 34.1(1.7) | 26.7 (1.6)† | 26.9 (1.5)† | 26.1 (1.5)† |
| % of predicted peak $VO_2$ | 99(5.0) | 100(5.0) | 98(5.0) | 80(2.0)* | 81(3.0)† | 79(3.0)* |
| $VO_2$ at AT (ml/kg/min) | 16.8(1.0) | 16.5(0.9) | 17.5(1.2) | 13.7(0.7)* | 13.6(0.8)* | 14.1(0.8)* |
| Peak workload (W) | 241.3(12.7) | 242.7(11.6) | 241.3(11.2) | 201.3(11.4)* | 204.0(11.5)* | 198.7(11.4)* |
| Peak RER | 1.16 (0.01) | 1.14(0.01) | 1.15(0.01) | 1.15(0.02) | 1.13(0.01) | 1.14(0.02) |
| Peak HR (bpm) | 162.3(5.4) | 165.2(4.9) | 166.1(4.8) | 150.2(4.8) | 155.9(3.7) | 157.4(5.0) |
| Peak $O_2$ pulse (ml/beat) | 17.2(0.6) | 17.0(0.7) | 16.6(0.6) | 15.4(0.9) | 15.1(1.0) | 14.4(0.8)‡ |
| $\Delta VO_2/\Delta WR$ (ml/min/W) | 9.5(0.1) | 9.6(0.2) | 9.4(0.1) | 8.3(0.3)† | 8.4(0.3)† | 8.2(0.2)† |
| Exercise time (min) | 13.1(0.6) | 13.2(0.5) | 13.2(0.5) | 11.3(0.5)* | 11.4(0.5)* | 11.2(0.5)* |

**Table 5.5. Cardiopulmonary exercise testing data after placebo, sildenafil and hydralazine.**
AT, anaerobic threshold; W, watts; RER, respiratory exchange ratio; HR, heart rate; WR, work rate; Δ, change. *P<0.05 *vs* normotensives; †P<0.01 *vs* normotensives; ‡P< 0.05 *vs* placebo.

| | Normotensives | | | Hypertensives | | |
|---|---|---|---|---|---|---|
| | **placebo** | **sildenafil** | **hydralazine** | **placebo** | **sildenafil** | **hydralazine** |
| | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) | Mean (SEM) |
| Peak exercise systolic BP, absolute values (mmHg) | 197(3.6) | 190(4.1) | 197(3.1) | 220(2.4)† | 214(3.6)† | 216(3.2)† |
| Peak exercise systolic BP, Δ from before exercise (mmHg) | 72 (4.2) | 70(3.6) | 75(4.5) | 71(3.5) | 71(5.2) | 72(4.0) |
| Peak exercise systolic BP, Δ% from before exercise (%) | 58.8(4.7) | 59.0(3.3) | 63.8(4.7) | 47.7(2.9) | 51.1(4.4) | 50.3(3.4) |

**Table 5.5.**
Continued from previous page

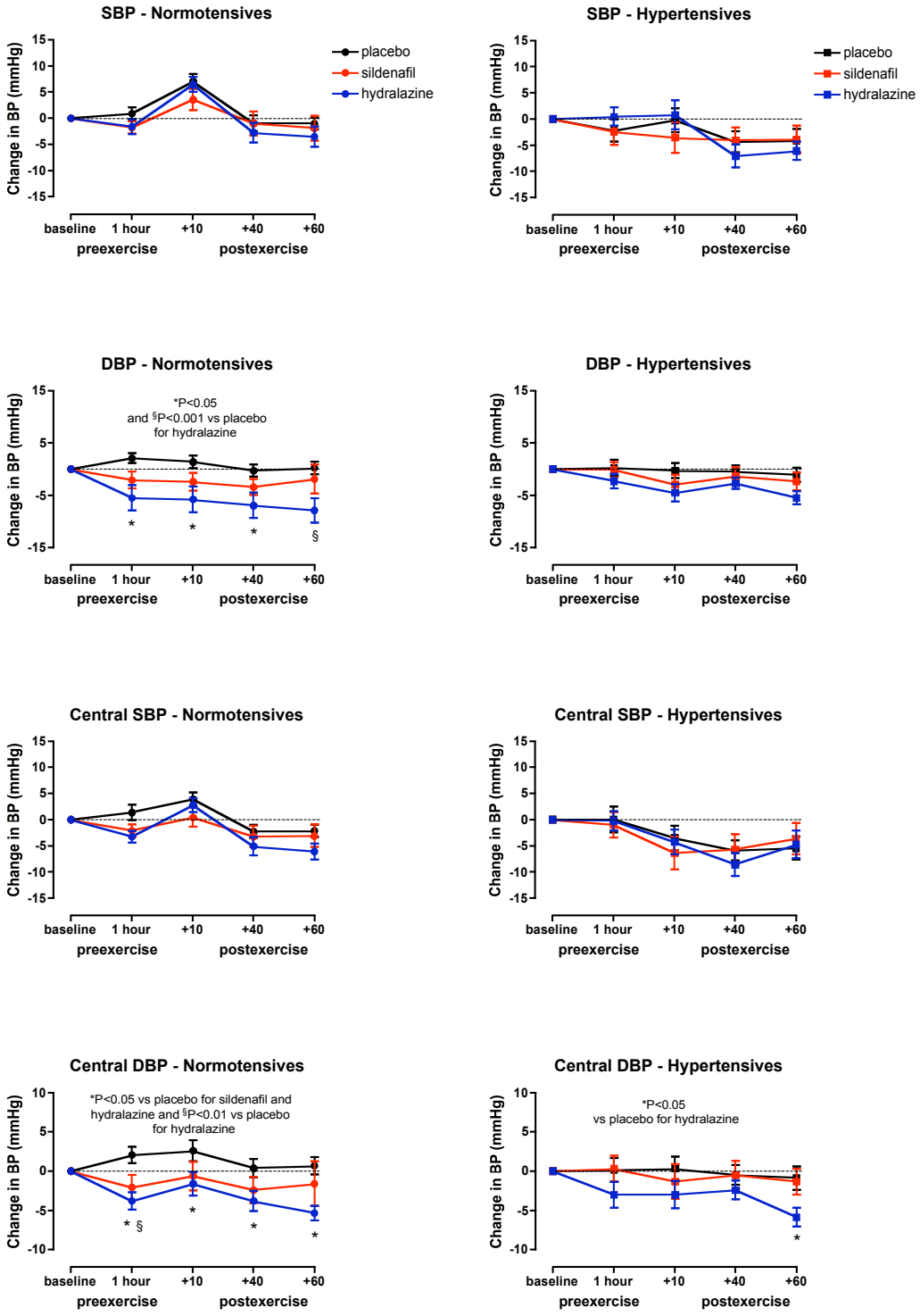


**Figure 5.2. Changes from baseline in peripheral and central systolic and diastolic BP in normotensive and hypertensive subjects**.
SBP, systolic blood pressure; DBP, diastolic blood pressure.

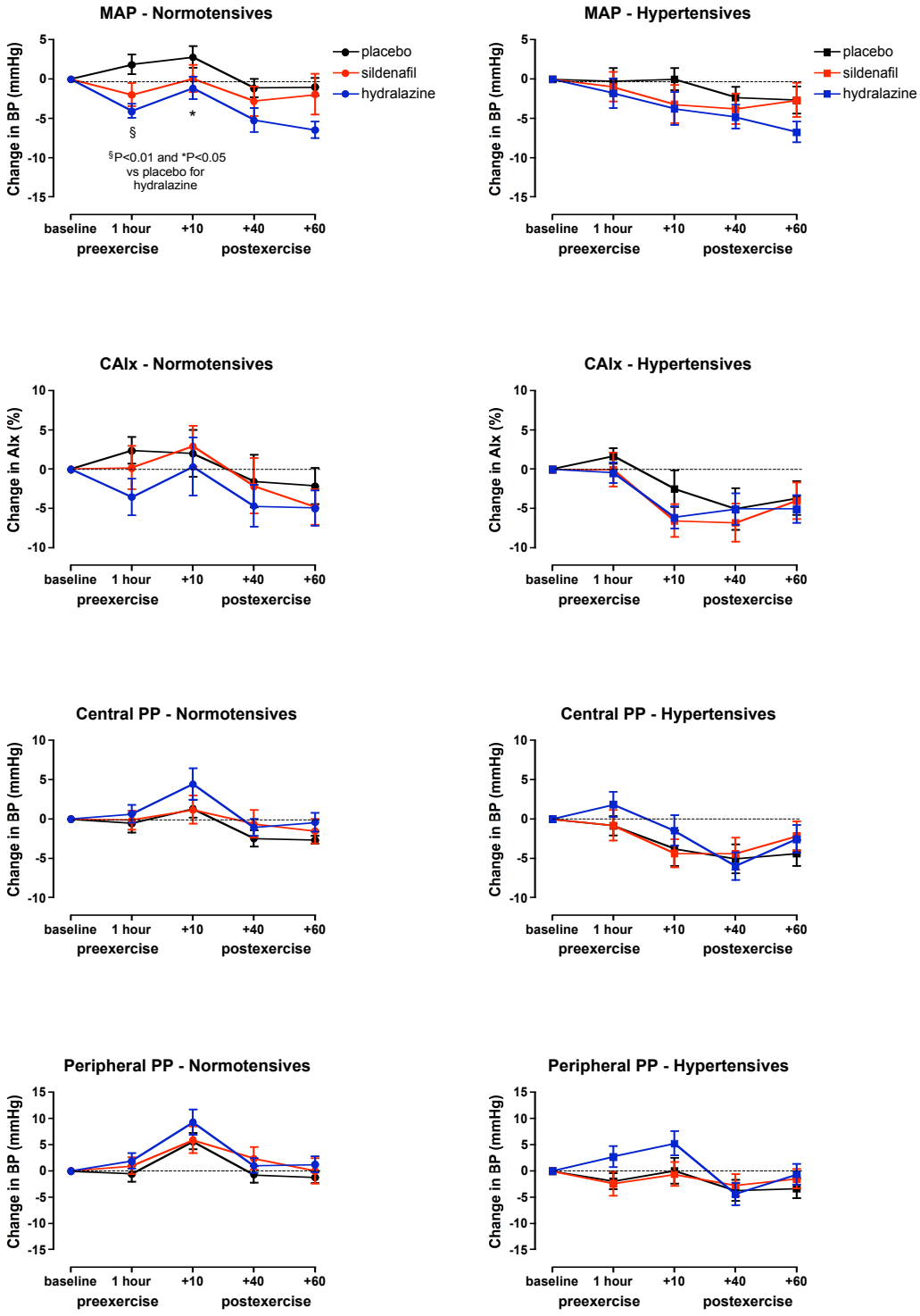


**Figure 5.3. Changes from baseline in mean arterial pressure, peripheral and central PP and CAIx in normotensive and hypertensive subjects.**
MAP, mean arterial pressure; CAIx, central augmentation index; PP, pulse pressure.

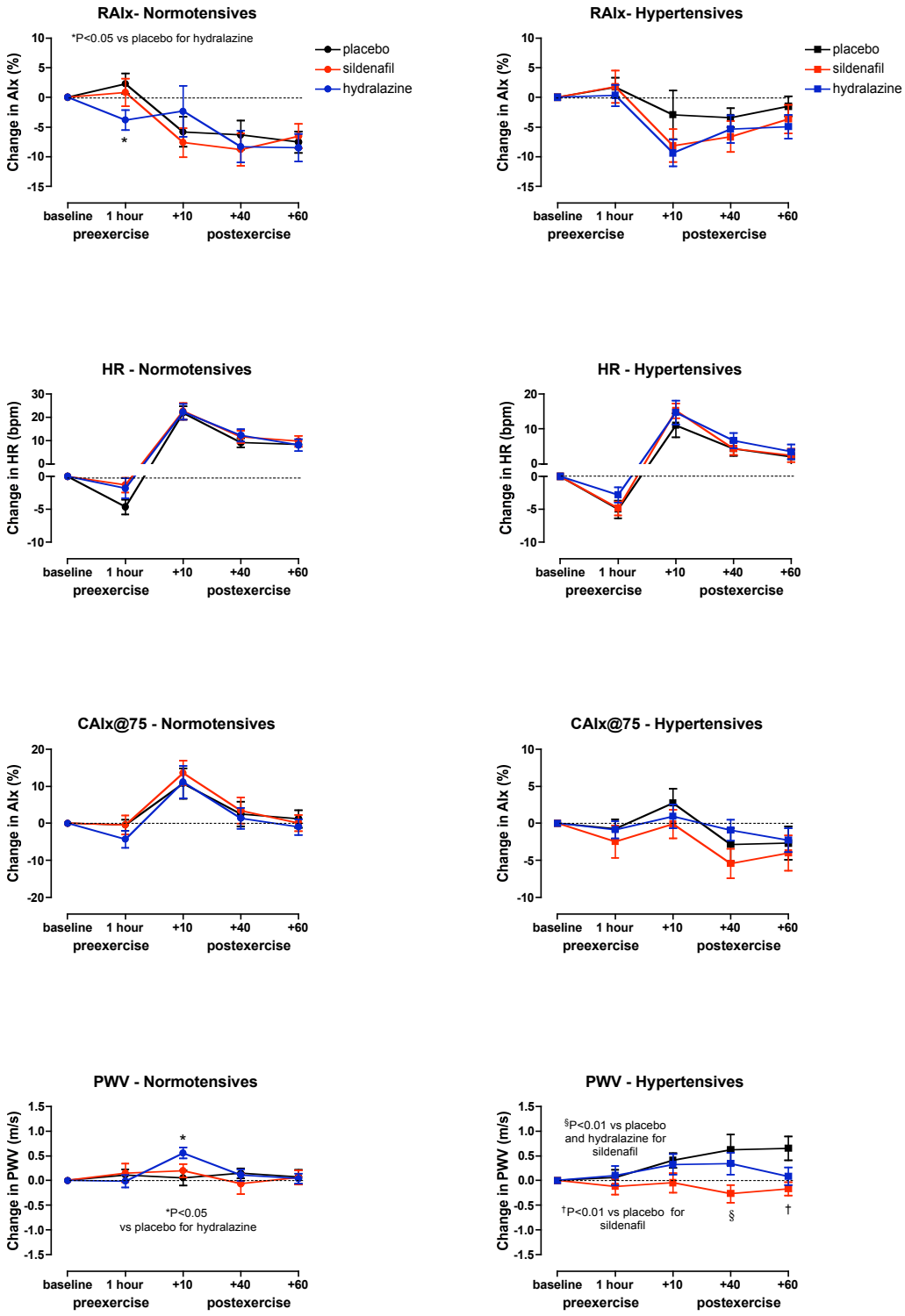


**Figure 5.4. Changes from baseline in RAIx, HR, CAIx@75 and PWV in normotensive and hypertensive subjects.**

RAIx, radial augmentation index; HR, heart rate; CAIx@75, CAIx adjusted to standard HR of 75bpm; PWV, pulse wave velocity.

### 5.3.6 Subjects withdrawals and adverse effects

Two subjects were withdrawn from the study because of severe headache, one while taking sildenafil and the other while on placebo. A list of the adverse effects experienced by hypertensive and normotensive subjects is given in Table 5.6 and Table 5.7, respectively. Dyspepsia was the most common symptom reported in the hypertensive group while on sildenafil, followed by headache and low back pain. Nasal congestion, headache and dry throat were reported while on hydralazine. In the normotensive group, headache was the most common symptom reported while taking sildenafil, followed by dyspepsia and low back pain. Headache was also the most common symptom reported while on hydralazine.

Among the subjects who experienced dyspepsia, 9 took over-the-counter acid suppression medications, with resolution of the symptoms in few days. The next most common symptom was mild to moderate headache, for which 11 subjects took paracetamol; however, in 2 subjects, one from the hypertensive and the other from the normotensive group, headache was severe and led to discontinuation of the treatment and withdrawn from the study. A number of subjects also experienced low back/buttock/leg pain of mild to moderate intensity at the beginning of treatment with sildenafil. These symptoms usually lasted for the first 2 to 3 days and were responsive to paracetamol.

| Hypertensives (n=16) | Sildenafil | | | | Hydralazine | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | Mild | Mod | Severe | **Total** | Mild | Mod | Severe | **Total** | Mild | Mod | Severe |
| **Indigestion/heartburn** | **8 (50)** | 5 (31) | 3 (19) | | **2 (12)** | 1 (6) | 1 (6) | | **1 (6)** | 1 (6) | | |
| **Headache** | **6 (37)** | 3 (19) | 2 (12) | 1 (6) | **2 (12)** | | 2 (12) | | **1 (6)** | 1 (6) | | |
| **Back/buttock/leg ache** | **4 (25)** | 2 (12) | 2 (12) | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Fatigue** | **0** | | | | **1 (6)** | 1 (6) | | | **1 (6)** | | | |
| **Facial flushing** | **2 (12)** | 2 (12) | | | **0** | | | | **0** | | | |
| **Cramp** | **1 (6)** | 1 (6) | | | **0** | | | | **1 (6)** | | 1 (6) | |
| **Dry throat** | **0** | | | | **2 (12)** | 1 (6) | 1 (6) | | **0** | | | |
| **Nasal congestion** | **0** | | | | **3 (19)** | 1 (6) | 2 (12) | | **0** | | | |
| **Insomnia** | **1 (6)** | | 1 (6) | | **0** | | | | **2 (12)** | 1 (6) | 1 (6) | |
| **Joint pain** | **1 (6)** | | 1 (6) | | **0** | | | | **0** | | | |
| **Loin pain** | **2 (12)** | | 2 (12) | | **0** | | | | **0** | | | |
| **Nausea** | **1 (6)** | | 1 (6) | | **0** | | | | **1 (6)** | 1 (6) | | |
| **Neck pain** | **1 (6)** | 1 (6) | | | **0** | | | | **0** | | | |
| **Urinary frequency** | **0** | | | | **1 (6)** | | 1 (6) | | **0** | | | |
| **Anxiety** | **0** | | | | **1 (6)** | | 1 (6) | | **0** | | | |
| **Diarrhoea** | **0** | | | | **0** | | | | **1 (6)** | | | 1 (6) |

**Table 5.6. Symptoms experienced with sildenafil, hydralazine and placebo in all hypertensive subjects recruited.**
Values are numbers (percentages) of subjects. Mod, moderate.

| Normotensives (n=16) | Sildenafil | | | | Hydralazine | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Total** | Mild | Mod | Severe | **Total** | Mild | Mod | Severe | **Total** | Mild | Mod | Severe |
| **Indigestion/heartburn** | **3 (19)** | 1 (6) | 2 (12) | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Headache** | **4 (25)** | 2 (12) | 2 (12) | | **4 (25)** | 3 (19) | 1 (6) | | **1 (6)** | | | 1 (6) |
| **Back/buttock/leg ache** | **2 (12)** | 2 (12) | | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Fatigue** | **1 (6)** | | 1 (6) | | **1 (6)** | 1 (6) | | | **1 (6)** | | | |
| **Facial flushing** | **0** | | | | **0** | | | | **0** | | | |
| **Cramp** | **1 (6)** | 1 (6) | | | **0** | | | | **1 (6)** | 1 (6) | | |
| **Dry throat** | **0** | | | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Nasal congestion** | **0** | | | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Insomnia** | **1 (6)** | | 1 (6) | | **0** | | | | **1 (6)** | 1 (6) | | |
| **Joint pain** | **0** | | | | **0** | | | | **0** | | | |
| **Loin pain** | **1 (6)** | | 1 (6) | | **0** | | | | **0** | | | |
| **Nausea** | **0** | | | | **1 (6)** | 1 (6) | | | **0** | | | |
| **Neck pain** | **0** | | | | **0** | | | | **0** | | | |
| **Urinary frequency** | **0** | | | | **0** | | | | **0** | | | |
| **Anxiety** | **0** | | | | **1 (6)** | | 1 (6) | | **0** | | | |
| **Diarrhoea** | **0** | | | | **0** | | | | **0** | | | |

**Table 5.7. Symptoms experienced with sildenafil, hydralazine and placebo in all normotensive subjects recruited.**
Values are numbers (percentages) of subjects. Mod, moderate.

## 5.4 DISCUSSION

This study confirmed, as anticipated, that hypertensive patients have reduced exercise capacity compared with normotensive subjects. However, PDE5 inhibition did not affect peak $VO_2$ or the systolic BP response to exercise. Nevertheless, the results shown that PDE5 inhibition reduces the post-exercise increase in PWV found in hypertensive but not in normotensive subjects, an effect that was not observed with placebo or hydralazine treatment.

### 5.4.1 Blood pressure, pulse wave analysis and pulse wave velocity in normotensive and hypertensive subjects

After placebo, central and peripheral BP, parameters of arterial wave reflection and arterial stiffness were significantly higher in hypertensive patients than normotensive subjects at all time points. Furthermore, in the recovery period after exercise, PWV significantly increased compared with baseline values in hypertensive patients, whereas this increase was not observed in the normotensive group. Studies performed in healthy subjects report either a reduction (Naka *et al*., 2003) or no change (Munir *et al*., 2008) in PWV after exercise, but no data are available for hypertensive individuals. Findings from this study indicate that, unlike healthy subjects, in hypertensive patients arterial distensibility is reduced in the recovery period after exercise, consistent with an impaired vascular response to exercise.

### 5.4.2 Maximal exercise capacity in normotensive and hypertensive subjects

Hypertensive subjects exhibited a lower exercise capacity, measured as peak $VO_2$, than normotensive individuals, which is in agreement with previous evidence (Fagard *et al*., 1988; Lim *et al*., 1996). Not only peak $VO_2$ but also $VO_2$ at anaerobic threshold was significantly lower in hypertensive individuals, as well as the changes in the $VO_2$/work rate relationship, which reflects a smaller increase in $VO_2$ with work rate compared with normotensive individuals. Overall, this suggests an earlier occurrence of anaerobic metabolism during exercise, leading to early fatigue and reduced exercise time. This response may be the result of impaired oxygen delivery to the working muscles and

central and peripheral factors may play an important role. Indeed, there is evidence of an impaired left ventricular relaxation and suboptimal ventricular filling with reduced stroke volume during exercise from the earliest stages of hypertension (Lund-Johansen, 1980; Lim *et al*., 1996). Stroke volume may also be reduced due to the higher afterload present in hypertension (Goodman *et al*., 1992; Modesti *et al*., 1999).

### 5.4.3 Effects of sildenafil on blood pressure, pulse wave analysis and pulse wave velocity before exercise

In hypertensive patients, at baseline, BP was lower after drug treatment compared with the placebo arm, indicating a BP lowering effect of both drugs after 1 week of treatment. However, on the study day, 1 hour after drug administration, BP was not significantly different from baseline, in agreement with findings reported in the study by Oliver and coworkers (Oliver *et al*., 2006), thus suggesting an attenuation of the acute BP lowering effect of sildenafil after chronic administration. This may be possibly related to the activation of counterregulatory mechanisms, such as the renin-angiotensin system, stimulated by the initial vasodilatation-mediated reduction in BP.

In the current study sildenafil did not significantly reduce parameters of arterial wave reflection, measured as CAIx, CAIx@75 or RAIx, either after 1 week of drug treatment or 1 hour after drug administration; the same was observed for PWV. However, although not significant, there was a trend to a progressive reduction of CAIx and RAIx in the hypertensive group, with P values of 0.07 and 0.06, respectively. Therefore, the possibility of a significant effect on arterial wave reflection should not be dismissed, and it is possible that, with a larger sample size, or a longer duration of treatment, a significant effect could have been observed. Other studies have reported an effect of sildenafil on arterial wave reflection (see section 1.6.4), mainly single-dose studies in which interactions with other drugs could have affected, at least in part, the observed effect. In the study by Oliver and coworkers (Oliver *et al*., 2006), acute administration of sildenafil reduced arterial wave reflection and a similar, smaller effect was observed after chronic administration (16 days), although this was not different from placebo, in

agreement with findings reported in the current study. In addition, hypertensive individuals in the study by Oliver and coworkers were significantly older than the hypertensive subjects recruited in the study presented here (mean age 60 *vs* 48 years, respectively) and, as such, most likely to have stiffer arteries. Indeed, they had substantially higher baseline values of all indexes of arterial wave reflection, thus making the detection of a small effect of sildenafil on wave reflection more likely.

### 5.4.4 Effects of sildenafil on exercise systolic BP

The effects of sildenafil on systolic BP response to exercise were investigated, on separate days, during a low-intensity exercise test (step test) and during maximal exercise effort, and compared with placebo and hydralazine. On each occasion, hypertensive subjects had a higher resting and exercise systolic BP, but the magnitude of the exercise-induced increase in systolic BP was not significantly different from normotensive subjects, and the type of treatment made no difference overall. The evidence for an exaggerated systolic BP response to exercise is not uniform in the literature, and there are data showing that hypertensive patients, despite a higher resting BP, may present an absolute increase in exercise BP similar to normotensive individuals (Palatini, 1994), in agreement with findings reported in the current study. Alternatively, with regard to the step test, it is possible that its low intensity and short duration (3 minutes) were not sufficient to detect a different BP response to exercise between normotensive and hypertensive subjects. With respect to systolic BP at peak exercise, it is worth noting that, unlike the step test, in which BP was measured after 3 minutes of exercise in both groups, during cardiopulmonary exercise testing normotensive subjects were able to exercise for longer and reached a higher peak workload than hypertensive subjects, and this may contribute to explaining the lack of difference in peak exercise systolic BP observed between the two groups.

### 5.4.5 Effects of sildenafil on parameters of exercise capacity

In the study presented, PDE5 inhibition did not affect peak $VO_2$ or any of the other parameters measured at peak exercise in hypertensive patients. This suggests that,

unlike conditions such as pulmonary hypertension and heart failure, an impaired NO-cGMP pathway does not significantly contribute to the reduced systemic exercise capacity in arterial hypertension. One potential explanation for this difference may derive from studies conducted in patients with pulmonary hypertension and heart failure (Galie *et al*., 2005; Lewis *et al*., 2007a). In these studies, the favourable effects observed with PDE5 inhibition seem to be consistently related to a reduction of pulmonary vascular resistance. Indeed, the improved exercise capacity reported with sildenafil in patients with heart failure is mainly observed in those with secondary pulmonary hypertension (Lewis *et al*., 2007b). In support of this, and in agreement with the findings observed in the control group in the present study, there is current evidence showing that, in healthy subjects, sildenafil improves exercise capacity under hypoxia, which elicits a pulmonary hypertensive response, but not under normoxia (Hsu *et al*., 2006). Other factors may also have influenced the ability to detect an effect of sildenafil on peak $VO_2$ in the hypertensive patients recruited for the current study. As a consequence of the inclusion criteria, they represented a carefully selected group with a single risk factor, and it cannot be excluded that in hypertensive individuals with additional risk factors such as hypercholesterolaemia, diabetes or cigarette smoking, PDE5 inhibition might improve exercise capacity. However, the main aim of the study was to investigate the effects of PDE5 inhibition in hypertension, without such confounding risk factors.

The study presented in Chapter 4 showed that sildenafil improves the vasodilator response to handgrip exercise in hypertensive patients, suggesting that the NO-cGMP system contributes to exercise hyperaemia in arterial hypertension, and its impairment affects the vasodilator response to exercise. However, in the current study, the hypothesized effect of sildenafil on exercise capacity was not observed. How can these apparently contrasting findings be explained? First, it is important to note the different exercise modalities employed in the studies, i.e. small muscle mass exercise *vs* whole body exercise. Exercise involving large muscle groups produces changes in systemic haemodynamics that are not observed with local exercise involving the upper limb,

which evokes only small haemodynamic changes. Second, most of the evidence supporting an important role for the NO-cGMP system in exercise hyperaemia derives from studies performed using local exercise (Gilligan *et al*., 1994; Schrage *et al*., 2004), including the forearm study presented in Chapter 4, while evidence derived from studies involving large muscle groups seems conflicting (Radegran, 1997; Bradley *et al*., 1999). Alternatively, it may be possible that forearm blood flow is not a reliable tool to investigate exercise-induced changes in blood flow, and may not reflect exercise responses at the systemic level. Furthermore, the different methodologies used to measure blood flow in those studies (femoral thermodilution and Doppler/ultrasound *vs* plethysmography) may account for the differences observed. In fact, when using venous occlusion plethysmography, flow is measured during the rest periods between contractions, representing 'post-exercise' rather than 'exercise' hyperaemia. When all these elements are considered, a more consistent agreement emerges on the contribution of the NO-cGMP system in the post-exercise period rather than during exercise (Radegran & Saltin, 1999; Green *et al*., 2004; Joyner & Wilkins, 2007).

### 5.4.6 Effects of sildenafil on pulse wave velocity and pulse wave analysis after exercise

After exercise, sildenafil reduced CAIx and RAIx in hypertensive patients, an effect that was not observed in healthy subjects. However, a similar reduction was observed after hydralazine, with no difference between the two treatments. With respect to PWV, when the effects of the 3 interventions after exercise were compared, no significant difference was observed in healthy controls. By contrast, in the hypertensive patients, a significant difference was detected amongst the 3 treatments in favour of sildenafil, which reduced PWV after exercise, an effect that was not observed with the control vasodilator hydralazine. Between 10 and 60 min after exercise sildenafil did not cause any significant change in BP, therefore changes in distending pressure cannot explain the observed increase in arterial distensibility, suggesting that this may be due to a direct effect of the drug on the arterial wall. Heart rate has also been reported to influence PWV (Lantelme *et al*., 2002), but this has been considered by others as an

artefact of the methodology used to measure PWV (Hayward *et al*., 2002). In any case, in this study, changes in HR were not significantly different amongst treatment arms in the recovery period after exercise. Because pharmacologic inhibition of NOS abolishes the vasodilator effect of PDE5 inhibitors (Kass *et al*., 2007), findings observed in the recovery period after exercise are likely to be explained by the increased activity of cGMP, acting as a second messenger for NO. In this context, PDE5 inhibition could exert a beneficial effect by improving arterial distensibility and reducing cardiac workload after exercise. This is particularly relevant when considering that, unlike healthy individuals, in whom exercise training has been shown to improve dynamic arterial compliance (Tanaka *et al*., 2000), exercise does not seem to improve arterial stiffness in hypertension (Stewart, 2002). Combining PDE5 inhibition with exercise may therefore provide additional benefits in active hypertensive individuals.

### 5.4.7 Tolerability

A number of side effects related to sildenafil treatment were experienced by the study participants, headache and dyspepsia in particular. Headache was the most common side effects reported by normotensive individuals when on sildenafil (25%), followed by dyspepsia (19%) and low back pain (12%). In the hypertensive group, dyspepsia was reported by 50% of subjects, a frequency substantially higher than the frequency reported by Goldstein and coworkers (16% of individuals taking sildenafil 100mg) (Goldstein *et al*., 1998). However, in that study, sildenafil was used intermittently and at a lower dose, whereas the frequency of dyspepsia reported by Oliver and coworkers, in which the dose of sildenafil used was the same as the current study, was 40% (Oliver *et al*., 2006). A possible mechanism for the dyspeptic symptoms may be attributed to the inhibitory effect of sildenafil on the contractile activity of the oesophageal smooth muscle cells, resulting in decreased lower oesophageal sphincter tone and residual pressure as well as contraction amplitude, so that gastric contents reflux into the oesophagus (Eherer *et al*., 2002). This might increase the risk of chronic reflux oesophagitis and Barrett's oesophagus, warranting further investigation of the effects of chronic PDE5 inhibition on the lower oesophagus.

Headache was reported by 37% of hypertensive subjects, a frequency higher than reported by Goldstein and coworkers (30% of subjects taking sildenafil 100mg) and by Oliver and coworkers (32%). In another study by Galie and coworkers, in which sildenafil was given regularly for 12 weeks to patients with pulmonary hypertension, headache occurred in 42% of subjects taking 40 mg three times daily and in 49% of subjects taking 80 mg three times daily (Galie *et al*., 2005). Low back pain and buttock/leg muscle ache was reported by 4 subjects (25%) in the current study. These symptoms were not reported as side effects of sildenafil in the study by Goldstein and coworkers (only symptoms that occurred in 5% or more subjects were reported). However, other studies have reported myalgia as a side effect of sildenafil (Olsson *et al*., 2000; Osegbe *et al*., 2003; Eardley *et al*., 2005). In the pulmonary hypertension study, myalgia was reported by 14% of subjects taking 80 mg three times daily (Galie *et al*., 2005), and in the study by Oliver and coworkers, low back/buttock/leg muscle ache was reported by 28% of subjects (Oliver *et al*., 2006). In the latter study, plasma creatine kinase levels were measured in 4 subjects and were within the normal laboratory range, suggesting that the muscle aches experienced by some subjects were not due to an underlying myositis.

It is clear that side effects from sildenafil were relatively frequent in the current study, and one subject was withdrawn because of severe headache. This could represent a limit for the potential use of sildenafil in arterial hypertension. However, in most cases, side effects were transient and self-limiting and, if patients were adequately informed of their possible occurrence, the likelihood of long-term compliance might increase.

### 5.4.8 Summary

In this study, PDE5 inhibition with sildenafil produced no changes in exercise capacity in hypertensive subjects. However, by reducing arterial stiffness in the recovery period after exercise, PDE5 inhibition may offer a therapeutic benefit in active, hypertensive individuals.

# CHAPTER 6

## CONCLUSIONS AND FUTURE DIRECTIONS

## 6.1 THE EFFECTS OF PDE5 INHIBITION ON FOREARM EXERCISE-INDUCED VASODILATATION

A number of studies have attempted to determine the role of the NO system during exercise hyperaemia in the human forearm, with different results, either in favour (Gilligan *et al*., 1994; Dyke *et al*., 1995; Maxwell *et al*., 1998; Duffy *et al*., 1999) or against (Wilson & Kapoor, 1993; Endo *et al*., 1994) its major involvement. However, none of these studies have specifically addressed the role of the NO-cGMP pathway in exercise hyperaemia. In the study presented in Chapter 4, sildenafil was used as a pharmacological probe to test the hypothesis behind the studies presented in this thesis, supported by data showing that sildenafil improves vasomotor response in smokers (Kimura *et al*., 2003), in patients with heart failure (Katz *et al*., 2000) and Raynaud's phenomenon (Fries *et al*., 2005), and also improves exercise capacity in patients with pulmonary hypertension (Michelakis *et al*., 2003; Galie *et al*., 2005) and heart failure (Lewis *et al*., 2007a). Findings from the study suggest that an important role is played by the NO system during exercise in hypertensive patients, and that an intact endothelium is necessary for a normal response to the haemodynamic changes induced by exercise. Importantly, the present data indicate that, in hypertensive patients, the reduced vasodilator response to handgrip exercise is susceptible to improvement with PDE5 inhibition, while this benefit is not observed in healthy subjects, where the functional integrity of the endothelium is able to fully respond to the increased blood flow and shear stress that occur during exercise.

The reduced activity of the NO-cGMP pathway *per se* is probably not the only explanation for the findings observed in the study, as vascular function is also influenced by the powerful vasoconstrictor endothelin-1 (ET-1). In a healthy endothelium, the NO system counterbalances the potent and long-lasting vasoconstrictor effects of ET-1 (Haynes & Webb, 1994) and seems also to regulate ET-1 production via a cyclic GMP-dependent pathway (Boulanger & Luscher, 1990; Kelly *et al*., 2004). In endothelial dysfunction, a large body of evidence suggests that an augmented ET-1 activity is

involved, reflecting a shift in the balance between vasodilators and vasoconstrictors (Kedzierski & Yanagisawa, 2001). In agreement with this, and with particular regard to vascular responsiveness during exercise, are previous findings showing that an enhanced vasoconstrictor response to ET-1 limits exercise-induced vasodilatation in hypertensive subjects (McEniery *et al*., 2002). Together with the results shown here, this suggests that the vasodilator response to exercise is likely to be the result of a crosstalk between increased vasodilator influences and decreased vasoconstrictor influences. In support of this are physiological studies in animals showing that ET-1 mediated vasoconstriction at rest decreases during exercise (Merkus *et al*., 2003), therefore contributing to vasodilatation, and that pre-treatment with a NO synthase inhibitor enhances the vasodilator response to ET receptor blockade during exercise (Houweling *et al*., 2005). Thus, under conditions of endothelial dysfunction, vasoconstrictor influences might prevail, leading to a reduced vasodilator response during exercise. In summary, it is likely that more than one substance is involved in the vasodilatation observed in skeletal muscles during exercise, each contributing to a variable extent, depending on physiological factors, such as muscle fibre type, exercise intensity, time after initiation of exercise, and the presence of pathophysiological conditions such as endothelial vasomotor dysfunction. In this context, future studies investigating the combined effects of PDE5 inhibition and ET-1 antagonism in hypertensive individuals could be particularly useful, and contribute to our understanding of the complex mechanisms involved in the vascular response to exercise and overall exercise capacity in health and disease status.

### 6.1.1 Forearm plethysmography and exercise hyperaemia

Strain gauge plethysmography, while representing a reliable method to assess changes in blood flow in resting muscle beds, does not allow measurements of blood flow during contractions, but only during brief rest periods between contractions. For this reason, it might be argued that this flow represents ‘postexercise’ and not ‘exercise’ hyperaemia. However, the majority of data available on exercise hyperaemia are the results of studies performed with this technique (Joyner *et al*., 2001) and, although postexercise

hyperaemia may not exactly mimic exercise hyperaemia, the effect observed with the PDE5 inhibitor sildenafil was not observed with verapamil, providing justification for further investigation of this approach. For this reason, a similar study in which exercise-induced vasodilatation was measured by other methodologies such as dilution techniques and Doppler/ultrasound (Saltin *et al*., 1998) would allow for a comparison of the effects of PDE5 inhibition on exercise hyperaemia. Furthermore, the importance of differences related to the type of fibre within and among skeletal muscles should be considered, as this may also contribute to explaining the conflicting reports in the literature about the role of NO in exercise-induced vasodilatation. Indeed, there is evidence suggesting that the relative importance of vascular control mechanisms during exercise varies as a function of skeletal muscle fibre type (Delp & Laughlin, 1998). Thus, future studies need to be designed to enhance our understanding of the various vascular control mechanisms in muscles composed of different fibre types, in which case the effect of PDE5 inhibition may vary depending on the type of muscle fibre investigated.

## 6.2 THE EFFECTS OF PDE5 INHIBITION ON SYSTEMIC EXERCISE CAPACITY AND ARTERIAL STIFFNESS

### 6.2.1 PDE5 inhibition and systemic exercise capacity

Perhaps surprisingly, given the literature for other cardiovascular conditions such as pulmonary hypertension and heart failure, PDE5 inhibition did not improve exercise capacity in the study presented in Chapter 5. However, hypertensive patients recruited for the study represented a highly selected group where hypertension was the only risk factor, and it is possible that, in presence of additional risk factors such as diabetes or hypercholesterolaemia, PDE5 inhibition might improve exercise capacity, something that would need to be investigated in future studies. Nevertheless, findings from the study show that sildenafil does not have a negative impact on exercise performance in hypertensive individuals. This is particularly relevant given current evidence showing that PDE5 inhibitors may be useful in the treatment of hypertension (Oliver *et al*., 2006; Wolk *et al*., 2009), and that hypertension is one of the most common comorbidities in patients with erectile dysfunction (Kloner *et al*., 2003b; Solomon *et al*., 2003), many of whom use sildenafil or other PDE5 inhibitors for an extended period of time.

### 6.2.2 PDE5 inhibition and arterial stiffness

To date, most of the available evidence on the effects of an acute bout of exercise on arterial stiffness derives from studies performed in healthy subjects (Kingwell *et al*., 1997; Naka *et al*., 2003; Heffernan *et al*., 2007), with very little evidence of studies of this kind being performed in hypertensive individuals and few investigating the pattern of changes in the recovery period following exercise. Unlike normotensive subjects, in the untrained hypertensive patients recruited for this study, exercise resulted in a significant increase in PWV during the recovery period, contributing to increasing left ventricular afterload in the context of an already stiff arterial system. PDE5 inhibition, by reversing these changes, may offer an additional beneficial effect in active, hypertensive individuals. Indeed, 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 in this study may also suggest a progressive reduction in the intrinsic stiffness of large arteries.

### 6.2.3 Duration of action and tolerability

The use of sildenafil in clinical practice may be limited by its short duration of action, because drugs with a duration of action long enough to allow once-daily administration are preferred in the management of hypertension, to maximise patient compliance with the treatment. Therefore, long-acting PDE5 inhibitors such as tadalafil, which can be administered once daily, might be more suitable. Alternatively, if modified release preparations of sildenafil or vardenafil were developed, these might also represent a valid option. Furthermore, research to identify new and more selective PDE5 inhibitors continues at a significant level, as shown by recent data (Wolk *et al*., 2009), and studies comparing both efficacy and tolerability of PDE5 inhibitors with established antihypertensive medications would help to determine their place in clinical practice.

Side effects from oral sildenafil were relatively frequent, and this could represent a limit for the potential use of this drug in arterial hypertension. Dyspepsia in particular was one of the most common symptoms reported with regular treatment. As such, a thorough investigation of the chronic effects of PDE5 inhibitors on the lower oesophagus would be warranted, because of the possibility of an increased risk in oesophageal neoplasia subsequent to chronic oesophagitis.

# CHAPTER 7

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# APPENDIX

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# Phosphodiesterase type 5 inhibition reverses impaired forearm exercise-induced vasodilatation in hypertensive patients

Teresa M. Attinà[a], Lorenzo S. Malatino[b], Simon R. Maxwell[a], Paul L. Padfield[c] and David J. Webb[a]

***Objective*** **Established hypertension is characterized by increased peripheral vascular resistance and endothelial dysfunction, features that may underlie the reduced exercise-induced vasodilatation seen in hypertensive patients. Sildenafil citrate is a phosphodiesterase type 5 (PDE5) inhibitor used clinically for the treatment of male erectile dysfunction. Its vasodilating properties are due to the inhibition of cyclic guanosine monophosphate (cGMP) breakdown and prolongation of the signalling actions of the nitric oxide (NO)–cGMP pathway in vascular smooth muscle cells. Sildenafil has beneficial effects on endothelial function and exercise tolerance in congestive heart failure and pulmonary hypertension, and we hypothesized that it would improve exercise-induced vasodilatation in hypertensive patients.**

***Methods and results*** **Ten hypertensive patients and ten matched normotensive subjects were studied in a three-way, randomized, single-blind and placebo-controlled study. On each study day, forearm blood flow (FBF) responses to handgrip exercise were assessed before and after intra-arterial (brachial) infusion of sildenafil, verapamil (a control, cGMP-independent vasodilator), and saline (placebo). Preinfusion exercise-induced vasodilatation was significantly reduced in hypertensive patients compared to normotensive controls. Sildenafil and verapamil infusions both caused a similar increase in baseline FBF. However, while verapamil did not affect the vasodilator response to handgrip exercise in either group, sildenafil substantially enhanced this response in hypertensive patients, but not in normotensive subjects.**

***Conclusions*** **Our data suggest that sildenafil, through an increase in cGMP levels in the vasculature, substantially and selectively improves the vasodilator response to handgrip exercise in hypertensive patients. These findings represent an essential first step in support of further studies exploring the potentially beneficial effects of PDE5 inhibition on impaired exercise capacity in hypertension.** ***J Hypertens*** **26:501–507 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

**Journal of Hypertension** 2008, **26**:501–507

**Keywords: cGMP, exercise, hypertension, nitric oxide, phosphodiesterase 5, sildenafil (Viagra)**

**Abbreviations: ANOVA, analysis of variance; BMI, body mass index; cGMP, cyclic guanosine monophosphate; DBP, diastolic blood pressure; ET-1, endothelin-1; FBF, forearm blood flow; FMD, flow-mediated dilatation; MAP, mean arterial pressure; MVC, maximum voluntary contraction; NO, nitric oxide; PDE5, phosphodiesterase type 5; SBP, systolic blood pressure; SEM, standard error of the mean; sGC, soluble guanylate cyclase; SWG, standard wire gauge**

[a]Clinical Pharmacology Unit, Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, UK, [b]Unit of Internal Medicine and Hypertension Centre, Teaching Hospital of Ragusa, University of Catania, Italy and [c]Department of Medical Sciences, Western General Hospital, Edinburgh, UK

Correspondence to Teresa Attinà, Clinical Pharmacology Unit and Research Centre, University of Edinburgh, Western General Hospital, Crewe Road, Edinburgh, EH4 2XU, Scotland, UK
Tel: +44 131 537 1826; fax: +44 131 343 6017;
e-mail: tattina@ed.ac.uk

**Received** 7 May 2007 **Revised** 19 September 2007
**Accepted** 19 October 2007

## Introduction

Nitric oxide (NO) is a powerful vasodilating substance, released by the endothelium, which plays a major role in the maintenance of normal vascular function and tone [1,2]. The main physiological stimulus to endothelial NO production is increased blood flow through the vessel lumen [3,4], resulting in increased vascular wall shear-stress that is sensed by the endothelium and translated into a vasodilator response [5,6]. NO has been demonstrated to be essential for this flow-mediated dilatation in human peripheral conduit and resistance vessels [7,8], raising the possibility that it may also contribute to exercise-induced vasodilatation. This is supported by evidence from animal [9,10] and human studies [11,12] showing that NO release in response to increased vascular wall shear stress contributes to exercise hyperaemia, and inhibition of NO synthesis reduces exercise-induced vasodilatation in healthy subjects. Endothelial dysfunction is characterized by alterations of the NO system and reduced NO-dependent vasodilatation, and is found in association with many cardiovascular conditions [13], including arterial hypertension, in which it has been demonstrated at the level of both resistance and conduit arteries [14,15]. Hence, endothelial dysfunction could contribute to the impaired exercise-induced vasodilatation [16] and reduced exercise capacity observed in hypertensive patients [17].

This work was presented at the 16th European Meeting on Hypertension (Madrid, Spain; June 2006) and the 21st Scientific Meeting of the International Society of Hypertension (Fukuoka, Japan; October 2006).

NO exerts its vasodilator effect on blood vessels through the activation of intracellular soluble guanylate cyclase (sGC) and consequent formation of cyclic guanosine monophosphate (cGMP), the main second messenger for NO, which ultimately leads to relaxation of smooth muscle [18]; this NO-cGMP pathway is regulated by the activity of synthesizing enzymes (sGC) and catabolizing enzymes (phosphodiesterase, PDE). Sildenafil citrate is a selective inhibitor of PDE type 5 (PDE5), the predominant isozyme responsible for the degradation of cGMP in smooth muscle cells. It acts by decreasing the rate of cGMP breakdown, thus enhancing its effects on vascular smooth muscle cell tone [19]. We, therefore, hypothesized that, in hypertensive patients, in whom endothelial dysfunction is a feature, the impaired vasomotor response to exercise might be improved by sildenafil and, if confirmed, this would be a key, first step in support of further studies exploring the effects of PDE5 inhibition on exercise capacity in arterial hypertension. To test our hypothesis, we assessed the forearm blood flow (FBF) response to a previously validated handgrip exercise task [16,20] in hypertensive and normotensive subjects before and after the local (brachial artery) administration of sildenafil, and compared this response with the one obtained after the infusion of the calcium-channel blocker verapamil, a cGMP-independent vasodilator (control), and placebo (saline). All drugs were infused at locally active doses.

## Methods

### Subjects

Ten hypertensive men were recruited from the Cardiovascular Risk Clinic, Western General Hospital, Edinburgh. Participants were enrolled if they had sustained systolic blood pressure (BP) >160 mmHg and/or diastolic BP >90 mmHg measured in a sitting position on at least three different occasions. All were newly diagnosed hypertensives who had not previously received antihypertensive treatment. Those with evidence of a secondary form of hypertension or with other risk factors were excluded, thus representing a highly selected group. At the same time, 10 healthy subjects were recruited from the community as a control group, on the basis of a systolic BP < 140 mmHg and diastolic BP < 80 mmHg; they were age and cholesterol matched with the hypertensive patients. All participants underwent physical examination and screening laboratory tests. Subjects with hypercholesterolaemia (total cholesterol > 6.5 mmol/l) and diabetes mellitus were excluded. All subjects were non-smokers and non-obese (body mass index; BMI < 30 kg/m$^2$). The study was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki. Written informed consent was obtained from each subject before entry into the study.

### Handgrip exercise

The handgrip task was performed with a calibrated handgrip dynamometer (MLT 003 Hand Dynamometer; ADInstruments Pty Ltd, Chalgrove, Oxfordshire, UK), as described previously [16,20]. In brief, subjects rhythmically squeezed the device using the non-dominant arm in 15-s cycles, consisting of 5 s of steady handgrip pressure alternating with 10 s of rest, and they were instructed to avoid Valsalva-like manoeuvres during the task. The exercise was performed for 5 min at 45% of maximum voluntary contraction (MVC), which was determined for each subject during the screening visit. FBF was determined in the last 3 min of exercise, during the 10-s period of relaxation between contractions, immediately before the next handgrip contraction.

### Drugs

The brachial artery of the non-dominant arm of each subject was cannulated with a 27-SWG needle, under local anaesthesia (1% lidocaine; Hameln Pharmaceuticals Gmbh, Hameln, Germany) for drug infusions. Sildenafil citrate, a kind gift from Pfizer (Pfizer Ltd, Sandwich, Kent, UK), was infused at 50 μg/min; the cGMP-independent control vasodilator verapamil (Abbott Laboratories Ltd, Queenborough, Kent, UK) was infused at 5 μg/min; normal saline (Baxter Healthcare Ltd, Thetford, UK) was infused during a control, non-vasodilating study. Drugs were infused for 6 min at 1 ml/min; the doses of sildenafil and verapamil used in the study were selected on the basis of previously published literature [21–23]. In preliminary dose-ranging studies we conducted in healthy subjects, the selected doses produced a similar increase in FBF, and the effects reached a plateau around the period in which handgrip exercise was performed. These preliminary studies also confirmed the local (forearm) effect of the doses used, and no changes in either BP or heart rate following the infusions were reported (data not shown).

### Measurements

#### *Forearm venous plethysmography*

Arterial vasodilatation in the forearm was measured as change in FBF using venous occlusion plethysmography, with mercury-in-silastic strain gauges securely applied around the widest part of the forearm. The hand was excluded from the blood flow determination through inflation of a wrist cuff to 220 mmHg. An upper arm cuff was intermittently inflated to 40 mmHg for 10 s in every 15 s to temporarily prevent forearm venous outflow and obtain plethysmographic recordings. FBF recordings were made over a 3-min period. The mean of the final five measurements was used for analysis. Blood flow was measured simultaneously in both arms by use of a dual-channel strain gauge plethysmograph (D.E. Hokanson, Bellevue, Washington, USA), as described previously [24].

#### *Blood pressure*

Blood pressure was monitored, with an appropriate-sized cuff, in the non-infused arm by use of a validated

oscillometric sphygmomanometer (HEM-705CP; Omron Healthcare UK Ltd, Milton Keynes, UK) [25].

#### *Forearm vascular resistance*

Forearm vascular resistance was calculated as mean arterial pressure (MAP) divided by forearm blood flow [26].

### General study design

The study was conducted with a three-way, randomized, single-blind and placebo-controlled design (Fig. 1). Subjects attended our research centre at 0900 h on three different occasions, at least one week apart, and after an overnight fast (>12 h). They were required to abstain from alcohol and caffeine-containing food and beverages from 24 h before the study. All visits were performed in a quiet, draught-free, temperature-controlled room (22–24°C). On each study day, subjects rested quietly for 30 min and during this time FBF measurements were recorded. Preinfusion handgrip exercise was then performed, with FBF assessed in the final 3 min of the exercise, during each of the 10-s relaxation periods between contractions. After a recovery period of 30 min, the brachial artery of the exercised arm was cannulated and, following a 30-min saline infusion, subjects received a 6-min infusion of sildenafil, verapamil or placebo (saline), in random order, with FBF recordings during the last 3 min of infusion. The cannula was then removed and handgrip exercise and FBF measurements repeated.

### Statistical analyses

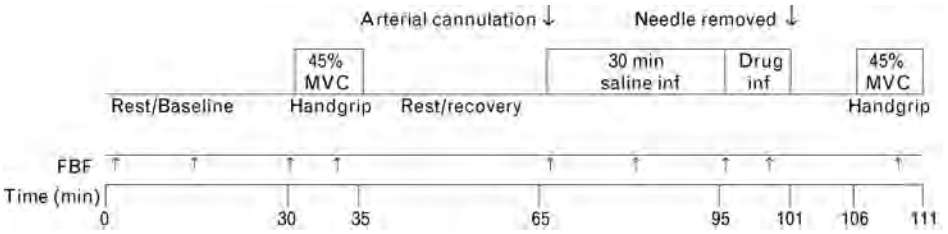
Plethysmographic data were extracted from Chart data files and analysed by an independent observer blinded to the treatment. All values are expressed as mean ± SEM. FBF data are presented as changes in absolute blood flow (ml/min per 100 ml of forearm volume) in the infused (exercised) arm. Preinfusion and postinfusion FBF responses to handgrip exercise for the three treatments were then compared within each group. Data were analysed with repeated-measures analysis of variance (ANOVA) with post-hoc Bonferroni corrections and two-tailed Student's *t*-test as appropriate. Statistical analysis was performed with Graph-Pad Prism (GraphPad Software, Inc., San Diego, California, USA). Significance was accepted at the 5% level in all cases.

## Results

The clinical characteristics of the two groups are shown in Table 1, and they differ only by BP. As shown in Table 2, resting FBF was similar in hypertensives and normotensives on each study day. Preinfusion handgrip exercise produced an increase in FBF in both groups; however, the increase observed in hypertensive patients was significantly less than that in the control group ($P < 0.001$) (Fig. 2). BP tended to increase in hypertensive patients, but not in normotensive controls, on each of the three study visits during handgrip exercise, but this did not reach statistical significance (Table 3). In the hypertensive group, changes in forearm vascular resistance after handgrip exercise did not differ significantly between sildenafil and verapamil, whereas a significant difference, in favour of sildenafil, was detected between sildenafil and saline ($P < 0.01$) (data not shown). Heart rate did not change in either group.

The effects of the three intra-arterial infusions on resting FBF are presented in Table 2. FBF did not change significantly in either group after the infusion of saline. The infusion of sildenafil and verapamil produced significant vasodilatation compared to saline, resulting in increased FBF in both groups ($P < 0.001$). The extent of vasodilatation was similar between the two treatments and with no significant difference between hypertensive and normotensive subjects. BP and heart rate did not change after any of the infusions. None of the subjects reported adverse effects related to the local infusion of the study drugs. In the normotensive subjects, vasodilator response to handgrip exercise did not change significantly after the infusions when compared to preinfusion values, and no significant difference was detected amongst the three treatments ($P = 0.35$) (Fig. 3a). In the hypertensive group, a significant difference in the response to handgrip exercise was observed amongst the three treatments ($P = 0.0167$); indeed, while no significant changes were detected in the vasodilator response to exercise following the infusion of saline or verapamil, a significant

**Fig. 1**




Schematic representation of the experimental protocol. Drug infusion (inf) refers to 6-min intrabrachial infusion of sildenafil (50 μg/min), verapamil (5 μg/min) or saline. FBF, forearm blood flow; MVC, maximum voluntary contraction.

**Table 1 Clinical characteristics of the hypertensive patients and normotensive subjects**

| Parameter | Hypertensive patients ($n=10$) | Normotensive subjects ($n=10$) | $P^a$ |
|---|---|---|---|
| Age (years) (range) | 46 ± 4 (31–67) | 43 ± 3 (28–59) | NS |
| Body mass index (kg/m$^2$) | 27.4 ± 0.9 | 25.7 ± 0.8 | NS |
| Total cholesterol (mmol/l) | 4.8 ± 0.2 | 4.7 ± 0.2 | NS |
| Systolic blood pressure (mmHg) | 170 ± 2 | 123 ± 4 | < 0.05 |
| Diastolic blood pressure (mmHg) | 97 ± 3 | 68 ± 2 | < 0.05 |
| Creatinine (μmol/l) | 91.7 ± 4.2 | 86.8 ± 2 | NS |
| Urea (mmol/l) | 5.1 ± 0.3 | 5.2 ± 0.4 | NS |
| Sodium (mmol/l) | 142 ± 0.7 | 141 ± 0.6 | NS |
| Potassium (mmol/l) | 4.2 ± 0.09 | 4.4 ± 0.08 | NS |
| Fasting glucose (mmol/l) | 5.4 ± 0.1 | 5.1 ± 0.1 | NS |

Data are mean ± SEM; NS, not significant. [a]Differences between groups were evaluated by unpaired Student's *t*- test (patients versus controls).

improvement was observed after sildenafil, which significantly increased exercise-induced vasodilatation compared to both verapamil and saline ($P < 0.05$) (Fig. 3b).

## Discussion

The present study provides evidence in support of a beneficial effect of the PDE5 inhibitor sildenafil citrate on the vascular response to handgrip exercise in hypertensive patients. We have confirmed previous work showing that the forearm vasodilator response to handgrip exercise is reduced in hypertensive patients compared with normotensive subjects [16]. Also, and the major novel observation from this work, we have shown that sildenafil substantially enhances exercise-induced vasodilatation in hypertensive patients but not in normotensive subjects, an effect that is not seen with the control dilator verapamil. These findings suggest that cGMP signalling is critical in regulating this flow limitation, given that it can be selectively improved by PDE5 inhibition.

### Vascular responses to intra-arterial infusions

The effects of local sildenafil infusion in our study are consistent with previous data, which demonstrate an increase in FBF after intra-arterial administration of sildenafil in healthy subjects [23]. With regard to the control vasodilator used in this study, verapamil, we recognize that no perfect control exists, especially in studies comparing hypertensive and normotensive subjects, whose starting conditions differ. However, if anything, studies show an increased response to verapamil in hypertensive subjects [21]. We chose verapamil because of its cGMP-independent effect [22], and selected a dose that produced a comparable vasodilator effect to that of sildenafil on FBF (Table 2). As resting FBF was similar in both groups before the second bout of exercise, as it was for preinfusion exercise, potential influences of different resting FBF, and inherent vascular tone, on the subsequent vasodilator response to exercise in each group were eliminated.

### Vasodilator response to handgrip exercise

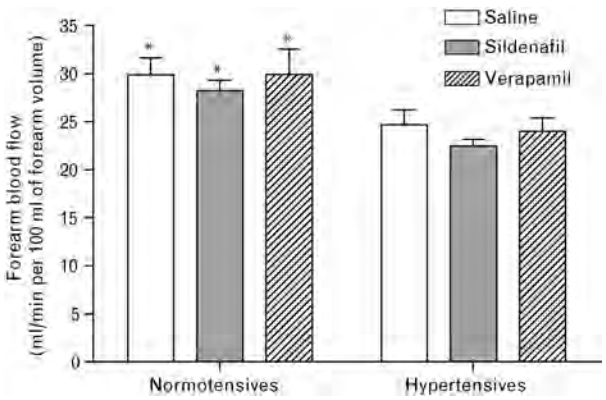
On each study day, maximum preinfusion vasodilator response to handgrip exercise was significantly less in patients with hypertension than in healthy controls. We did not directly address endothelial function in our subjects, but these findings are consistent with results obtained in a previous study from our group [16], and suggest that reduced endothelium-mediated vasodilatation may limit vascular responsiveness to shear stress, contributing to increased vascular resistance during exercise. In healthy controls, when we compared the effects of infusions on the vasodilator response to handgrip exercise, no significant difference was observed amongst the three treatments. By contrast, in the hypertensive patients, we detected a significant and substantial difference amongst the three treatments in favour of sildenafil, which selectively improved the response, whereas this

**Table 2 Forearm blood flow (FBF) values before and during infusions**

| | Hypertensive subjects | | | Normotensive subjects | | |
|---|---|---|---|---|---|---|
| | Infused | Non-infused | Ratio | Infused | Non-infused | Ratio |
| Before infusion | | | | | | |
| Saline | 4.05 ± 0.39 | 3.80 ± 0.36 | 1.10 ± 0.08 | 3.22 ± 0.37 | 3.45 ± 0.43 | 0.98 ± 0.08 |
| Sildenafil | 3.34 ± 0.25 | 3.10 ± 0.30 | 1.14 ± 0.09 | 3.35 ± 0.26 | 4.04 ± 0.62 | 0.91 ± 0.07 |
| Verapamil | 3.93 ± 0.59 | 3.71 ± 0.35 | 1.05 ± 0.07 | 3.96 ± 0.54 | 4.06 ± 0.59 | 0.99 ± 0.06 |
| During infusion[a] | | | | | | |
| Saline | 4.07 ± 0.33 | 3.71 ± 0.32 | 1.14 ± 0.08 | 3.35 ± 0.36 | 3.53 ± 0.38 | 0.98 ± 0.08 |
| Sildenafil | 5.58 ± 0.53* | 3.30 ± 0.32 | 1.79 ± 0.12 | 5.92 ± 0.55* | 3.86 ± 0.42 | 1.62 ± 0.15 |
| Verapamil | 6.44 ± 0.68* | 3.68 ± 0.40 | 1.78 ± 0.11 | 6.20 ± 0.50* | 3.59 ± 0.40 | 1.82 ± 0.13 |

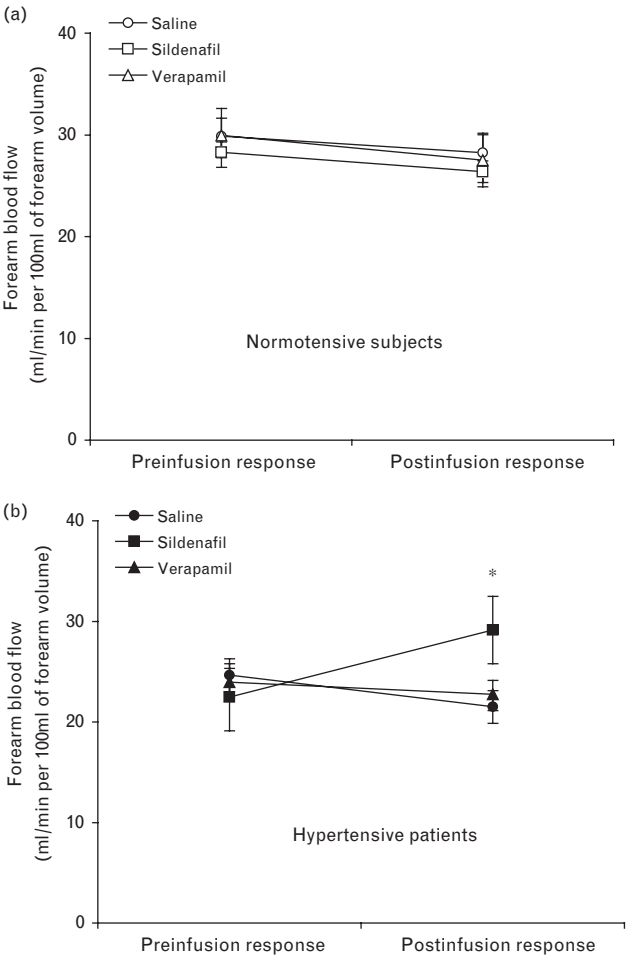
Data are mean ± SEM absolute FBF (ml/min per 100 ml of forearm volume) in the infused and non-infused arm, and FBF ratio (infused/non-infused) before and during the infusion of saline, sildenafil and verapamil. [a]FBF measurements during the last 3 min of drug infusion. * $P < 0.001$ infused versus non-infused arm.

**Fig. 2**




Preinfusion exercise vasodilator responses. Changes in absolute blood flow (ml/min per 100 ml of forearm volume) in the infused (exercised) arm in response to handgrip exercise before saline, sildenafil and verapamil infusion. Data are mean ± SEM. $*P < 0.001$ in normotensive subjects versus hypertensive patients.

**Fig. 3**




Preinfusion and postinfusion exercise vasodilator responses. Changes in absolute blood flow (ml/min per 100 ml of forearm volume) in the infused (exercised) arm in response to exercise after 6-min intra-arterial infusion of saline, sildenafil (50 μg/min) or verapamil (5 μg/min) in (a) normotensive subjects and (b) hypertensive patients. Data are mean ± SEM. $*P < 0.05$ versus saline and verapamil in the hypertensive patients.

was not observed with the control, cGMP-independent, vasodilator verapamil. We consider this the most important finding of our study, supporting the involvement of the NO-cGMP pathway in the vasodilator response to exercise. A number of studies have attempted to determine the role of the NO system during exercise hyperaemia in the human forearm, with different results, either in favour of [11,12,27,28] or against [29,30] its major involvement; however, none of these studies have addressed specifically the role of the NO-cGMP pathway in exercise hyperaemia. It has been shown previously that inhibition of NO synthase abolishes the vasodilatation mediated by PDE5 antagonism [31,32], so our observations are likely to be explained by the increased activity of cGMP, which acts as a second messenger for NO and is ultimately responsible for smooth muscle cell relaxation. Further support for this explanation is provided by a recently published study in which sildenafil, through an increased level of cGMP in the vasculature, reversed vascular alterations in an experimental model of chronic NO deprivation [33]. Therefore, in situations associated with alterations of the NO-cGMP pathway, PDE5 inhibition could exert beneficial effects by increasing the intracellular level of cGMP and potentially contribute to restoring physiological responses.

**Table 3 Blood pressure values before and after handgrip exercise**

| | Hypertensive subjects | | | Normotensive subjects | | |
|---|---|---|---|---|---|---|
| | SBP | DBP | MAP | SBP | DBP | MAP |
| Before handgrip | | | | | | |
| Saline | 154 ± 3 | 75 ± 3 | 99 ± 3 | 125 ± 5 | 67 ± 3 | 85 ± 3 |
| Sildenafil | 153 ± 4 | 78 ± 4 | 105 ± 4 | 123 ± 4 | 65 ± 2 | 84 ± 2 |
| Verapamil | 154 ± 2 | 79 ± 4 | 106 ± 2 | 127 ± 5 | 70 ± 3 | 90 ± 3 |
| After handgrip | | | | | | |
| Saline | 159 ± 5 | 77 ± 4 | 102 ± 4 | 128 ± 5 | 70 ± 3 | 89 ± 3 |
| Sildenafil | 157 ± 4 | 82 ± 4 | 109 ± 3 | 125 ± 4 | 66 ± 2 | 87 ± 2 |
| Verapamil | 160 ± 3 | 81 ± 3 | 110 ± 4 | 131 ± 5 | 71 ± 3 | 92 ± 3 |

DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure. Data are mean ± SEM, mmHg.

Previous studies have shown conflicting results of the effects of sildenafil on brachial artery flow-mediated vasodilatation (FMD) [34–36]. In particular, in a previous study from our group [37], sildenafil had no effect on FMD in hypertensive patients. However, it should be noted that the stimulus for FMD is (passive) reactive hyperaemia, which occurs in response to a temporary occlusion of the vessel, whereas the present study investigated the effects of sildenafil on (active) exercise hyperaemia. This is a more complex phenomenon, in which the pattern of blood flow changes observed is the result of an

integrated response also involving the skeletal muscle and the resistance arterioles. Furthermore, as blood flow progressively increases during sustained exercise, the involvement of the NO-cGMP pathway may become more prominent, and this might contribute to the explanation of the effect of sildenafil on exercise-induced vasodilatation in our hypertensive subjects.

The reduced activity of the NO-cGMP pathway *per se* is probably not the only explanation for our observations, as other factors might contribute to the reduced exercise-induced vasodilatation in hypertensive patients, such as the powerful vasoconstrictor endothelin-1 (ET-1). Indeed, in endothelial dysfunction, a large body of evidence suggests that ET-1 activity is augmented, reflecting a shift in the balance between vasodilators and vasoconstrictors [38]. Consistent with this, and with particular regard to vascular responsiveness during exercise, are previous findings from our group demonstrating that an enhanced vasoconstrictor response to ET-1 limits exercise-induced vasodilatation in hypertensive subjects [16].

In this study, we used sildenafil as a pharmacological probe to test our hypothesis, supported by data showing that sildenafil improves vasomotor response in smokers [39], in patients with heart failure [40] and Raynaud's phenomenon [41], and also improves exercise capacity in patients with pulmonary hypertension [42,43] and heart failure [44]. Furthermore, PDE5 inhibition is a promising therapeutic approach in arterial hypertension, as evidenced by a recently published study in which regular sildenafil treatment effectively lowered BP compared to placebo [37]. Although the use of sildenafil in clinical practice may be limited by its short duration of action, longer-acting PDE5 inhibitors are already available and others are currently in development. Results from this study represent a preliminary and essential step in support of a more complete exploration of the role of PDE5 inhibition on exercise capacity in hypertension, which will more fully elucidate the mechanisms underlying our observations and determine their clinical impact.

### Study limitations

Strain gauge plethysmography, while representing a reliable method to assess changes in blood flow in resting muscle beds, does not allow measurements of blood flow during contractions, but only during brief rest periods between contractions. For this reason, it might be argued that this flow represents 'postexercise' and not 'exercise' hyperaemia; however, the majority of data we have on exercise hyperaemia are the results of studies performed with this technique [45] and, although postexercise hyperaemia may not exactly mimic exercise hyperaemia, the effect we observed with the PDE5 inhibitor sildenafil was not observed with verapamil, providing justification for further investigation of this approach.

Hypertensive patients recruited for the study represented a highly selected group where hypertension was the only risk factor. We did not address endothelial dysfunction in these patients (as in previously published studies) [16], but the BP cut-off point (160/90 mmHg) was chosen in order to enhance the differences between the hypertensive and normotensive subjects, presumably including endothelial function.

In our study we did not measure local cGMP levels, as venous cannulation would have interfered with handgrip exercise. However, measurement of cGMP levels after PDE5 inhibition has a high intersubject variability [23] and seems not to correlate well with vasodilatation [46].

## Acknowledgements

The study was supported by a scholarship from the Italian Society of Hypertension and by an unrestricted grant from Pfizer United Kingdom Ltd (>US$10 000).

There are no conflicts of interest.

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