EFFECTS OF SINGLE-DOSE AND SHORT-TERM ORAL CAPTOPRIL ON HEPATIC BLOOD FLOW AND HAEMODYNAMICS IN MILD TO MODERATE HYPERTENSIVE PATIENTS

by

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ABSTRACT

The angiotensin converting enzyme inhibitor, captopril has been extensively used in the treatment of mild to moderate hypertension and congestive heart failure. Although the haemodynamic changes associated with acute and/or chronic captopril therapy have been extensively studied, there is conflicting information of its influence on hepatic blood flow (Q_H). Most of the human studies have been investigated the influence of captopril on Q_H after single-dose administration and very few data are available on its effects after chronic therapy. The most frequently used method to estimate Q_H is based on the hepatic clearance of indocyanine green (ICG) from the blood.

This thesis reports i) the effects of single-dose (100 mg) and two-weeks (100 mg/day) captopril treatment on Q_H, assessed by ICG clearance, in six mild to moderate hypertensive male subjects, ii) the changes in the area under the serum concentration-time curve (AUC) and plasma clearance (Cl_{pICG}) of ICG, iii) the changes in blood pressure, heart rate and splanchnic vascular resistance (SVR) before and after acute and short-term captopril therapy, iv) the effects of postural change from sitting to upright on Cl_{pICG} and Q_H, blood pressure, heart rate and SVR before and after two-weeks captopril therapy, v) the effects of acute and two-weeks captopril treatment on some important pharmacokinetic parameters of unchanged captopril. There were two study days two-weeks apart (day 1 and day 14). Q_H was estimated at baseline seated, upright, reseated and 1 hour after captopril dosing on the first study day. The same procedures were repeated in the same patients following 14 days captopril therapy. The serum concentration of ICG was

determined by spectrophotometric analysis following intravenous ICG dose of 0.45 mg/kg.

There was a slight ≈ 5% and ≈ 6% decrease, but, no significant change in Cl_{pICG} and Q_H, respectively, whether expressed in absolute terms or per unit body weight or body surface area, following the initial and terminal doses of captopril. A significant 25%-29% ($p = 0.04$) increase in ICG plasma clearance after two-weeks captopril treatment was observed in all of the four study phases (seated, upright, reseated and post-captopril) as compared to control values. Liver blood flow increased substantially, in the four study phases, in the range from 21% to 25% ($p = 0.06$) after 14 days treatment with captopril.

Systolic and diastolic blood pressures were reduced significantly after the initial dose of captopril from 160.5 ± 5.3 and 103.3 ± 1 mm Hg to 132.4 ± 9.3 mm Hg ($p < 0.005$) and 86.6 ± 6.3 mm Hg ($p<0.05$), respectively, with peak reduction 3 hours after captopril dosing. Treated baseline systolic and diastolic blood pressures on day 14 were ≈ 15 and ≈ 11 mm Hg lower than the pretreatment values ($p < 0.005$ and $p < 0.05$, respectively). The reduction in blood pressure after two-weeks captopril treatment was sustained for 20 hours post-dose. The absolute decrease in systolic and diastolic blood pressures, 3 hours after the terminal captopril dose, was 31.7 ± 6.6 mm Hg (19.7%) and 20.4 ± 8.0 mm Hg (19.8%), respectively ($p < 0.005$), as compared to pretreatment values.

Heart rate remained unaltered after the acute doses of captopril and decreased slightly ≈ 3 beats/ min after two-weeks captopril therapy.

Despite the significant reduction in systolic and diastolic blood pressures, SVR was unaffected by the acute doses of captopril. In contrast, after two-weeks captopril therapy SVR decreased as an average by 19% ± 3% (p

= 0.125).

Postural change from sitting to upright significantly decreased Cl_{pICG} by ≈ 25% ($p = 0.005$) and 27% ($p = 0.001$) on day 1 and 14, respectively. Similarly, Q_H significantly decreased by ≈ 23% ($p = 0.008$) and ≈ 25% ($p = 0.003$) during upright posture before and after two-weeks captopril therapy, respectively. There was a slight increase in diastolic blood pressure (≈ 5-7 mm Hg) in upright position on both study days. A significant increase in heart rate of 4 and 6 beats/min ($p \leq 0.025$) was observed on standing before and after captopril. The SVR increased in upright position by 31.0% ($p < 0.05$) and 33.3% (non-significant), before and after two-weeks captopril treatment, respectively, as compared to seated values.

In agreement with previous studies, the serum concentration of unchanged captopril increased rapidly after captopril administration. Peak serum concentration (C_{max}) of unchanged captopril was 697.2 ± 192.8 ng/ml (mean ± SEM) and the time required to reach C_{max} (t_{max}) was ≈ 76 minutes after a single captopril dose. The C_{max} after the terminal dose of two-weeks captopril treatment was 870.5 ± 85.9 ng/ml which is ≈ 25% (non-significant) larger than that after the initial dose and was reached ≈ 52 minutes following drug administration.

In conclusion, despite the fact that captopril has no apparent acute effect on Q_H as measured 1 hour post-dose, there is a substantial chronic increase in Q_H on continued captopril administration in patients with mild/moderate hypertension. Acute and short-term administration of captopril does not interfere with the homeostatic responses to postural change. The decrease in SVR on continued therapy may suggest an important antihypertensive mechanism of captopril. The captopril induced increase in Q_H after prolonged therapy should be considered when captopril is

coadministered with high-clearance drugs, because systemic availability, hence, therapeutic effect may be altered.

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ABBREVIATIONS

ACE	angiotensin-converting enzyme
ANOVA	analysis of variance
AUC	area under the serum concentration *vs* time curve
AUC_0^{∞}	area under the serum concentration *vs* time curve from time zero to infinity
AUC_0^{t}	area under the serum concentration *vs* time curve from time zero to time t
AUTOAN	decision making pharmacokinetic modelling program
B	baseline
BP	blood pressure
BSA	body surface area
BW	body weight
°C	degrees Celsius
C_a	hepatic arterial plus portal venous drug concentration
CA	post-captopril phase of the study
CHF	congestive heart failure
Cl	clearance
Cl_H	hepatic clearance
Cl_{int}	hepatic intrinsic clearance
Cl_{pICG}	plasma clearance of indocyanine green
C_{max}	Peak serum concentration of drug
C_{p3Hr}	serum concentration of drug 3 hours after administration
$C_p(t)$	serum concentration at time t
$C_p(0)$	serum concentration at time zero

CR	micro-capillary reader
C_V	hepatic venous concentration
C.V.	coefficient of variation
ECG	electrocardiogram
EDTA	ethylenediamin-tetraaceticacid dihydrate
E_H	hepatic extraction ratio
et al	*et alii* and other people
f_B	free fraction in blood
g	gram
GI	gastrointestinal
Hr	hour
h	hour
H	height
Hct	hematocrit
HP	Hewlett-Packard
HPLC	high-performance liquid chromatograph
HV	hepatic venous pressure
ICG	indocyanine green
i.v.	intravenous
K_E	apparent elimination rate constant
kg	kilogram
KH_2PO_4	monopotassium phosphate
M	molar
MANOVA	multivariate analysis of variance
MAP	mean arterial pressure
μg	microgram
μl	microliter

μm	micrometer
mg	milligram
min	minute
ml	milliliter
m^2	square meter
mm	millimeter
mM	millimolar
mm Hg	millimeters of mercury
n	sample size
Na_2HPO_4	disodium phosphate
ng	nanogram
nm	nanometer
NPM	N-(3-Pyrenyl) maleimide
NS	statistically not significant
pH	negative logarithm (base 10) of the hydrogen ion concentration
PTFE	polytetrafluoroethylene
Q_H	hepatic blood flow
r^2	r-square(and r is the Pearson product-moment correlation coefficient)
RAS	renin-angiotensin system
rpm	revolutions *per* minute
s1	first seated phase of the study
s2	second seated (reseated) phase of the study
SD	standard deviation
sec	second
SEM	standard error of the mean

SH	spontaneously hypertensive
SVR	splanchnic vascular resistance
SPSS	Statistical Package for Social Sciences
t-test	test of a statistical hypothesis concerning the Student-t distribution
$t_{1/2}$	elimination half-life
t_{max}	time to reach maximum serum concentration
T	treated value
U.B.C.	University of Brirtish Columbia
U	unit
up	upright phase of the study
USP	United States Pharmacopeia
UV	ultraviolet
vs	*versus*
W	body weight

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my husband who lived through all the
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love carried me through this project.

1. INTRODUCTION

1.1. General Background

Hypertension is a major risk factor in the development of cardiovascular diseases such as coronary artery disease, cerebrovascular disorder and renal disease (Dzau, 1989). Hypertension is generally defined as systolic and diastolic blood pressures persistently exceeding 160 and 95 mm Hg, respectively (Shepherd and Vanhoutte, 1980). Primary or essential hypertension, is the most common form of systemic hypertension. Although its cause is unknown, the elevated blood pressure is generally assumed to be caused by an increase in peripheral resistance to blood flow (Walter, 1982). The linear relationship between elevated blood pressure and development of cardiovascular disorders begins at the level classified as mild hypertension (diastolic pressure 90-104 mm Hg) (McMahon, 1984). Angiotensin-converting enzyme (ACE) inhibitors represent a relatively new type of antihypertensive drugs which have been recommended as first-line drugs in the United States (Dzau, 1989). Although, ACE inhibitors are widely used in Europe their acceptance as first-line drugs has not been uniform (Zannad and Gilgenkrantz, 1989).

1.2. Renin-Angiotensin System

The renin-angiotensin system (RAS) plays an important role in the maintenance of cardiovascular homeostasis in both, healthy and hypertensive subjects (Brogden *et al.*, 1988). The schematic representation of the RAS is

shown on Figure 1. According to the classical concept, the enzyme renin is released from the kidney circulates in the plasma where it converts angiotensinogen (produced in the liver) to the inactive decapeptide angiotensin I (Dzau, 1988). Subsequently, ACE cleaves angiotensin I to form the octapeptide angiotensin II, a potent vasoconstrictor, which is the main active compound in the system. Angiotensin II is transported by arterial blood to peripheral tissues (blood vessel, kidney, heart, brain etc.) and exerts its action on angiotensin II receptors (Campbell, 1987). Recently, this classical concept of the circulating RAS has been revised based on evidence which suggest that angiotensin II is produced mainly in tissues by the action of local ACE to convert the locally produced angiotensin I to angiotensin II (Campbell, 1987). Therefore, the RAS cannot be considered simply as a circulating endocrine hormone system, but also as a tissue generated regulator of vascular resistance and tissue function (Dzau, 1988). These local RASs may function, partly or completely, independently of the circulating RAS (Campbell, 1987). The existance of locally produced angiotensin has been demonstrated in a number of tissues which are involved in the regulation of cardiovascular homeostasis such as the blood vessel wall, kidney, heart, brain, adrenal glands and intestine (Unger *et al.*, 1989; Dzau *et al.*, 1988). Most recently, the contribution of peripheral and splanchnic vascular tissues to the systemic angiotensin II disposition and the regulatory function of the splanchnic vascular bed has been demonstrated in humans (Gasic *et al.*, 1991).

The vasoconstrictor effects of angiotensin II are primarily exhibited on the smooth muscle of arterioles, hence, leads to an increase in systemic vascular resistance. Angiotensin II also stimulates the secretion of aldosterone by the adrenal cortex, which facilitates sodium and water

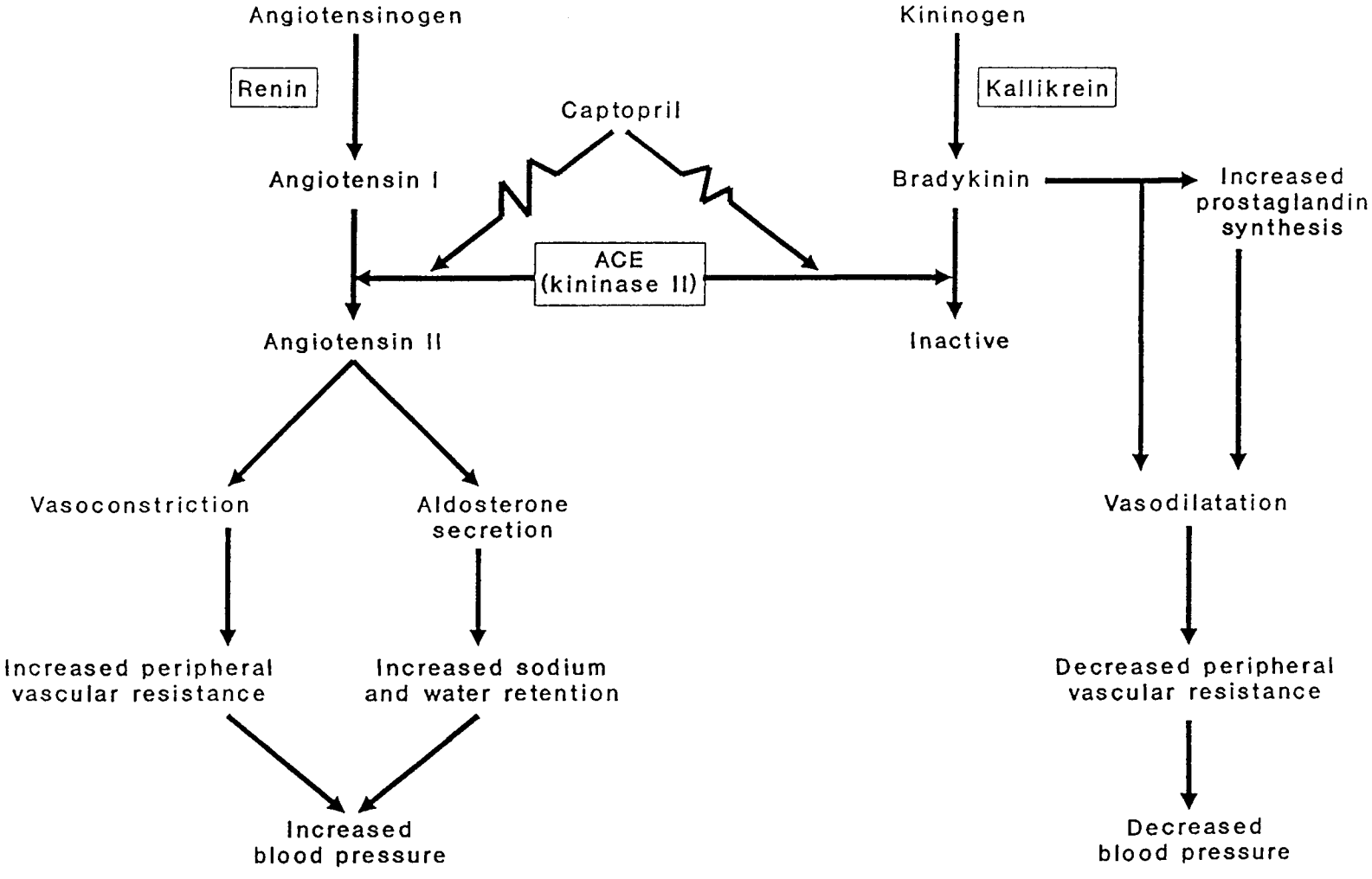


Figure 1. Sites of action of captopril (modified from Benowitz and Bourne, 1989).

retention and potassium excretion (Brogden *et al.*, 1988; Raia *et al.*, 1990). Therefore, angiotensin II formation produces vasoconstriction and increased blood volume which, in turn, result in an increased systemic blood pressure. Under physiological conditions the increase in blood pressure, blood volume and angiotensin II level cause an inhibitory action on the release of renin by negative feedback mechanisms to maintain homeostasis (Brogden *et al.*, 1988). Since ACE is identical to kininase II it also inactivates the potent vasodilator bradykinin, which has been shown to enhance the formation of vasodilatory prostaglandins (Figure 1) (Raia *et al.*, 1990).

1.3. Captopril

Captopril, (D-3-mercapto-2-methyl-1-oxopropyl-L-proline) (Capoten^{R}), is an orally active angiotensin-converting enzyme (ACE) inhibitor frequently used in the treatment of mild to moderate hypertension and congestive heart failure (Kadin, 1982; Brogden *et al.* 1988). Chemically, captopril is a dipeptide thiol (Figure 2), containing a sulfhydryl moiety and two optically active centers. In plasma captopril undergoes oxidation at the thiol group to yield captopril disulfide (Kadin, 1982). Therefore, the immediate protection of the thiol group by the derivatization with N-ethylmaleimide is essential to prevent loss of captopril after blood sampling (Funke, 1980).

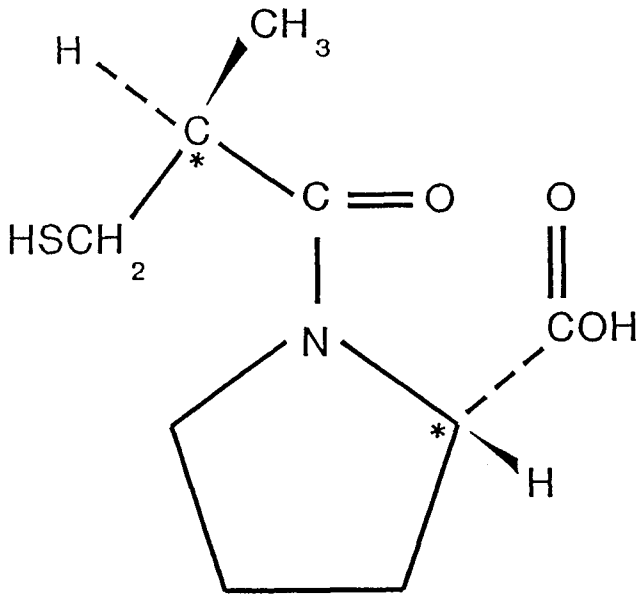


Figure 2. Chemical structure of captopril

1.3.1. Mechanism and Sites of Action

The inhibitory effects of captopril on the RAS in patients with essential hypertension have been demonstrated in a number of studies (Atlas *et al.*, 1979; Muiesan *et al.*, 1982; Wenting *et al.*, 1982). The sulfhydryl group enables the captopril molecule to reversibly interact with the zinc ion at the active site of ACE (Ondetti, 1988). It is this inhibitory action on ACE that determines, partly, the pharmacological effects of captopril. The precise mechanism by which captopril reduces blood pressure and peripheral resistance has not, yet, been fully elucidated (Brogden *et al.*, 1988). Acute inhibition of ACE by captopril result in a decrease in angiotensin II and plasma aldosterone (Atlas *et al.*, 1979; Tarazi *et al.*, 1980), an increase in plasma renin activity and angiotensin I (Tarazi *et al.*, 1980). The increased production of renin is a result of feedback mechanisms in response to the decreased level of angiotensin II (Brogden *et*

al., 1988). Captopril has been shown to increase the plasma levels of bradykinins in hypertensive patients after acute dosing (Mookherjee *et al.*, 1983), but, these levels have remained unchanged in other studies (Johnston *et al.*, 1979). An increase in tissue levels of bradykinin after captopril treatment has been demonstrated in the dog; further, such changes have been postulated to contribute to the hypotensive effects of captopril (Johnston *et al.*, 1982). Swartz and Williams (1982) have demonstrated the increased production of vasodilatory prostaglandins after acute and chronic administration of captopril in hypertensive subjects. The hypotensive effects of captopril, in part, may be related to the increased release of prostaglandins (Swartz and Williams, 1982).

In addition to the inhibitory effects on circulating ACE, captopril has been shown to interfere with local ACE activity (Unger *et al.*, 1989). It has been demonstrated, that captopril effectively inhibited ACE activity in several tissues of spontaneously hypertensive (SH) rats (lung, kidney, aorta, brain, heart) 15 minutes after administration (Cohen and Kurtz, 1982). The extent and duration of blood pressure reduction seemed to correlate better with the inhibition of ACE in certain tissues, for example, kidney or arterial wall, than with the plasma ACE activity (Cohen and Kurtz, 1982; Dzau, 1988). There is clinical evidence which shows that, following chronic therapy with captopril, blood pressure remained decreased long after the discontinuation of the drug even though plasma ACE activity was found to be at pretreatment levels (Waeber *et al.*, 1989). Whether this mechanism of action can be related to the local RAS has not yet been established (Waeber *et al.*, 1989).

In addition to the hormonal effects of captopril, there are other mechanisms which may contribute to the overall effects of the drug. Large

doses of captopril caused smooth muscle relaxation in the portal veins of SH rats (Ljung *et al.*, 1981). In humans evidence of smooth muscle relaxation in the arterial wall may be the observed dilation and improved distensibility of large arteries after acute and chronic treatment with captopril in hypertensive patients (Simon *et al.*, 1985). Further, captopril may interact with the sympathetic nervous system as demonstrated by the decrease in plasma noradrenaline concentrations in hypertensive patients (Wenting *et al.*, 1983). It has been demonstrated that at high concentrations captopril may depress the responsiveness of vascular smooth muscle to sympathetic nerve stimulation (Vanhoutte *et al.*, 1989). In another study, no direct evidence that ACE inhibitors decrease the activity of sympathetic nervous system has been reported, in patients with hypertension or cardiac failure (Ball, 1989). Lastly, the sulfhydryl group of captopril can serve as an antioxidant and can protect the potent vasodilator substance, the endothelium-derived relaxing factor, from superoxide mediated inactivation in the blood vessel wall of the rabbit (Goldshmidt *et* al. 1991).

In summary, captopril exerts pharmacological effects on both plasma and tissue level. The acute administration of captopril leads to the inhibition of ACE activity, the reduction and increase of systemic angiotensin II and angiotensin I, respectively, the increase and decrease of plasma renin and aldosterone concentration. In addition, it could facilitate the release or action of vasodilator substances such as bradykinin and prostaglandin both in plasma and in tissues. It may cause direct relaxation of the vascular smooth muscle and reduce the responsiveness of vascular smooth muscle to vasoconstrictor stimuli. Captopril may interact with the sympathetic nervous system and reduce the release of noradrenaline. Finally, captopril

may serve as an antioxidant and protect the vasodilator endothelium-derived relaxing factor from degradation.

1.3.2. Haemodynamic Effects in Hypertension

Numerous trials have demonstrated the blood pressure reducing effects of captopril in mild to moderately hypertensive patients (Veterans Administration Cooperative Study Group on Antihypertensive Agents, 1984). Many studies have reported adequate control of mild to moderate hypertension with once daily administration of captopril (Parati *et al.*, 1989). The blood pressure reduction is always associated with the lowering of total peripheral resistance after acute or chronic treatment (Tarazi *et al.*, 1980). Maximum blood pressure reduction (10 to 20%) occurs about 1 hour after the administration of a single oral dose of captopril; higher doses may increase the duration of its antihypertensive action (Brogden *et al.*, 1988). Despite the significant decrease in blood pressure and total peripheral resistance, captopril treatment is usually not associated with changes in cardiac output and heart rate (Tarazi *et al.*, 1980; Wenting *et al.*, 1982). However, a slight increase in cardiac output and heart rate after a single oral dose of captopril has also been reported (Fagard *et al.*, 1982). The fact, that blood pressure reduction by captopril does not cause a reflex increase in heart rate suggests that the baroreceptor reflexes may be blunted by captopril (Tarazi *et al.*, 1980). However, no alterations in baroreceptor response has been reported due to postural change (Vandenburg *et al.*, 1983) or to carotid baroreceptor stimulation (Mancia *et al.*, 1982).

1.3.3. Effects on Liver Blood Flow

Liver blood flow is an important determinant of drug metabolism particularly of drugs that are characterized with a high hepatic clearance (Nies *et al.*, 1976). It has been demonstrated that angiotensin II, the potent vasoconstrictor in the RAS, alters liver blood flow and splanchnic vascular resistance (Messerli *et al.*, 1977). Further, the splanchnic vessels have been shown to be more sensitive to angiotensin than other vessels in the circulation (Messerli *et al.*, 1975). In healthy subjects, angiotensin infusion produced vasoconstriction in the splanchnic vascular bed and reduced liver blood flow by about 18% and 33% with subpressor and pressor doses, respectively (Messerli *et al.*, 1977). In addition, angiotensin II, in doses which decreased hepatic blood flow by 18%, decreased the metabolic clearance rates of those steroids, that are highly cleared in the liver, by 14-33% (Messerli *et al.*, 1977). Gasic *et al.*, (1989) has reported that angiotensin I dose-dependently decreased splanchnic blood flow and increased splanchnic vascular resistance in normotensive subjects. These effects of angiotensin I on splanchnic blood flow were attenuated by the administration of an ACE inhibitor, cilazapril (Gasic *et al.*, 1989). These data suggest that the RAS may play an important role in the regulation of blood flow in the splanchnic vascular bed.

In a number of studies, involving animals and human subjects, the effect of ACE inhibitors on hepatic blood flow, however, remains rather controversial (Gavras *et al.*, 1978; Echtenkamp *et al.*, 1983; Stadeager *et al.*, 1989). Studies in sodium-depleted normotensive, conscious dogs have shown a decrease in liver blood flow measured by radioactive microspheres

following the inhibition of ACE with teprotide (Gavras *et al.*, 1978). In contrast, Echtenkamp *et al.*, (1983) demonstrated a pronounced (18%) increase in hepatic blood flow assessed by the bromsulphalein (BSP) clearance method in conscious, sodium-depleted dogs after the inhibition of angiotensin II with intravenously administered saralasin. In the same study, after saralasin administration splanchnic vascular resistance decreased by 35% compared to control values. Richer *et al.*, (1983) have noted a significant, 2 fold increase in liver blood flow and 56% decrease in splanchnic vascular resistance after 8 days treatment with 100 mg/kg oral doses of captopril in SH rats. Most recently, Rozsa *et al.*, (1991) reported a significant (33.5%) increase in the mesenteric blood flow after 6 weeks oral treatment with 100 mg captopril in normotensive and SH rats. It has been suggested, that this increase in liver blood flow was due to the inhibition of ACE in the splanchnic vascular tissue (Rozsa *et al.*, 1991). Stadeager *et al.*, (1989) have reported a significant increase in splanchnic blood flow and decrease in splanchnic vascular resistance after the i.v. injection of 10 mg enalapril (ACE inhibitor) in 19 healthy human subjects.

Most of the human studies using captopril as the ACE inhibitor have been designed to investigate its influence of captopril on the redistribution of regional blood flow in patients with either congestive heart failure (Faxon *et al.*, 1984, Levine *et al.*, 1984, Craeger *et al.*, 1981) or essential hypertension (Ventura *et al.*, 1985). Although it has been observed in these studies that redistribution of regional blood may occur after captopril administration and that captopril has heterogeneous effects in different vascular beds, there are conflicting reports of the effects of captopril on liver blood flow. Levine *et al.*, (1984) and Craeger *et al.*, (1981) have observed a non-significant (30%) and a significant (18%) decrease in

splanchnic blood flow, respectively, after a single oral dose of captopril (25-150 mg) in patients with congestive heart failure. Other studies investigated the effects of captopril on liver blood flow in patients with liver cirrhosis (Eriksson *et al.*, 1984) and healthy subjects (Shepherd *et al.*, 1985) to establish its potential contribution in the treatment of portal hypertension. In these patients, mean hepatic blood flow remained unaltered by 12.5-25 mg single oral doses of captopril. In spite of the relatively large number of studies conducted in order to investigate the haemodynamic changes associated with captopril treatment in patients with essential hypertension, only a few examined its effects on liver blood flow. Crossley *et al.*, (1984) reported a uniform, significant 25% reduction in hepatic blood flow about 60 minutes after a single 50 to 100 mg oral dose of captopril in patients with essential hypertension. In contrast, Ventura *et al.*, (1985), who investigated the regional blood flow distribution after captopril treatment (25 mg/day), noted no change in splanchnic blood flow 90 minutes and 12 weeks after captopril dosing, despite a reduction in splanchnic vascular resistance in patients with essential hypertension.

1.3.4. Captopril Pharmacokinetics in Hypertension

The pharmacokinetics of captopril in healthy subjects and in hypertensive patients have been reviewed by Kubo and Cody (1985). Most recently, the pharmacokinetics of captopril has been reviewed in relation to other ACE inhibitors (Burnier, 1989). The pharmacokinetic properties of captopril in hypertensive patients are similar to those of healthy subjects, except in those cases, when hypertension is associated with secondary renal dysfunction (Kubo and Cody, 1985). Captopril is characterized by rapid

absorption from the GI tract after oral administration with 60-75% of the administered dose being absorbed (Kripalani *et al.*, 1980; Duchin *et al.*, 1982). The time to reach maximum plasma concentration (t_{max}) after a single-dose is between 30-80 minutes in hypertensive subjects and somewhat less, 30-50 minutes after chronic (6 month) administration (Jarrott *et al.*, 1982). There are controversial reports on the effects of food on the bioavailability of captopril (Raia *et al.*, 1990). Earlier reports suggested that concurrent consumption of food has reduced the bioavailability of captopril about 30-50% (Shingvi *et al.*, 1982). Öhman *et al.*, (1985) noted only a slight decrease in the AUC of captopril and no alteration in blood pressure response when captopril was taken with food for 5-weeks in patients with hypertension. Maximum plasma concentration (C_{max}) after a single 100 mg oral captopril dose have been noted to be 361 ± 11 ng/ml in hypertensive subjects (Jarrott *et al.*, 1982). In another study involving patients with essential hypertension, C_{max} of unchanged captopril has been found to be 1310 ± 20 ng/ml after a single 1 mg/kg oral captopril dose (Richer 1984). In one study (Jarrott *et al.*, 1982) chronic captopril administration in hypertensive subjects (100 mg 3 times a day for 6 month) increased C_{max} of unchanged captopril about 3 fold (361 *vs* 1080 ng/ml), as compared to the acute dosing. In another study, C_{max} of unchanged captopril remained the same after 2-weeks captopril treatment (Öhman *et al.*, 1985). In most of the studies, the AUC of total captopril plasma concentration, which represents a pool of captopril and mixed disulfides (Pereira *et al.*, 1988), has been found to be about 20% higher after multiple drug administration than after a single-dose (Brogden *et al.*, 1988). Captopril plasma concentration declines rapidly after acute administration with an elimination half-life ($t_{1/2}$) from 0.7 (Richer *et al.*, 1984) to 1.7 hours (Jarrott *et al.*, 1982). The $t_{1/2}$ of

captopril remained unchanged after chronic treatment with the drug (Jarrott *et al.*, 1982). Due to its short $t_{1/2}$, 6 hours after dosing captopril was not detectable in plasma (Richer *et al.*, 1984). It has been shown (Richer *et al.*, 1984) that despite the rapid decline in captopril plasma concentration, the blood pressure lowering effects of the drug persist much longer. Once absorbed, captopril is partially oxidized at the thiol group and undergoes reversible modifications by interacting with endogenous sulfhydryl-containing substances forming various disulfides such as disulfide dimer of captopril, cysteine captopril disulfides and S-methyl-disulfides (Ondetti, 1988; Drummer *et al.*, 1984). These disulfide metabolites can be reconverted to captopril (Ondetti, 1988) and may contribute to the prolonged antihypertensive activity of the drug (Jarrott *et al.*, 1982). Further, Drummer *et al.*, (1984) reported that some of the disulfide metabolites of captopril effectively inhibit ACE which may explain the long blood pressure lowering effect of the drug. Captopril and its metabolites are excreted mainly in the kidneys (Kubo and Cody 1985). Impaired renal function increased the antihypertensive effect of captopril and this has been shown to be the consequence of the accumulation of captopril metabolites (Drummer *et al.*, 1987).

1.4. Estimation of Liver Blood Flow

Several attempts have been made in the past years to develop a relatively non-invasive method to estimate liver blood flow in man (Bradley, 1974). Difficulty arises from the double blood supply of the liver, one by the hepatic artery and one by the portal vein (Ohnhaus, 1979). The portal vein is the confluence of veins from the splanchnic organs (spleen,

intestine, pancreas and stomach) (Greenway and Lautt, 1989). In addition, capillary connections between the hepatic artery and portal vein exist which provide functional interrelationships between the two systems (Richardson and Withrington, 1981). Therefore, shunts of the blood flow between the two systems may occur (Ohnhaus, 1979). Under these physiological circumstances the accurate measurement of liver blood flow is difficult in man and none of the currently available methods provide completely satisfactory measurement of liver blood flow under all circumstances (Huet, 1981). Direct measurement of liver blood flow requires surgical intervention and anaesthesia which, besides being an invasive technique, also may influence hepatic blood flow (Huet, 1981).

1.4.1. Indocyanine Green Clearance Method

The most widely used techniques to estimate liver blood flow in man are based on the hepatic clearance of substances such as bromsulphalein (Bradley *et al.*, 1945), indocyanine green (Caesar *et al.*, 1961), galactose (Tygstrup and Winkler, 1958) that are highly cleared from the blood when perfused through the liver. This method has been developed by Bradley *et al.*, (1945) and involves the constant intravenous infusion of bromsulphalein and the simultaneous measurements of its concentration in the arterial blood (portal vein) entering the liver and in the venous blood (hepatic vein) leaving the liver, based on the Fick principle (Bradley *et.al.*, (1945). Using indocyanine green (ICG) as a test substance, this method has been frequently used to establish hepatic blood flow rates in man (Wynne *et al.*, 1990; Crossley *et al.*, 1984; Grainger *et al.*, 1983; Caesar *et al.*, 1961). Hepatic extraction ratio of ICG was in the range from 0.63-0.85 (mean 0.78) (Wynne

et al., 1990), 0.68-0.89 (mean 0.78) (Grainger *et al.*, 1983). Crossley *et al.*, (1984) reported that the hepatic extraction ratio of ICG was 0.68 ± 0.08 in patients with essential hypertension and this ratio did not change significantly 1 hour after captopril (0.71 ± 0.07). This method, however, is a highly invasive technique since it requires hepatic venous blood sampling which can only be performed by the catheterization of the hepatic vein. Therefore, the original technique has been modified to a single i.v. injection of ICG and peripheral venous blood sampling with the purpose to avoid venous catheterization and make the method less invasive and easier to perform in clinical practice (Wynne *et al.*, 1989; Shepherd *et al.*, 1985; Geneve *et al.*, 1990; Robson *et al.*, 1990). In this simplified method the plasma clearance of ICG is equal to liver plasma flow, based on the assumptions that ICG is cleared only by the liver and hepatic extraction of ICG is complete (Burczynski *et al.*, 1987). Since in this simplified method hepatic extraction ratio of ICG is not measured, an hepatic extraction of 100% is usually assumed (Soons *et al.*, 1990). The clearance methods were recently reviewed (Greenway and Lautt, 1987; Bradley, 1974; Huet *et al.*, 1981). The principles of these clearance techniques are discussed as follows.

1.4.2. Hepatic Clearance

In the case of a substance eliminated primarily by the liver, that is by metabolism and/or biliary excretion, the hepatic clearance estimates total body clearance (Morgan and Smallwood, 1990). Thus, hepatic clearance (Cl_H) refers to the efficiency of the liver to eliminate a substance from the body by the liver and is equal to the product of liver blood flow (Q_H) and

extraction ratio (E_H):

$$Cl_H = Q_H \, E_H$$

E_H of a substance can be calculated as $(C_a - Cv)/C_a$, the hepatic arterial plus portal venous (C_a) and hepatic venous (C_v) concentration difference across the liver (Wilkinson and Shand, 1975). Since E_H is dependent on the blood flow to the liver (Q_H), the unbound fraction of substance in the blood (f_b) and the hepatic intrinsic clearance of the substance (Cl_{int}) the previous equation 1 has been extended as follows (Rowland *et al.*, 1976):

$$Cl_H = Q_H \frac{f_b \, Cl_{int}}{Q_H + f_b \, Cl_{int}}$$

This is the most frequently applied model of hepatic drug clearance which considers the liver as a "well-stirred" compartment assuming that the drug in the liver compartment is in equilibrium with blood leaving the liver (Nies *et al.*, 1976). This model describes quantitatively the influence of physiological factors such as liver blood flow, enzyme activity and protein binding on the hepatic clearance of drugs (Wilkinson and Shand, 1975).

1.4.3. Effects of Liver Blood Flow on Hepatic Clearance

Based on the previously mentioned model of hepatic clearance, when the liver exhibits very inefficient elimination of the drug (*i.e.* extraction ratio is low and no change in the protein binding of the drug assumed ($Cl_{int} << Q_H$)), then hepatic clearance of the drug depends on the intrinsic ability

of the liver to eliminate the drug ($Cl_H \approx Cl_{int}$) (Rowland *et al.*, 1976).

Conversely, when the liver efficiently eliminates drugs (*i.e.* extraction ratio is high) and plasma protein binding of the drug remains unchanged ($Cl_{int} >> Q_H$), then hepatic clearance of the drug depends entirely on liver blood flow ($Cl_H \approx Q_H$) (Rowland *et al.*, 1976). For these drugs, an increase in hepatic blood flow will cause a proportional increase in hepatic clearances (Wilkinson and Shand, 1975). Experimental evidence has been provided in the dog to substantiate this relationship between hepatic blood flow and drug clearance using the high-clearance drug propranolol (Nies *et al.*, 1976). Several drugs, acting on the cardiovascular system, have been shown to exhibit hepatic blood flow dependent kinetics in man, for example, the antiarrhythmic drug lidocaine and the lipophilic β-adrenoreceptor antagonists *viz.* metoprolol, labetolol and propranolol (George, 1979). Propranolol has been demonstrated to undergo extensive presystemic (first-pass) metabolism after oral administration, as a result of the high hepatic extraction passing through the liver (Routledge and Shand, 1979). Also, the bioavailability of propranolol has been shown to increase by 61% in healthy subjects when administered together with the vasodilatory agent hydralazine (McLean *et al.*, 1980; Schneck and Vary, 1984). The increase in the oral bioavailability of propranolol by hydralazine has been attributed to the transient increase in liver blood flow caused by the vasodilatory action of hydralazine (McLean *et al.*, 1980; Schneck and Vary, 1984).

1.4.4. Indocyanine Green

1.4.4.1. Pharmacokinetics of ICG

Indocyanine green (ICG), is a sulfonic acid dye, which has been extensively used to evaluate hepatic function and estimate liver blood flow in man and animals (Ceasar *et al.*, 1961; Svensson *et al.*, 1982; Heintz *et al.*, 1986; Modi *et al.*, 1988; Burns *et al.*, 1989; Dorr *et al.*, 1989; Wynne *et al.*, 1990). A review of ICG in relation to the liver has been made by Paumgartner (1975). ICG has a high molecular weight of 775 and is rapidly and completely bound to plasma proteins and distributed into the vascular system (Paumgartner 1975). Meijer *et al.*(1988) reported almost complete (85%) recovery of unchanged ICG in the bile of humans. ICG is removed from the circulation specifically by the liver and there does not appear to be any significant enterohepatic recirculation or extrahepatic elimination of the dye (Caesar *et al.*, 1961; Paumgartner, 1975). The hepatic extraction ratio of ICG is over 0.70 obtained by direct measurement in healthy man (Caesar *et al.*, 1961; Wynne *et al.*, 1990; Grainger *et al.*, 1983). In addition, ICG is relatively safe (Huet *et al.*, 1981) and sensitive analytical methods are available to measure its concentration in plasma or serum (Caesar *et al.*, 1961; Rappaport and Thiessen 1982; Svensson *et al.*, 1982). These characteristics make ICG a suitable test substance to estimate liver blood flow in man, particularly, to assess relative changes in liver blood flow in patients with no liver disease (Soons *et al.*, 1990).

1.4.4.2. Analysis of ICG in Human Serum

Traditionally, ICG has been measured in human plasma by absorbance spectrophotometry at 800-805 nm (Caesar *et al.*, 1961; Grainger *et al.*, 1983; Hollins *et al.*, 1987). This method has been widely accepted due to the fact that it is simple, fast and relatively sensitive. Recently, the specificity of the spectrophotometric assay has been questioned due to an interfering compound which absorbs light at the same wavelength as the parent compound (Rappaport and Thiessen, 1982). This impurity and/or degradation product formed the basis to develop several high-performance liquid chromatographic (HPLC) assays which circumvent the possible nonspecificity of the spectrophotometric assay. These HPLC methods employ either fluoresence detection (Dorr *et al.*, 1989; Hollins *et al.*, 1987;) or UV detection (Rappaport and Thiessen, 1982; Heintz *et al.*, 1986) and their specificity for parent ICG has been demonstrated.

Several studies were conducted to compare the spectrophotometric and HPLC analysis of ICG in plasma (Svensson *et al.*, 1982; O'Reilly *et al.*, 1987; Grasela *et al.*, 1987). These comparisons revealed that the differences in ICG plasma concentrations determined by HPLC and spectrophotometry are apparent only at late time points (>20 min) and the two methods have been shown to give similar results if blood sampling for ICG were restricted between 2 and 15 minutes (Heintz *et al.*, 1986). Grasela *et al.*, (1987) found that the spectrophotometric method overestimated ICG plasma concentration compared to the HPLC method, however, the statistical inferences made from the clinical study were identical regardless which method was used for ICG analysis. Svensson *et al.*, (1982) reported no

detectable levels of ICG in human plasma 1 hour after the administration of multiple i.v. doses of 0.5 mg/kg ICG in two healthy subjects.

1.5. Upright Posture: A Physiological Stimulus to Alter Liver Blood Flow

Several factors, including exercise, heat, food, age and upright posture are known to alter hepatic blood flow (Nies *et al.*, 1976; George, 1979). In addition, many disease states such as congestive heart failure, liver and thyroid disease and haemorrhagic shock may cause changes in liver blood flow (Nies *et al.*, 1976; George, 1979). Splanchnic blood flow has been reported to be significantly reduced (15%) in patients with hypertension, particularly in those with renal stenosis (Messerli *et al.*, 1975).

A change in posture to standing is a well known physiological factor altering liver blood flow (Wilkinson, 1976). Due to the redistribution of blood from the upper portion of the body (heart and lungs) to the legs, reflex vasoconstriction occur in the splanchnic vascular bed to compensate for the decrease in cardiac output and to maintain blood pressure (Shepherd and Vanhoutte, 1980). These reflex changes cause a significant decrease in liver blood flow and an increase in heart rate (Shepherd and Vanhoutte, 1980). The vasoconstriction is mediated through the sympathetic nervous system, since the response is abolished by splanchnic sympathectomy in hypertensive subjects (Wilkins *et al.*, 1951). The increased hydrostatic pressure in the legs facilitates the ultrafiltration of plasma to the interstitial space, hence decreases plasma volume and increases the concentrations of blood constituents such as hematocrit, haemoglobin and proteins (Hagan *et al.*, 1978; Dixon and Paterson 1978). The stabilization of plasma volume requires about 40-60 minutes in the assumed posture (Hagan

et al., 1978). The decrease in liver blood flow upon standing has been demonstrated in a number of studies involving healthy subjects (Daneshmend *et al.*, 1981; Modi *et al.*, 1988) and patients with hypertension (Culbertson *et al.*, 1951). The magnitude of change in liver blood flow due to upright posture in healthy subjects has been found to be 30 to 40% (Daneshmend *et al.*, 1981; Modi *et al.*, 1988; Culbertson *et al.*, 1951) and 30% in patients with essential hypertension (Culbertson *et al.*, 1951).

Possible interference of the inhibition of ACE with the reflex cardiovascular responses to upright posture have been investigated in a number of studies (Stadeager *et al.*, 1989; Vandenburg *et al.*, 1983; Muiesan *et al.*, 1982; Tarazi *et al.*, 1980). Most of the studies conducted in patients with essential hypertension and using captopril an ACE inhibitor agreed that captopril does not seem to interfere with the circulatory reflexes induced by standing and mediated through the sympathetic nervous system (Vandenburg *et al.*, 1983; Muiesan *et al.*, 1982).

1.6. Rationale and Objectives

1.6.1. Rationale

There are a number of studies which examined the pharmacological effects of captopril in patients with essential hypertension. However, very few studies measured the changes in liver blood flow after captopril administration in patients with mild to moderate hypertension. In these studies, most often the acute effects of captopril on liver blood flow have been investigated and only a few studies were conducted to examine its effects after prolonged therapy. Since hypertension is a chronic disease

and the control of mild/moderate hypertension requires long-term therapy, to study the effects of captopril on liver blood flow after repeated administration may be more important than after acute dosing. Captopril has been frequently used in combination with other drugs, for example, the β-adrenoreceptor blocker propranolol (McAreavey and Robertson, 1990) in which hepatic and systemic clearance is largely dependent on the rate of drug delivery to the liver (Routledge and Shand, 1979). A clean understanding of captopril induced alterations in liver blood flow would be expected to clarify the potential for drug/drug interactions when flow dependent drugs are coadministered with captopril. Clinically, captopril induced changes in hepatic blood flow may lead to a change in hepatic clearance necessitating a change in drug dosage in order to maintain steady-state blood concentration.

The assessment of ICG clearance after an intravenous bolus of 0.5 mg/kg has been widely used to estimate relative changes in liver blood flow in man (Ohnhaus, 1979). It has been demonstrated that repeated measurement of ICG clearance in the same subject under the same conditions has been reproducible with a coefficient of variation 5% (Daneshmend *et al.*, 1981). The spectrophotometric method used for the analysis of ICG in human serum has been proved to provide similar results to that of the HPLC method, if blood sampling was restricted to 2 to 15 minutes (Svensson *et al.*, 1982; Grasela *et al.*, 1987).

1.6.2. Objectives

1. To examine the acute effects of captopril on ICG clearance and liver blood flow in mild to moderate hypertensive patients.
2. To investigate the prolonged effects of captopril on ICG clearance and

liver blood flow in mild to moderate hypertensive patients.

3. To reproduce the well established decrease in liver blood flow during postural change from sitting to standing and use this as guide in interpreting any changes in ICG clearance or liver blood flow seen with captopril treatment.
4. To examine the effects of two-weeks captopril treatment on liver blood flow during postural change.
5. To study the changes in the haemodynamic parameters (blood pressure, heart rate, splanchnic vascular resistance) of patients after acute and short-term administration of captopril.

2. EXPERIMENTAL

2.1 Materials and Supplies

2.1.1. Drugs

Captopril tablets (CAPOTEN^{R}) (50 mg) were purchased from Bristol-Myers Squibb Canada Inc., Montreal, Canada.

Sterile Indocyanine Green, USP (CARDIO-GREEN^{R} (CG)) (50 mg vial) was purchased from Becton Dickinson Microbiology Systems, Cockeysville, Maryland, U.S.A.

Sodium Chloride Injection USP (30 ml, 0.9%) was purchased from Abbott Laboratories Ltd., Montreal, Canada.

Heparin Sodium Injection USP (10 ml, 1000 U/ml) was obtained from Allan and Hanbury, Glaxo Canada Ltd., Toronto, Ontario, Canada.

2.1.2. Chemicals and Reagents

Captopril was donated by Bristol-Myers Squibb Canada Inc., Montreal, Quebec, Canada. ACS reagent grade disodium ethylenediaminetetraacetic acid dihydrate (EDTA) and L-Ascorbic Acid (Vitamin C) were obtained from Sigma Chemical Company, St.Louis, Montana, U.S.A.; N-(3-Pyrenyl) maleimide (NPM) was purchased from Fluka, Hauppauge, New York, U.S.A. and was purified in the laboratory of Dr. Y.K.Tam (University of Alberta, Faculty of Pharmacy and Pharmaceutical Sciences, Edmonton, Alberta, Canada). ACS reagent grade monopotassium phosphate, disodium phosphate were purchased from BDH

Chemicals, Toronto, Ontario, Canada.

2.1.3. Solvents

ACS reagent grade methanol was obtained from BDH Chemicals, Toronto, Ontario, Canada. Ethanol (95%) was supplied by StanChem, Vancouver, B.C., Canada. Deionized water was produced on site *via* a Milli-Ro^{R} system, Millipore Corp., Bedford, MA., U.S.A. HPLC grade acetonitrile (ultraviolet (UV) cutoff 190 nm) was purchased from Caledon Laboratories Ltd., Georgetown, Ontario, Canada.

2.1.4. Supplies for Human Experiments

Indocyanine green was administered intravenously (i.v.) through a sterile three way stopcock obtained from Medex Inc., Hilliard, Ohio, U.S.A. The sterile Jelco Striped i.v. catheter needles (20 g, 32 mm) were obtained from Criticon, Canada Inc., Markham, Ontario, Canada. Plastic 5 ml syringes, 22 and 19 gauge needles and Vacutainer^{R} blood collection tubes (without additive) were obtained from Becton Dickinson Canada Inc., Mississauga, Ontario, Canada. Following centrifugation the serum was stored frozen in amber vials (4 ml) with polytetrafluoroethylene (PTFE) lined screw caps (Kimble, Division of Owens-Illinois, U.S.A.

Captopril serum samples were stored frozen in conical tipped polypropylene tubes (15 ml) with screw caps which were purchased from Sarstedt Canada, St. Laurent, Quebec, Canada.

2.1.5. Other Supplies

SlickSeal^{R} micro-centrifuge polypropylene tubes with flat, permeable caps (1.7 ml) were obtained from Island Scientific, Bainbridge Island, Washington, U.S.A. Maple Leaf^{R} disposable glass culture tubes (3 ml), were obtained from Johns Scientific Inc., Toronto, Ontario, Canada.

2.2 Equipment

2.2.1. Spectrophotometer

A Hewlett Packard (HP) 8452 A diode-array ultraviolet/visible (UV) spectrophotometer equipped with a HP Vectra^{R} computer interface was used to measure indocyanine green in human serum.

2.2.2. High-Performance Liquid Chromatograph

The liquid chromatographic analysis of unchanged captopril was carried out in the laboratory of Dr. Y.K. Tam, Division of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta. A high-performance liquid chromatograph equipped with Partisil^{R} 5 ODS-3 C_{18} column (5 μm, 100 mm x 4.6 mm I.D., Whatman, Clifton, NJ, U.S.A.), a Model U6K sample injector and an FS 970 fluorometer (Schoeffel, Oakville, Canada) with excitation and emission wavelength of 340 and 389 nm, respectively, was used.

2.2.3. Miscellaneous

Other equipment used included: Vortex-Genie^{R} mixer (Fisher Scientific Co., Springfield, MA, U.S.A).; Fisher Accumet^{R} Model 912 pH meter and electrode (Fisher Scientific Co., Springfield, MA, U.S.A.); a Wrist Action^{R} laboratory shaker at about 1400 rpm (Burrel Corp., Pittsburg, PA, U.S.A.); a Fisher^{R} Model 235C micro-centrifuge at 13,000 x g, (Fisher Scientific Co., Springfield, MA, U.S.A.); a Silencer^{R} table top general purpose centrifuge with temperature control set at 4°C (Western Scientific Ltd., London, Ontario, Canada); a Criticon^{R} 1900 Dinamap 845 x T vital signs monitor with heart rate, systolic and diastolic blood pressure (BP), mean arterial pressure measurement capability; a Canlab IEC^{R} Model CR micro-capillary reader and IEC MB centrifuge, (Damon/IEC Division, International Equipment Corp., Needham, Heights, MA., U.S.A.) for hematocrit measurement; SpeedVac^{R} automatic sample concentrator and dryer, Model A290, (Savant Instruments, Inc., Farmingdale, NY., U.S.A.).

2.3. Preparation of Stock and Reagent Solutions

2.3.1. Indocyanine Green

Indocyanine green was accurately weighed and dissolved in methanol to obtain a final concentration of ≈ 10 μg/ml. This solution was protected from light by wrapping the volumetric flask in aluminium foil and storage at 4°C for up to 48 hours.

2.3.2. Captopril

Approximately 5 mg of captopril was accurately weighed and transferred to a 50 ml volumetric flask and dissolved in an aqueous 1 mM solution of disodium EDTA. A 2.5 ml aliquot of this solution was diluted to 50 ml in a volumetric flask with aqueous 1 mM disodium EDTA solution (final concentration 5 μg/ml). Captopril stock solution was prepared daily for analysis.

2.3.3. Reagent Solutions

Disodium EDTA solution (1 mM) was prepared by dissolving $\approx$ 37.22 mg disodium EDTA in deionized water in a 100 ml volumetric flask.

Disodium EDTA (37.22 mg/ml) and ascorbic acid (19.80 mg/ml) solution was prepared by dissolving 186.12 mg and 99.05 mg disodium EDTA and ascorbic acid, respectively, in deionized water in a 5 ml volumetric flask. This solution was freshly prepared on the day of analysis.

Sorensen phosphate buffer (pH 7.0) was prepared using the following procedures. Monopotassium phosphate (KH_2PO_4, 1.81 g) was accurately weighed and dissolved in deionized water to a final volume of 200 ml (solution 1). Disodium phosphate (Na_2HPO_4, 1.90 g) was accurately weighed and dissolved in deionized water to a final volume of 200 ml (solution 2). An accurate volume (41.30 ml) of solution 1 was transferred to a 100 ml volumetric flask and was adjusted to the final volume with solution 2 to yield a buffer

solution of 0.067 M. The pH of the final solution was measured and adjusted, if necessary, to 7.0 with aliquots of solution 1 or 2.

N-(3-Pyrenyl) maleimide (NPM) solution was prepared by dissolving 7.5 mg purified NPM in HPLC grade acetonitrile. This solution was stored at 4°C after preparation for up to 24 hours.

2.4. UV Spectrophotometric Analysis of Indocyanine Green (ICG) in Human Serum

2.4.1. Preliminary Experiments

2.4.1.1. Stability of ICG in Deionized Water

The degradation of ICG in deionized water was determined as follows: ICG was dissolved in deionized water to produce a concentration of 10 μg/ml. The absorbance of the ICG solution was repeatedly measured at the wavelength of 778 nm over 24 hours following preparation. ICG samples were stored at 4°C in a glass container wrapped in aluminium foil to protect from light catalyzed degradation.

2.4.1.2. Stability of ICG in Human Serum

A methanolic solution of ICG was prepared to obtain a concentration of 0.01 mg/ml. Volumes (0.02 and 0.6 ml) of this solution, with concentrations of 0.2 and 6 μg/ml were evaporated to dryness at 40°C under a gentle stream of nitrogen (N=7 for each concentration). The ICG residue was reconstituted

by adding 0.4 ml blank human serum to the samples and by allowing them to stand 30 minutes with intermittent vortex mixing. All samples were stored at -5°C and were protected from light. Immediately prior to analysis, 0.6 ml volume of double distilled water was added to the samples to obtain ICG concentrations of 0.2 and 6.0 μg/ml. These samples were analyzed spectrophotometrically at 0, 4, 8, 14, 24, 36 and 48 hours following preparation as follows. Samples were stored at -5°C until analysis and were protected from light. All samples were analyzed against blank serum at the wavelength of 800 nm. Standard samples were prepared at the time of the analysis, identical to the ICG samples, to provide a concentration range from 0.2 to 6.0 μg/ml of ICG. The time dependent change of ICG in human serum was estimated by plotting the measured concentrations of the two samples against time and by calculating the coefficient of variation for each concentration.

2.4.2. Quantitative Spectrophotometric Analysis of ICG in Patient Serum

2.4.2.1. Preparation of Samples for Standard Curve

For the preparation of standard samples the modified methods of Rappaport Thiessen (1982) and Dorr *et. al.* (1989) were used as follows. Aliquots of 0.02, 0.06, 0.1, 0.2, 0.3 and 0.5 ml of methanolic ICG stock solution (0.01 μg/ml) were pipetted into clean amber vials. Each vial was made up to a volume of 0.5 ml with methanol. Each sample was evaporated to dryness using the Savant^{R} (40°C x 15 min). Vials were capped with teflon lined screw caps and were stored in a vacuum desiccator at -20°C until analysis for up to 48 hours. Immediately prior to analysis, a set of

standard samples was selected and the ICG residues were reconstituted with 50 μl ethanol (95%) and were gently vortex mixed for 5 seconds to ensure complete dissolution of ICG. To each vial, 0.95 ml blank human serum was pipetted to obtain concentrations of 0.2, 0.6, 1.0, 2.0, 3.0 and 5.0 μg/ml. Samples were gently vortex mixed for 15 seconds. A similarly prepared serum blank was used as reference. All standards were protected from light. A standard curve was constructed by plotting the absorbance against the concentration of ICG using linear regression. For each analysis a new standard curve was prepared.

2.4.2.2. Spectrophotometric Analysis

ICG in serum was analyzed by the modified UV spectrophotometric method described by Caesar *et al.* (1961). The absorbance of ICG in patient samples was measured at the wavelength of maximum absorbance (806 nm). The temperature of the samples was controlled by using temperature controlled sample cell holder. Patient samples were prepared immediately prior to analysis in a similar manner to the blank and standard samples. The analysis of the samples was carried out within 30 minutes following preparation. All standards and patient samples were measured in duplicate. The unknown concentrations of ICG in patient samples were determined by fitting the absorbances to the regression line of the standard curve.

2.4.3 Inter-day Reproducibility of the Spectrophotometric Assay

The inter-day reproducibility of the spectrophotometric method was estimated by preparing four sets of ICG solution in human serum with

different concentrations (0.2, 0.6, 1.0, 2.0, 3.0 and 5.0 μg/ml) on four consecutive days. Preparation of the samples was as was outlined in section 2.4.1.2. On the day of analysis the absorbance of one set of the samples was measured against a similarly prepared serum blank and the concentration of ICG determined. The precision of the spectrophotometric method was estimated as the relative standard deviation (coefficient of variation). The relationship between the actual and calculated ICG serum concentrations was established by plotting the mean calculated ICG concentrations *versus* the mean actual ICG concentrations.

2.4.4. Intra-day Reproducibility of the Spectrophotometric Method

The intra-day precision of the spectrophotometric assay was determined by preparing ICG solutions in human serum with concentrations of 0.2, 1.0 and 5.0 μg/ml. Preparation of the samples was the same as outlined in Section 2.4.2.1. Four replicate measurements were performed for each ICG concentration using standard samples and serum blank as reference. The coefficient of variation for each of the three ICG concentrations used was calculated.

2.5. High-Performance Liquid Chromatographic Analysis of Unchanged Captopril in Serum

Blood samples (3 ml) withdrawn for the measurement of unchanged captopril were immediately mixed with 50 μl of a solution of EDTA (0.1 M) and ascorbic acid (0.1 M) (Jarrott *et al.* 1981). After centrifugation at 13000 x g for 2 min a 0.5 ml aliquot of the supernatant was mixed with 2 ml

of 0.1 M phosphate buffer (pH 7) and 0.2 ml of the derivatizing agent, NPM. This mixture was shaken at room temperature for 15 min at ≈1400 rpm on a laboratory shaker. Samples were kept frozen at -20°C and then sent packed in dry ice to Dr.Y. K. Tam, (University of Alberta) for extraction and analysis by a method developed in their laboratory (Pereira *et al.* 1988). All samples were prepared in duplicate. Blank samples were withdrawn prior to captopril administration and were prepared similarly to the samples following captopril treatment.

2.6. Human Subjects

Seven non-smoking male patients (age 42-58 years, weight 84-118 kg) with mild to moderate hypertension (diastolic blood pressure >90 mm Hg) gave their written consent prior to the initiation of the study. The recruitment of the patients was made by the collaborating physician (Dr. R.E. Rangno). The protocol and procedures of the present study were approved by the University Human Ethics Committee, U.B.C. and by the Human Ethics Committee of St. Paul's Hospital. All patients were instructed not to take captopril for 1 month or any other medication for at least 2 weeks prior to the study. This was validated through measurement of the blank sample. All patients were required to abstain from alcohol or caffeine for at least 48 hours prior to the study. None of the patients had a history of hepatic or renal disease, or cardiac dysfunction (*e.g.* CHF) and all patients had normal ECG and biochemical/haematological laboratory results at the time of the study. Patients were prohibited from consumption of food for 12 hours prior to the study and during each study day, but were allowed to drink water (Spring Water^{R}) and/or apple juice (unsweetened and without preservatives) *ad*

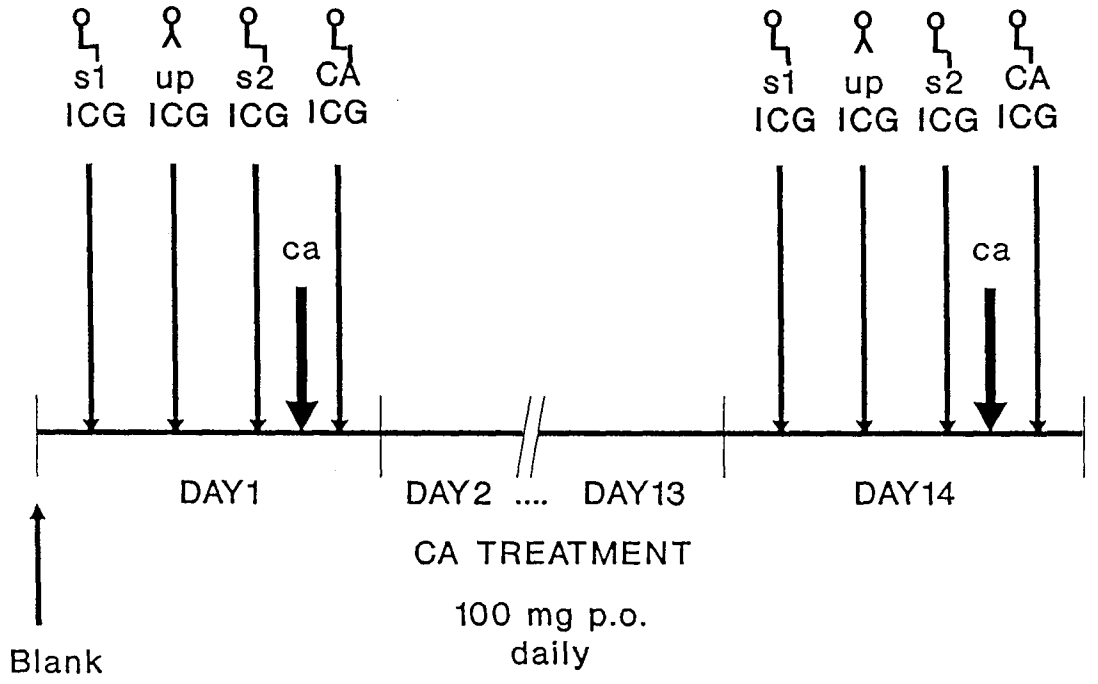
libitum. Room temperature was maintained at 24°C and body temperatures were recorded twice after arrival to the hospital and 1 hour after captopril administration during each study day.

2.7. Study Protocol

Scheme 1 shows a synopsis of the study protocol. There were two study days two-weeks apart. During both study days (day 1 and day 14) the patient was given indocyanine green (CARDIO-GREEN^R, 0.45 mg/kg i.v. bolus) four times in seated (s1), upright (up), reseated (s2) positions and after the 100 mg oral dose of captopril (CA). During the two-weeks captopril treatment period between the study days, the patients received 100 mg captopril (2 CAPOTEN^R 50 mg tablets) once daily. The second last dose was taken about 20 hours before the baseline measurements of the second study day were performed. Blood pressures and heart rate were recorded prior to and following the injection of ICG and after captopril dosing. The procedures and study conditions of day 1 were repeated on day 14. During the entire study period all patients were under the supervision of Dr. R. E. Rangno (Division of Clinical Pharmacology, St.Paul's Hospital).

2.7.1. Dye and Drug Administration, Blood Sample Collection

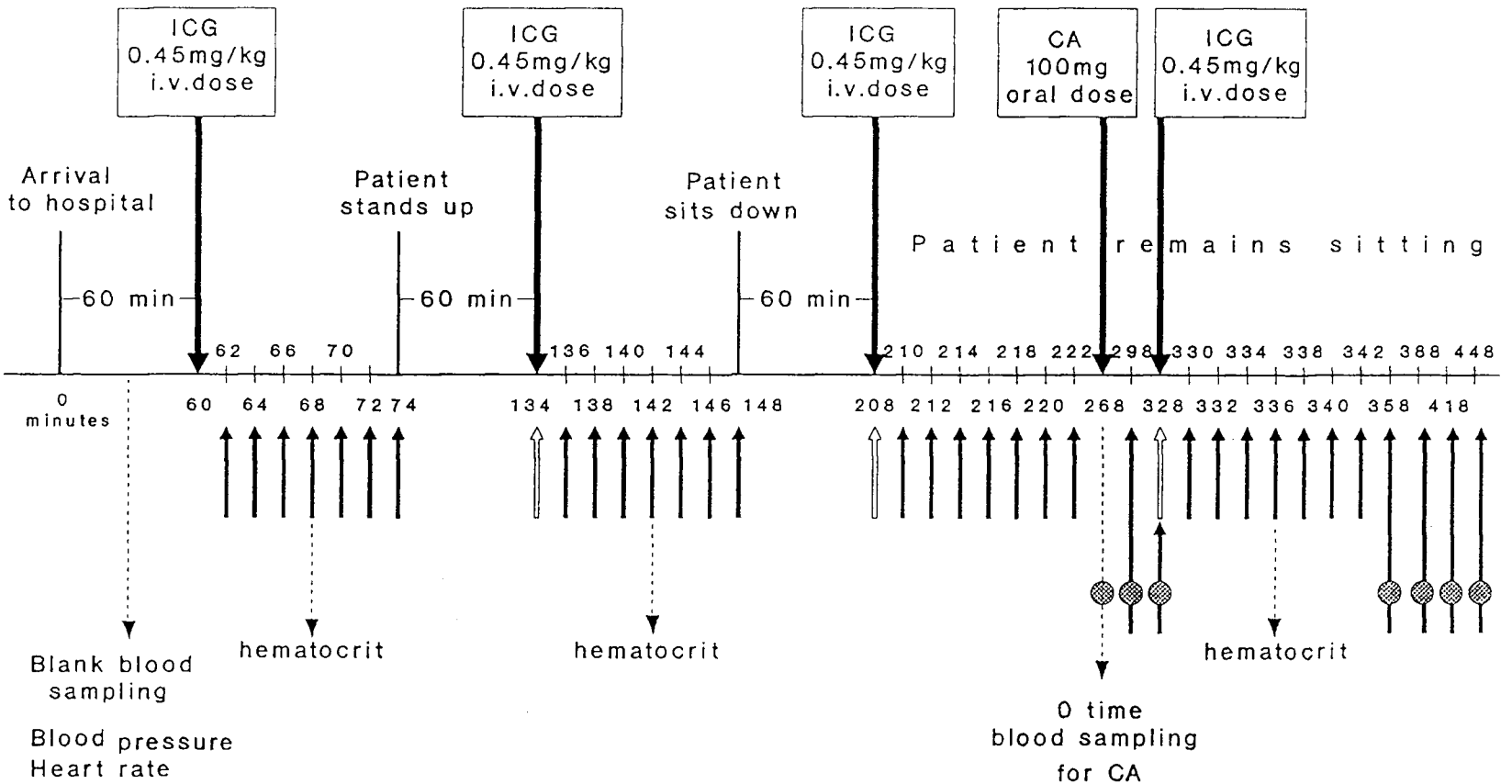
Scheme 2 shows the detailed study protocol of days 1 and 14. On each study day an indwelling teflon catheter was placed in a large forearm vein of the patient. A three-way stop cock was attached to facilitate blood sampling and drug administration. Blank blood samples were collected prior to the first i.v. injection of ICG. The patency of the catheter and blood



Abbreviations:

ca captopril
ICG indocyanine green
s1 first seated
up upright
s2 reseated
CA post-CA

Scheme 1. Study protocol.



Legend:

↑ blood sampling for ICG, ⇧ 0 time blood sampling for ICG, ⊕ blood sampling for CA

Scheme 2. Detailed study protocol.

sampling device was maintained during the experiment by using heparinized saline (50 U/ml) to flush the catheter. To minimize alterations in hepatic blood flow caused by postural changes all patients were required to remain seated for a minimum of 60 minutes prior to the injection of ICG except when ICG was injected to estimate hepatic blood flow during the upright position. The sterile ICG solution (CARDIO-GREEN^{R}) for intravenous injection was prepared immediately prior to injection by adding the supplied aqueous solvent (10 ml) to the sterile ICG powder. Each patient received the same ICG dose on day 1 and day 14. The 0.45 mg/kg ICG dose was given as an i.v. bolus over 15 seconds. Blood samples (7 ml) were drawn from the catheter, after the removal of heparinized saline, into Vacutainer^{R} tubes at -2, 2, 4, 6, 8, 10, 12, 14 minutes following the injection of ICG. After the i.v. injection of ICG the stop-cock was thoroughly rinsed with about 20 ml of normal saline or glucose (20%). Blood samples were allowed to clot at room temperature and serum was separated by centrifugation at 3000 rpm for 30 minutes at 4°C. The serum was transferred into amber glass vials with teflon lined screw caps and kept frozen at -20°C until analysis (maximum of 36 hours). The 100 mg captopril dose was taken with about 200 ml water. Blood samples (3 ml) for the captopril analysis were drawn from the catheter, following the removal of heparinized saline, into sterile, disposable syringes at -5, 30, 60, 90, 120, 150, 180 minutes following the administration of captopril. Blood samples ($\approx$ 1.5 ml) were immediately transferred into two 1.5 ml Eppendorf^{R} vials containing the 50 μl mixture of EDTA (0.1 M) and ascorbic acid (0.1 M). After centrifugation at 13000 g for 2 minutes the supernatant was processed as outlined in Section 2.5. Serum samples for captopril measurement were kept frozen (-20°C) in polypropylene tubes until analysis. In addition, blood samples (2 ml) were drawn into

Vacutainer^{R} tubes containing lithium heparin and the hematocrit was measured at the time indicated in Scheme 2.

2.7.2. Blood Pressure and Heart Rate Measurement

Baseline blood pressures and heart rate were measured at the beginning of each study day after the patients were seated position for ≈ 60 minutes. Four to six blood pressure and heart rate recordings were performed at 2 minute intervals before and following the injections of ICG. Subsequent to the administration of captopril, blood pressure and heart rate measurements were made at 10 minute intervals until the end of the study day. Except during the upright position, blood pressure and heart rate were measured in the seated position.

2.8. Data Analysis

2.8.1. Kinetic Parameters of ICG and Liver Blood Flow

The serum concentration *versus* time data of ICG were fit to a compartmental pharmacokinetic model using the computer programme AUTOAN (Sedman and Wagner, 1978). The estimated elimination rate constant (K_E) and the estimated zero time serum concentration of ICG were used for the calculation of the kinetic parameters of ICG. Formulae used in the pharmacokinetic parameter calculation were obtained from Gibaldi and Perrier (1975).

The area under the serum concentration *vs* time curve from time zero to infinity (AUC_0^{∞}) was calculated by using the trapezoidal approximation and

the following equation:

$$AUC_0^{\infty} = AUC_0^{t} + AUC_t^{\infty} \quad (1)$$

where AUC_0^{t} is the area under the serum concentration *vs* time curve from zero to the last drawn sample. AUC_t^{∞} was calculated by dividing the last ICG serum concentration (C_p) data by K_E.

The apparent elimination half-life ($t_{1/2}$) was calculated by dividing 0.693 by K_E.

The plasma clearance (Cl_{pICG}) was obtained by the following equation:

$$Cl_{pICG} = \frac{DOSE}{AUC_0^{\infty}} \quad (2)$$

Liver blood flow (Q_H) was estimated as:

$$Q_H = \frac{Cl_{pICG}}{E_H\ (1\text{-Hct})} \quad (3)$$

where E_H is the hepatic extraction ratio (assumed to be 1) and Hct is the measured hematocrit.

Peak unchanged captopril serum concentration (C_{max}) and the time of maximum captopril concentration (t_{max}) were obtained from each patient's serum data.

2.8.2. Physiological Calculations

Mean arterial pressure (MAP) was calculated by the automated device as:

$$MAP = \frac{\text{Systolic BP} + 2\ (\text{Diastolic BP})}{3} \qquad (4)$$

Body surface area (BSA) of each patient was calculated from the formula of Dubois and Dubois (1916):

$$BSA = W^{0.425} * H^{0.725} * 71.84 \text{ (a constant)} \qquad (5)$$

where BSA is the body surface area in cm^2, W is the body weight in kilograms, and H is the height in cm.

Splanchnic vascular resistance (SVR) was calculated as follows:

$$SVR = \frac{MAP}{Q_H} \qquad (6)$$

where MAP is the mean arterial pressure in mm Hg, Q_H is the estimated liver blood flow in ml/sec.

The study protocol was designed so that each patient served as his own control. The percent change (% change) in treated value (T) from the appropriate baseline (B) was calculated as follows:

$$\%\ \text{change} = \frac{T - B}{B} \times 100 \qquad (7)$$

The percent change (% change) in the absolute decrease in treated value (T) from baseline (B) on day 1 and day 14 was calculated by the following

equation:

$$\% \text{ change} = \frac{(\text{Day 14 B} - \text{Day 14 T}) - (\text{Day 1 B} - \text{Day 1 T})}{(\text{Day 1 B} - \text{Day 1 T})} \times 100 \quad (8)$$

2.8.3. Statistical Analyses

Statistical comparisons were made by multivariate analysis of variance (MANOVA) with repeated measures using the Software Package for Social Sciences computer program (SPSS-X, SPSS Inc., Chicago, Illinois, U.S.A.), analysis of variance (ANOVA) and two-tailed or one-tailed paired sample *t*-test, where appropriate. The level of significance chosen for all statistical analyses was $\alpha = 0.05$.

3. RESULTS

3.1. Spectrophotometric Analysis of Indocyanine Green (ICG)

3.1.1. Stability of ICG in Distilled Water

In vitro studies previously demonstrated the instability of ICG in distilled water (Gathje *et. al.* 1970). In our experiments the UV absorption of ICG, measured at 778 nm decreased by 23 % during the first 7 hours after preparation and by 63 % by the end of 24 hours, indicating significant instability in water. The rate of decline was apparently faster during the first 7 hours than between 7-24 hours.

3.1.2. Stability of ICG in Human Serum

The stability of ICG in human serum in low (0.2 μg/ml) and high (6.0 μg/ml) concentrations was determined by measuring the change in concentration as a function of time. The absorbance was measured at 806 nm. The results are shown in Figure 3. The best fit through the data points was obtained by linear regression and was described by $Y = 0.00003X + 0.197$ for the 0.2 μg/ml and $Y = - 0.0005X + 5.772$ for the 6.0 μg/ml ICG concentration. The p values of the two regression lines were larger than 0.25 suggesting that the slopes were not significantly different from zero and the ICG concentrations were stable with time in serum (ANOVA) unlike in distilled water. The mean (± SD, n=7) ICG concentrations were found to be 0.197 ± 0.025 and 5.762 ± 0.225 μg/ml for the 0.2 and 6.0 μg/ml ICG serum solutions, respectively. The coefficients of variation were 12.8% and 3.8% for the low

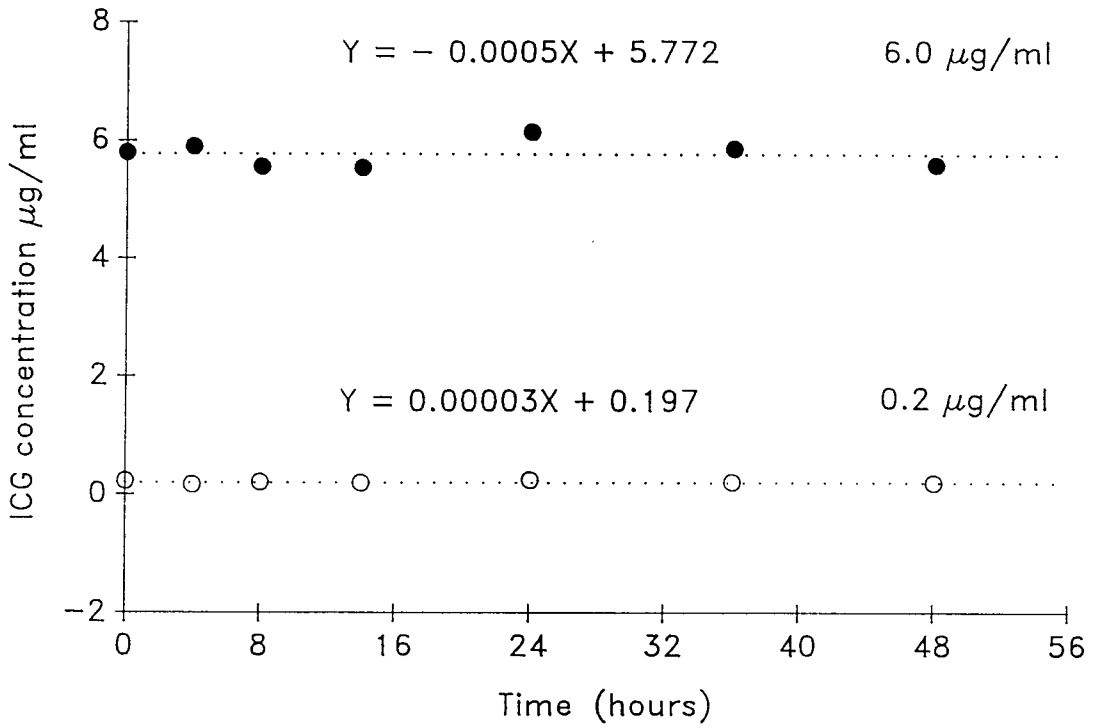


Figure 3. Stability test of ICG in human serum at high (6.0 μg/ml) and low (0.2 μg/ml) concentrations.

and high ICG concentrations, respectively. The slopes of the two regression lines were 0.00003 and - 0.0005 (μg/ml/hour) for ICG concentrations 0.2 and 6.0 μg/ml, respectively. These results indicate that ICG samples prepared in human serum were stable for 48 hours without any significant decline in ICG concentration when stored at -20°C.

3.1.3. Reproducibility of Standard Curves

The day to day reproducibility of the standard curves used for the quantitation of ICG in serum during the stability test is illustrated in Figure 4. In this figure, the slopes of the seven standard curves (0.2 - 6.0 μg/ml) were plotted against the time of their preparation. The linear regression line fitted through the data points is described by Y = -0.0002 + 1.007. The significance of regression was tested by ANOVA and the p value was found to be larger than 0.25 suggesting that the slopes do not change with time. The coefficients of variation for the slopes was found to be 1.6%. The absorbance spectra of the standard curve samples of ICG in serum, in the concentration range from 0.2-6.0 μg/ml are shown in Figure 5.

3.1.4. Standard Curves for the Quantitation of ICG in Patient Samples

Table 1 presents the data of a representative standard curve used for the quantitation of ICG in patient samples. The relationship between absorbance and concentration of ICG was linear in the range of 0.2-5.0 μg/ml with a correlation coefficient of r^2 = 0.999 (Figure 6). Coefficients of variation were less than 10% for all concentrations. The Y-intercept was less than 10% of the highest absorbance.

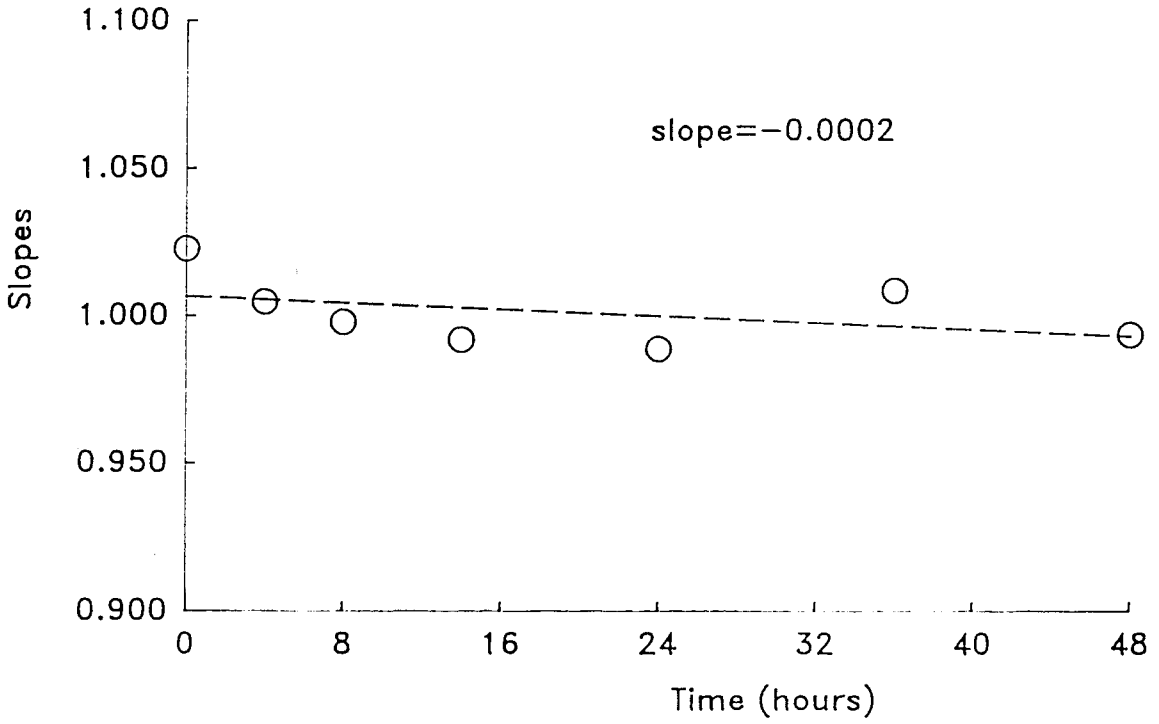


Figure 4. Variability in the slopes of the regression lines of standard curves used in the stability test of ICG in human serum.

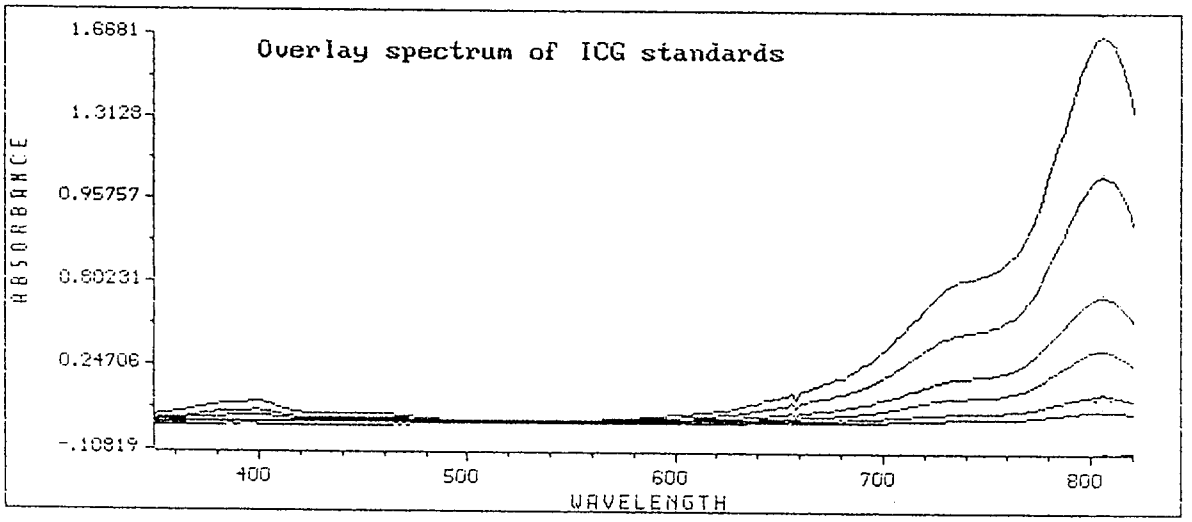


Figure 5. Spectrophotometric scans of ICG standard samples in human serum. Concentration range from 0.2 to 5.0 μg/ml.

Table 1. Standard curve data of ICG for patient serum samples

Number of samples, n = 4

Concentration of ICG (μg/ml)	Absorbance (mean ± SD)	Coefficient of variation %
0.2	0.0592 ± 0.002	3.6
0.6	0.1660 ± 0.003	1.8
1.0	0.2820 ± 0.007	2.6
2.0	0.5823 ± 0.011	1.9
3.0	0.8910 ± 0.016	1.8
5.0	1.4873 ± 0.009	0.6

Linear regression: $Y = 0.300X - 0.013$ $r^2 = 0.999$

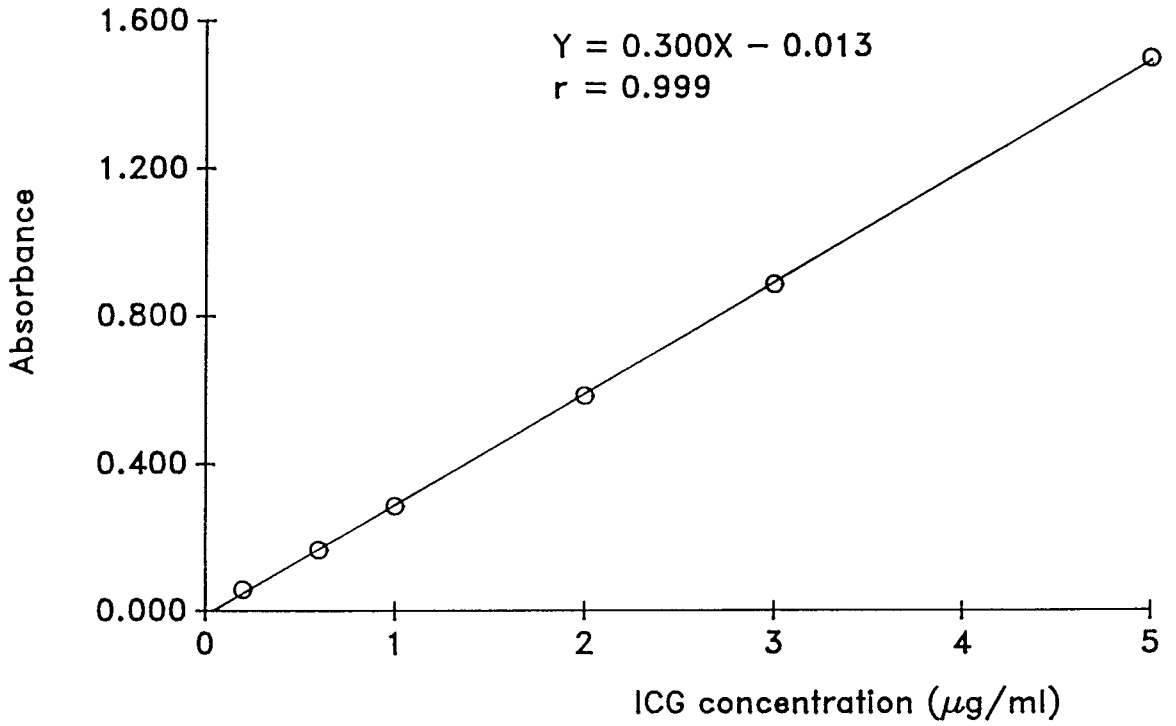


Figure 6. Representative standard curve in the concentration range from 0.2 to 5.0 μg/ml (n=4).

3.1.5. Inter-Day and Intra-Day Reproducibility of the Spectrophotometric Method

Figure 7 shows that there is a linear relationship between the mean (± SD, n=4) found and actual ICG concentrations with a correlation coefficient r^2 = 0.999. Table 2 presents the results of the inter-day recovery of ICG and the coefficients of variation in ICG concentrations. The recovery of ICG was found to be between 93.3 and 102.7%. The highest coefficient of variation (9.8 %) was found for the ICG concentration of 0.2 μg/ml. Similarly, the intra-day coefficients of variation for ICG concentrations 0.2, 1.0 and 5.0 μg/ml were 8.4%, 4.9% and 2.1%, respectively.

3.2. Human Subjects

Table 3 shows the characteristics of human subjects who participated in the study. The coefficients of variation for the age, body weight and body surface area were 13.6%, 12.6% and 4.5%, respectively. The coefficients of variation for the systolic and diastolic blood pressures were 8.4 and 2.2 mm Hg, respectively. One patient (R.S.) was withdrawn after the first study day due to his low hematocrit. Captopril was well tolerated in the patients studied. A mild irritating sensation in the throat developed in one patient after 7 days of treatment with captopril, but it did not require a stoppage in therapy. Hypotension without any symptoms was observed in one patient after the first dose of captopril, but resolved without discontinuation of the drug. Body temperatures were in the range from 35.8 to 36.7°C.

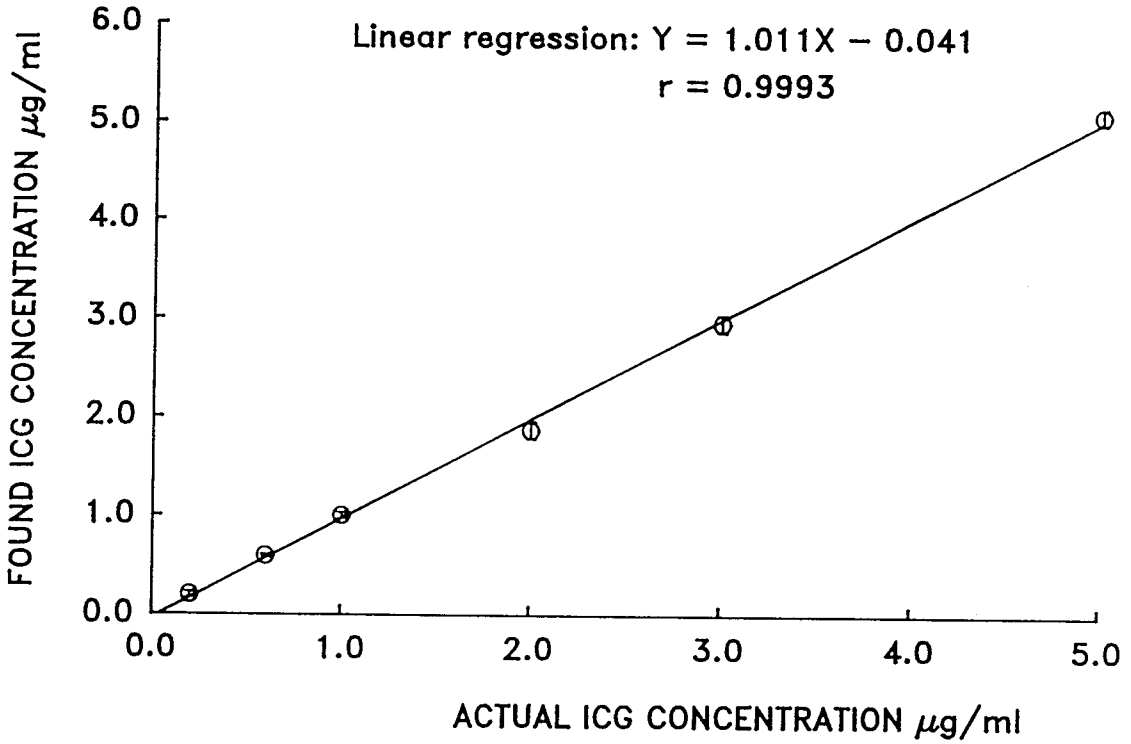


Figure 7. Relationship between the actual and found ICG concentrations. Data are presented as mean ± SD, n=4.

Table 2. Results of the inter-day reproducibility test of the spectrophotometric method for measuring ICG in human serum.

Actual ICG conc. μg/ml	Found ICG conc. μg/ml	Recovery[1] (%)	Coefficients of variation (%)
		(mean ± SD, n = 4)	
0.2	0.205 ± 0.020	102.7 ± 10.1	9.8
0.6	0.592 ± 0.017	98.6 ± 2.8	2.9
1.0	1.002 ± 0.031	100.2 ± 3.1	3.1
2.0	1.866 ± 0.088	93.3 ± 4.4	4.7
3.0	2.952 ± 0.087	98.4 ± 2.9	3.0
5.0	5.074 ± 0.082	101.5 ± 1.6	1.6

Linear regression: $Y = 1.011X - 0.041$ $r^2 = 0.999$

SD standard deviation

1 Calculated from the ratio of found and actual ICG concentration multiplying by 100

Table 3. Characteristics of Human Subjects.

Patient	Age (years)	BW (kg)	Height (cm)	BSA (m^2)	BP (mm Hg)	Baseline Hematocrit
OW	52	93	179	2.1	182 / 103	0.43
MO	44	119	188	2.4	162 / 102	0.44
MS	42	96	185	2.2	145 / 104	0.46
BH	58	92	173	2.1	152 / 104	0.50
DA	55	85	188	2.1	155 / 107	0.47
CB	58	84	173	2.0	168 / 100	0.41
RS[a]	44	101	180	2.1	143 / 102	0.38
Mean	51.5	95	181	2.2	160 / 103	
± SD:	± 7.0	± 12	± 7	± 0.1	± 13.5 / ± 2.3	
C.V.%	13.6	12.6	3.9	4.5	8.4 / 2.2	

BW body weight
BSA body surface area
BP pretreatment blood pressure
SD standard deviation
C.V. coefficient of variation

[a] Patient was withdrawn after day 1; data are excluded from the calculation of mean values

3.3. Changes in the Kinetic Parameters of ICG

3.3.1. Serum Concentration Data of Indocyanine Green

Representative semi-logarithmic plots of the serum concentration *versus* time curves of ICG obtained from a patient on day 1 and day 14 are shown in Figure 8. The semi-logarithmic plots of the serum concentration *versus* time curves from five patients are shown in Appendix 1. The four curves of each graph represent the four phases of day 1 and 14, such as seated (s1), upright (up), reseated (s2) positions and after captopril dosing (CA). For describing the changes in ICG serum concentrations with time after i.v. doses of 0.45 mg/kg of ICG a one compartment model was used as determined by AUTOAN. The monoexponential equation which describes the disposition of ICG in serum was as follows:

$$C_p(t) = C_p(0)\ e^{-K_E t} \qquad (9)$$

where $C_p(t)$ and $C_p(0)$ are the serum concentrations of ICG at time t and time zero, respectively and K_E is the elimination rate constant. In some instances, the decay of ICG serum levels was better fit to a two-compartment model suggesting an initial distribution phase of ICG after i.v. administration.

3.3.2. Kinetic Parameters of ICG ($t_{1/2}$, AUC_0^∞ and Cl_{pICG})

The elimination half-life ($t_{1/2}$) of ICG has been found to be 4.03 ± 0.68

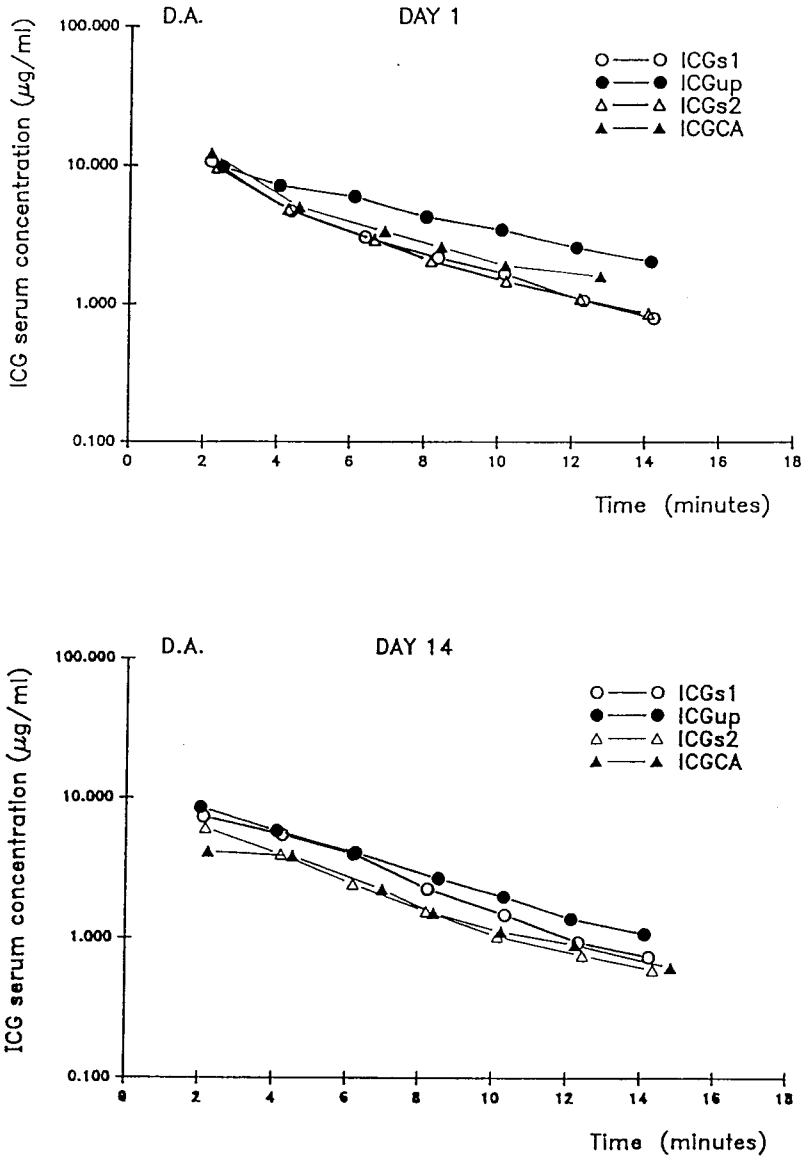


Figure 8. Representative serum concentration *versus* time curve of ICG obtained in a patient on day 1 and day 14 in seated (open circle), upright (closed circle), reseated (open triangle) positions and 1 hour after captopril (closed triangles).

minutes (mean ± SD, n = 24), on day 1 and 3.98 ± 0.52 minutes (mean ± SD, n=24), on day 14.

The total area under the serum concentration *vs* time curve (AUC_0^∞, referred to as AUC in the text) and plasma clearance (Cl_{pICG}) data of ICG for the six patients before and after captopril treatment on day 1 and 14 are shown in Table 4 and Table 5, respectively. In these tables, the absolute and percentage differences from baseline (s1) values during upright (up), reseated (s2) position and 1 hour after captopril administration (CA) for the six patients, on day 1 and 14 are also presented. The absolute and percentage differences from reseated (s2) values after captopril dosing (CA) are shown in the last columns of the tables. In addition, the last parts of Table 4 and Table 5 show the short-term effects of captopril on AUC and Cl_{pICG}, respectively, for the six patients, calculated as the absolute and percentage differences from day 1 data. Figure 9 illustrates the changes in Cl_{pICG}, expressed in absolute terms and per unit body weight and body surface area, for the six patients during the seated (s1), upright (up), reseated (s2) and post-captopril (CA) study phases, on day 1 and 14. Data of Cl_{pICG} normalized to body weight and body surface area are presented in Appendix 2.

3.3.2.1. Effects of Postural Change on AUC and Cl_{pICG} Before and After Captopril Treatment

The results of the comparisons of mean (± SEM, n=6) AUC and Cl_{pICG} data during postural change from seated (s1) to upright (up) and reseated (s2) positions before and after the administration of captopril on day 1 and 14 are shown on Table 6. Statistical comparisons were not performed for the

Table 4. Changes in AUC_0^∞ of ICG before and after captopril treatment in six mild/moderate hypertensive patients

Patient	AUC_0^∞ (min·μg/ml)				Difference from s1			Difference from s2
	s1	up	s2	CA	up	s2	CA	CA
D A Y 1								
MO	65.9	92.1	68.2	57.3	26.2 39.8%[1]	2.3 3.5%	-8.6 -13.0%	-10.9 -16.0%
OW	71.3	85.3	74.3	78.7	14.0 19.6%	3.0 4.2%	7.4 10.4%	4.4 5.9%
MS	57.3	77.2	74.8	73.5	19.9 34.7%	17.5 30.6%	16.2 28.3%	-1.3 -1.8%
BH	84.7	119.2	100.2	106.9	34.5 40.7%	15.5 18.3%	22.2 26.2%	6.7 6.7%
DA	67.4	98.6	64.3	80.4	31.2 46.2%	-3.1 -4.6%	13.0 19.2%	16.1 25.0%
CB	65.6	79.5	55.7	72.5	13.9 21.2%	-9.9 -15.1%	6.9 10.5%	16.8 30.3%
D A Y 1 4								
Patient	AUC_0^∞ (min·μg/ml)				Difference from s1			Difference from s2
	s1	up	s2	CA	up	s2	CA	CA
MO	48.5	59.5	57.6	54.7	11.1 22.9%	9.2 18.9%	6.2 12.9%	-2.9 -5.1%
OW	42.6	65.0	47.5	55.3	22.3 52.3%	4.8 11.4%	12.7 29.7%	7.8 16.5%
MS	45.4	58.5	49.1	61.3	13.1 28.8%	3.7 8.2%	15.9 35.0%	12.1 24.7%
BH	68.7	104.4	94.6	80.6	35.7 51.9%	25.9 37.7%	11.9 17.3%	-14.0 -14.8%
DA	61.1	69.3	45.2	46.0	8.2 13.4%	-15.9 -26.0%	-15.2 -24.8%	0.7 1.6%
CB	62.6	102.3	77.5	81.8	39.8 63.6%	14.9 23.9%	19.3 30.8%	4.3 5.6%

Continued on next page

Table 4. Cont'd

Patient	DAY 14 DIFFERENCE FROM DAY 1			
	AUC_0^∞ (min·μg/ml)			
	s1	up	s2	CA
MO	-17.4	-32.5	-10.5	-2.6
	-26.4%	-35.3%	-15.5%	-4.5%
OW	-28.7	-20.4	-26.8	-23.4
	-40.2%	-23.9%	-36.1%	-29.7%
MS	-11.9	-18.7	-25.7	-12.2
	-20.8%	-24.3%	-34.3%	-16.7%
BH	-16.0	-14.8	-5.6	-26.3
	-18.9%	-12.4%	-5.6%	-24.6%
DA	-6.3	-29.3	-19.1	-34.5
	-9.4%	-29.7%	-29.7%	-42.9%
CB	-3.1	22.8	21.8	9.3
	-4.6%	28.7%	39.2%	12.8%

1	% change in AUC
s1	data obtained in seated position
up	data obtained in upright position
s2	data obtained in reseated position
CA	data obtained 1 hour after captopril dosing
SEM	standard error of the mean

Table 5. Changes in Cl_{pICG} before and after captopril treatment in six patients with mild to moderate hypertension.

Patient	Cl_{pICG} (ml/min)				Difference from s1			Difference from s2
	s1	up	s2	CA	up	s2	CA	CA
D A Y 1								
MO	683.4	488.8	660.1	785.6	-194.5 -28.5%[1]	-23.2 -3.4%	102.3 15.0%	125.5 19.0%
OW	649.0	542.7	586.5	553.8	-106.3 -16.4%	-62.4 -9.6%	-95.2 -14.7%	-32.7 -5.6%
MS	785.3	583.1	601.3	612.1	-202.2 -25.8%	-184.0 -23.4%	-173.2 -22.1%	10.7 1.8%
BH	531.1	377.5	449.1	421.0	-153.6 -28.9%	-82.0 -15.4%	-110.1 -20.7%	-28.1 -6.3%
DA	519.1	355.0	544.1	435.3	-164.1 -31.6%	25.0 4.8%	-83.8 -16.1%	-108.8 -20.0%
CB	533.5	440.3	628.7	482.7	-93.2 -17.5%	95.2 17.9%	-50.8 -9.5%	-146.1 -23.2%
D A Y 1 4								
MO	928.6	755.9	780.8	822.6	-172.7 -18.6%	-147.9 -15.9%	-106.1 -11.4%	41.8 5.4%
OW	1055.2	692.8	947.5	813.4	-362.3 -34.3%	-107.6 -10.2%	-241.8 -22.9%	-134.2 -14.2%
MS	991.3	769.9	915.8	734.3	-221.4 -22.3%	-75.5 -7.6%	-257.0 -25.9%	-181.5 -19.8%
BH	654.9	431.2	475.6	558.3	-223.7 -34.2%	-179.3 -27.4%	-96.6 -14.8%	82.7 17.4%
DA	572.8	505.1	773.7	761.7	-67.7 -11.8%	200.9 35.1%	188.9 33.0%	-12.0 -1.6%
CB	559.5	342.0	451.7	427.8	-217.5 -38.9%	-107.8 -19.3%	-131.7 -23.5%	-23.9 -5.3%

Continued on next page

Table 5. Cont'd

Patient	DAY 14 DIFFERENCE FROM DAY 1 Cl_{pICG} (ml/min)			
	s1	up	s2	CA
MO	245.3 35.9%	267.1 54.6%	120.7 18.3%	36.9 4.7%
OW	406.2 62.6%	150.1 27.7%	361.0 61.6%	259.5 46.9%
MS	206.0 26.2%	186.8 32.0%	314.5 52.3%	122.2 20.0%
BH	123.8 23.3%	53.7 14.2%	26.5 5.9%	137.3 32.6%
DA	53.7 10.3%	150.1 42.3%	229.6 42.2%	326.4 75.0%
CB	26.0 4.9%	-98.3 -22.3%	-177.0 -28.2%	-54.9 -11.4%

1 % change in Cl_{pICG}
s1 data obtained in seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after captopril dosing
SEM standard error of the mean

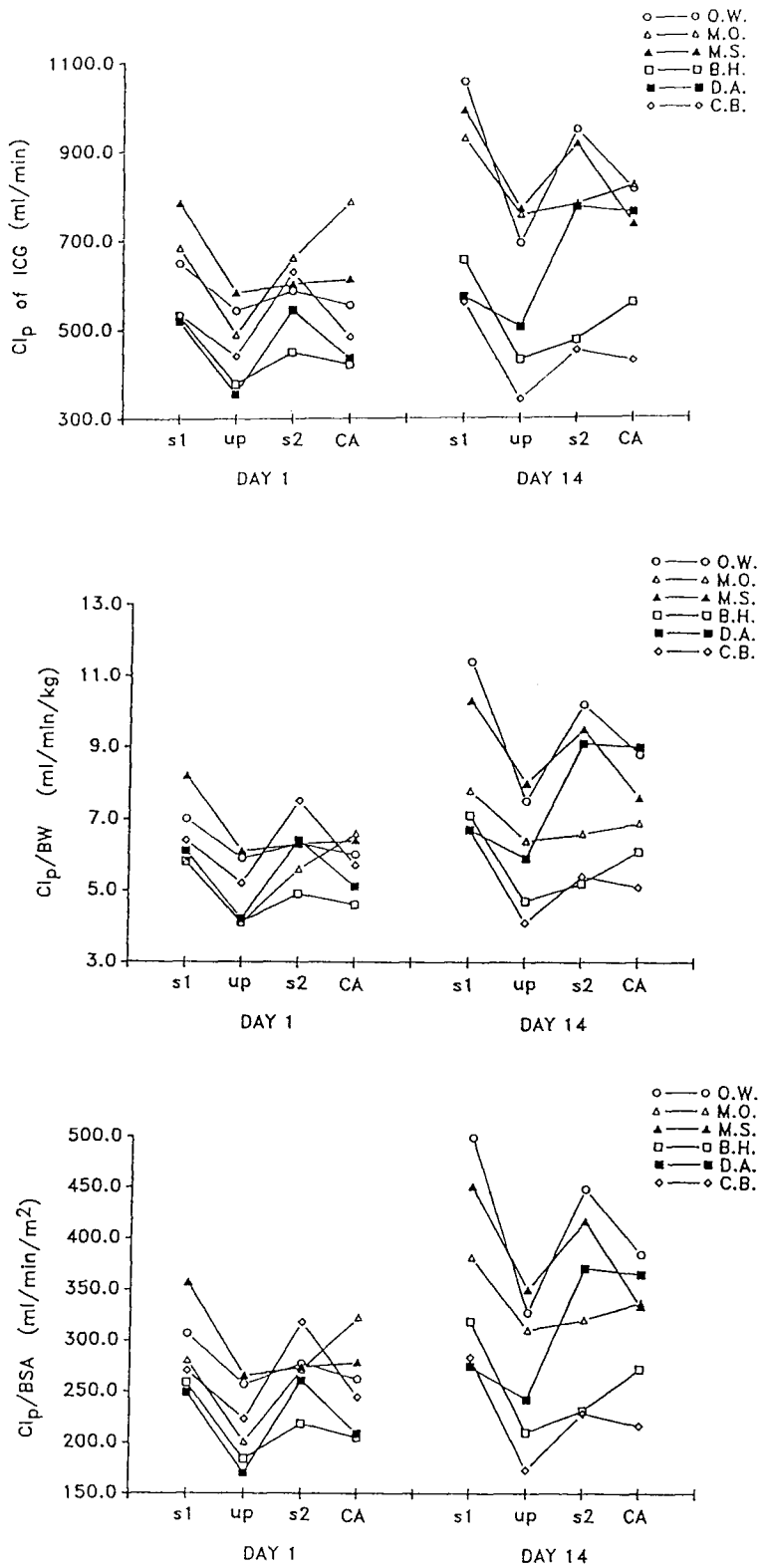


Figure 9. Cl_{pICG} (normalized to body weight and body surface area) in the seated (s1), upright (up), reseated (s2) and post-captopril (CA) phases obtained in six patients on day 1 and day 14.

Table 6. Changes in the mean AUC_0^∞ and Cl_{pICG} before and after captopril treatment on day 1 and day 14.

	Mean ± SEM, n = 6				Difference from s1			Difference from s2
	s1	up	s2	CA	up	s2	CA	CA
DAY 1								
AUC_0^∞	68.7	92.0	72.9	78.2	23.3[1]	4.2	9.5	5.3
min.µg/ml	±3.7	±6.3	±6.2	±6.6	±3.6	±4.3	±4.3	±4.3
					33.9%	6.1%	13.8%	7.3%
DAY 14								
AUC_0^∞	54.8	76.5	61.9	63.3	21.7[1]	7.1	8.5	1.3
min.µg/ml	±4.4	±8.6	±8.1	±6.0	±5.5	±5.7	±5.0	±3.8
					39.5%	13.0%	15.4%	2.2%
DAY 1								
Clp	616.9	464.6	578.3	548.4	-152.3**	-38.6	-68.5	-29.9
ml/min	±43.9	±36.9	±30.4	55.9	±18.3	±39.1	±37.9	±39.0
					-24.7%	-6.3%	-11.1%	-5.2%
DAY 14								
Clp	793.7	582.8	724.2	686.3	-210.9**	-69.5	-107.4	-37.8
ml/min	±91.0	±74.0	±87.2	±64.8	±38.8	±56.1	±65.6	±41.5
					-26.6%	-8.8%	-13.5%	-5.2%
DAY 1								
Clp/BW	6.5	4.9	6.2	5.7	-1.6**	-0.4	-0.8	-0.4
ml/min/kg	±0.4	±0.4	±0.4	±0.3	±0.2	±0.4	±0.4	±0.4
					-24.5%	-5.7%	-12.2%	-6.5%
DAY 14								
Clp/BW	8.3	6.1	7.7	7.2	-2.2**	-0.7	-1.1	-0.4
ml/min/kg	±0.8	±0.6	±0.9	±0.6	±0.4	±0.6	±0.7	±0.4
					-27.0%	-8.1%	-13.1%	-5.5%
DAY 1								
Clp/BSA	286.4	216.3	269.7	253.2	-70.4**	-17.1	-33.5	-16.4
ml/min/m^2	±16.3	±15.8	±13.0	±18.2	±7.3	±18.5	±16.7	±17.9
					-24.6%	-6.0%	-11.7%	-6.1%
DAY 14								
Clp/BSA	367.6	268.6	335.7	318.0	-99.0**	-31.9	-49.6	-17.7
ml/min/m^2	±37.7	±29.0	±37.8	±25.6	±18.8	±26.6	±30.9	±19.2
					-26.9%	-8.7%	-13.5%	-5.3%

1 no statistical comparisons were made for AUC data
s1 data obtained in first seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after captopril dosing
SEM standard error of the mean
BW body weight
BSA body surface area

** Statistically significant ($p \leq 0.005$) compared to s1, MANOVA

AUC data. Table 6 shows that postural change from sitting to upright increased AUC (mean ± SEM, n=6) from 68.7 ± 3.7 to 92.0 ± 6.3 min·μg/ml on day 1 and from 54.8 ± 4.4 to 76.5 ± 8.6 min·μg/ml on day 14. The absolute increase in AUC upon standing was 23.3 ± 3.6 min·μg/ml or 33.9% of control value before captopril treatment on day 1 and 21.7 ± 5.5 min·μg/ml or 39.5% of control value on day 14. Similarly, Cl_{pICG} (mean ± SEM, n=6) decreased in upright position from 616.9 ± 43.9 to 464.6 ± 36.9 ml/min (24.7%) on day 1 and from 793.7 ± 91.0 to 582.8 ± 74.0 ml/min (26.6%) on day 14. This decrease in Cl_{pICG} in upright position was statistically significant ($p = 0.001$), as compared to seated value and determined by MANOVA. When the data of Cl_{pICG} normalized to body weight and body surface area were used to assess the effects of postural shift from sitting to upright, Cl_{pICG} (mean ± SEM, n=6) normalized to body weight and body surface area decreased from 6.5 ± 0.4 to 4.9 ± 0.4 ml/min/kg (24.5%, $p = 0.002$, MANOVA) and 286.7 ± 16.3 to 216.3 ± 15.8 ml/min/m^2 (24.6%, $p = 0.003$, MANOVA), respectively, on day 1 and from 8.3 ± 0.8 to 6.1 ± 0.6 ml/min/kg (27.0%, $p = 0.002$, MANOVA) and 367.6 ± 37.7 to 268.6 ± 29.0 ml/min/m^2 (26.9%, $p = 0.002$, MANOVA), respectively, on day 14. These results show, that the percent decreases from seated values in Cl_{pICG} (mean ± SEM, n=6) normalized to body weight and body surface area during upright posture on day 1 and 14 were almost identical to those of the absolute Cl_{pICG} values. Further, there was no significant difference between the AUC values (mean ± SEM, n=6) obtained in the first seated (s1) and reseated (s2) periods of the study either on day 1 or day 14. The differences in AUC (± SEM, n=6) from seated (s1) values when patients were reseated were found to be 4.2 ± 4.3 min·μg/ml (6.1%) on day 1 and 7.1 ± 5.7 (13.0%) min·μg/ml on day 14. However, there was a tendency for an increase in AUC in reseated positions on both study days. Similarly,

Cl_{pICG} and Cl_{pICG} normalized to body weight and body surface area obtained during the reseated (s2) period were not statistically different from the first seated (s1) values either on day 1 or day 14, as determined by MANOVA ($p = 0.904$, $p = 0.964$ and $p = 0.985$, respectively, on day 1 and $p = 0.685$, $p = 0.759$ and $p = 0.698$, respectively, on day 14). There was, however, a slight decrease in mean Cl_{pICG} (± SEM, n=6) from 616.9 ± 43.9 to 578.3 ± 30.4 ml/min (6.3%) on day 1 and from 793.7 ± 91.0 to 724.2 ± 87.2 ml/min (8.8%) on day 14. The percent decrease in Cl_{pICG} in reseated positions were similar in magnitude when Cl_{pICG} normalized to body weight and body surface area were used for the comparison (5.7% and 6.0%, respectively, on day 1 and 8.1% and 8.7%, respectively, on day 14).

Figure 9 shows the individual data of Cl_{pICG}, expressed in absolute terms and per unit body weight and body surface area, during the seated (s1), upright (up), reseated (s2) positions and after captopril dosing (CA) on day 1 and 14. Data, for Figure 9 are presented in Table 5. The absolute decreases in Cl_{pICG} from seated (s1) values upon standing were in the range from -93.2 to -202.2 ml/min (2.1 fold difference) on day 1 and from -67.7 to -362.3 ml/min (5.3 fold difference) on day 14. The decreases in Cl_{pICG} in upright posture from seated (s1) values, expressed in percentage, were in the range from 16.4% to 31.6% (1.9 fold difference) on day 1 and 11.8% to 38.9% (3.3 fold difference) on day 14. All six patients responded to upright posture with a decrease in Cl_{pICG}, whether it expressed in absolute terms or per unit body weight or body surface area. In examining the individual data of the six patients on day 1, a decrease in Cl_{pICG} during the reseated (s2) period were observed in 4 of the 6 patients studied (range from 3.4% to 23.4%), when compared to the seated (s1) values and an increase in 2 patients (4.8% and 17.9%). On day 14, Cl_{pICG} decreased in 5

of the 6 patients during the reseated (s2) period (range from 7.6 to 27.4%), as compared to the seated (s1) values. In one patient a 35.1% increase in Cl_{pICG} was noted when patient reassumed seated position after standing. These results show large interpatient variation in Cl_{pICG} during the reseated (s2) period as compared to seated (s1) value.

Figure 10 (upright,narrow crosshatched bars) shows the absolute decrease from seated (s1) values in Cl_{pICG} and Cl_{pICG} normalized to body weight and body surface area (± SEM, n = 6) in upright position on day 1 and 14. The absolute decrease in Cl_{pICG} was -152.3 ± 18.3 or 24.7% on day 1 and -210.9 ± 38.8 or 26.6% on day 14, as compared to the seated (s1) values. These data suggest a 38.5% greater absolute decrease in Cl_{pICG} during upright position after two-weeks captopril treatment, when compared to the seated (s1) value and calculated by equation (8) (Section 2.8.2.). However, the apparently greater response to upright posture on day 14 is due to the higher control (s1) Cl_{pICG} values after two-weeks treatment with captopril. This can be shown by the almost similar results when the decrease in Cl_{pICG} upon standing on day 1 and 14 were expressed by the percent decrease from control (s1) values (24.7% *vs* 26.6%, respectively). Further, the mean (± SEM, n=6) absolute decrease in Cl_{pICG} in reseated (s2) position (wide left diagonal bars) was -38.6 ± 39.1 ml/min or 6.3% on day 1 and -69.5 ± 56.1 ml/min or 8.8% on day 14, as compared to the seated (s1) value.

3.3.2.2. Acute Effects of Captopril on AUC and Cl_{pICG}

To evaluate the acute effects of captopril on AUC and Cl_{pICG}, the data obtained 1 hour after the administration of the initial (day 1) and terminal (day 14) dose of captopril on day 1 and 14 were compared to those of the reseated (s2) measurements. Table 6 shows (last column, CA) that there was

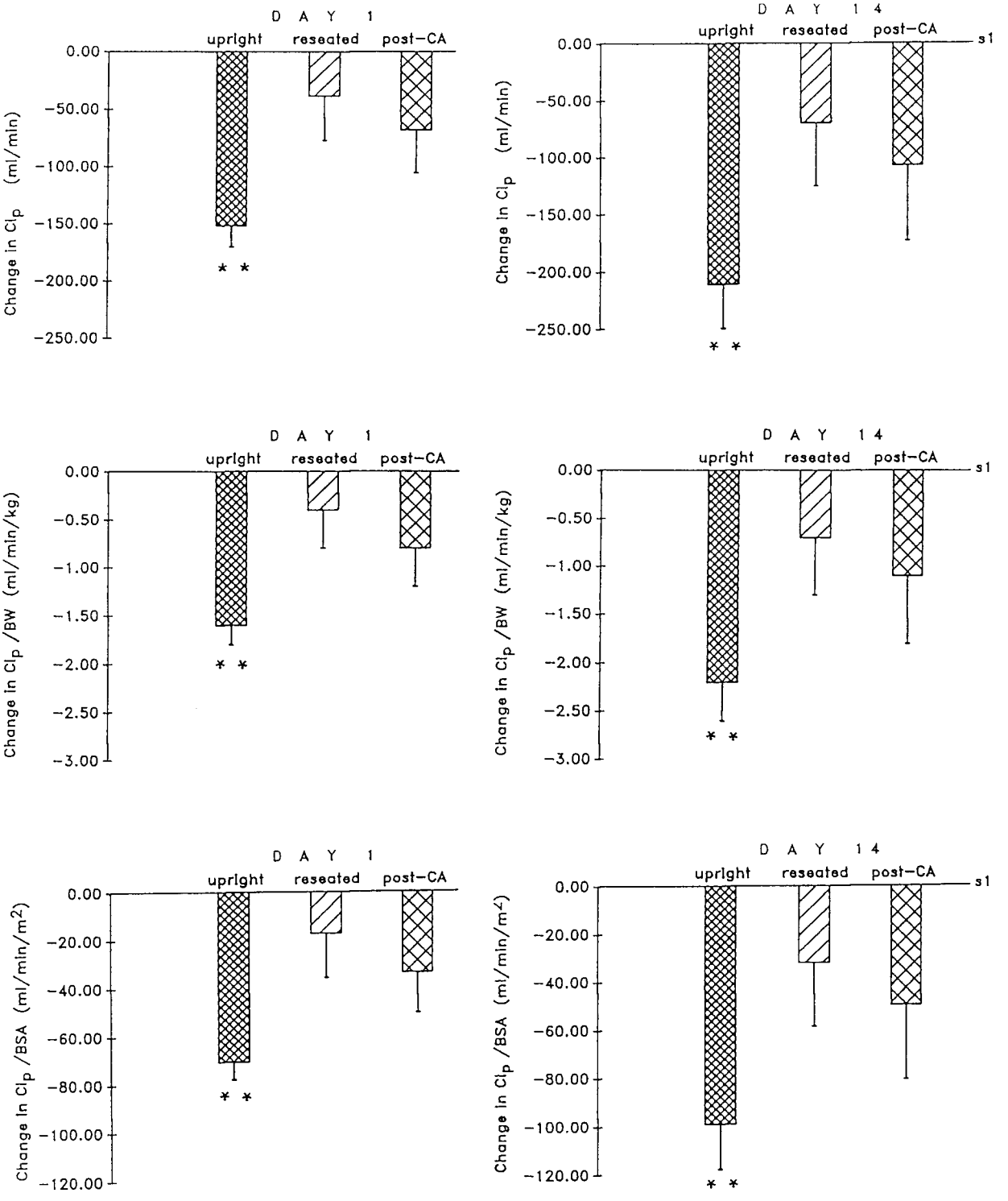


Figure 10. Effects of postural change and the initial (day 1) and terminal (day 14) dose of captopril on Cl_{pICG} (mean ± SEM, n=6). * * indicates significantly different from seated (s1), MANOVA, $p \leq 0.005$.

no significant change in AUC (mean ± SEM, n=6) of ICG 1 hour after the administration of the initial and terminal dose of captopril on day 1 and 14, when compared to the reseated (s2) values. The mean AUC (± SEM, n = 6) increased slightly from 72.9 ± 6.2 to 78.2 ± 6.6 min·μg/ml (7.3%) and from 61.9 ± 8.1 to 63.3 ± 6.0 min·μg/ml (2.2%) on day 1 and 14, respectively. Similarly, there was no significant change in Cl_{pICG} (± SEM, n=6) 60 minutes after captopril dosing either on day 1 or 14, as compared to reseated values. However, Cl_{pICG} (mean ± SEM, n=6) decreased slightly from 578.3 ± 30.4 to 548.4 ± 55.9 ml/min (5.2%) after the initial dose and from 724.2 ± 87.2 to 686.3 ± 64.8 ml/min (5.2%) after the terminal captopril dose on day 1 and 14, respectively, as compared to the reseated (s2) values. The absolute decreases from s2 in Cl_{pICG} (mean ± SEM, n=6) were found to be -29.9 ± 39.0 ml/min and -37.8 ± 41.5 ml/min on day 1 and 14, respectively. The percent changes from s2 in Cl_{pICG} were similar whether expressed in absolute terms or per unit body weight and body surface area (6.5 % and 5.5 % on day 1 and 6.1 % and 5.3 % on day 14, respectively).

In examining the individual Cl_{pICG} data, large interindividual variations were observed in the responses to the acute doses of captopril (post-CA), when data were compared to the reseated (s2) values (Figure 9). In two patients Cl_{pICG} increased by 19.0% and 1.8% and 5.4% and 17.4% on day 1 and day 14, respectively, and decreased in four others in the range from 5.6% to 23.2% and from 1.6% to 19.8% on day 1 and 14, respectively, when data were compared to reseated (s2) values.

In order to determine how the time difference between the seated (s1) and 1 hour post-captopril measurements affected the kinetic parameters of ICG, comparisons between these two measurements were made. Table 6 shows

that, when AUC data obtained 1 hour after the initial dose of captopril (CA, day 1) were compared with those of the seated (s1) values, an increase in AUC (mean ± SEM, n=6) occurred from 68.7 ± 3.7 to 78.2 ± 6.6 min·μg/ml (13.8%). A similar increase in AUC from 54.8 ± 4.4 to 63.3 ± 6.0 min·μg/ml (15.5%) was observed 1 hour after the terminal dose of captopril (CA, day 14) when data were compared to the first seated (s1) measurement of day 14. Likewise, Cl_{pICG} declined from 616.9 ± 43.9 to 548.4 ± 55.9 ml/min (11.1 %) and from 793.7 ± 91.0 to 686.3 ± 64.8 ml/min (13.5 %), 1 hour after the administration of the initial and terminal dose of captopril on day 1 and day 14, respectively, as compared to the Cl_{pICG} value obtained in the seated (s1) period of the study. The decrease in Cl_{pICG} after the initial and terminal doses of captoril were statistically not significant, as compared to seated (s1) ($p = 0.131$ and $p = 0.162$, MANOVA). Similar decreases in Cl_{pICG} were noted when data of Cl_{pICG} normalized to body weight and body surface area were used for the comparison (6.5 ± 0.4 *vs.* 5.7 ± 0.3 ml/min/kg, 12.2 % and 286.7 ± 16.3 *vs.* 253.2 ± 18.2 ml/min/m^2, 11.7 %, respectively). The difference from first seated (s1) in Cl_{pICG} normalized to body weight and body surface area is close to, but does not reach the established $p \leq 0.05$ level of significance ($p = 0.083$ and $p = 0.101$, respectively).

Figure 10 illustrates the absolute decrease in Cl_{pICG} after the initial captopril dose (post-CA, day 1, wide crosshatched bars) and after the terminal dose of two-weeks treatment with captopril (post-CA, day 14) as compared to seated s1 values. The absolute decrease (± SEM, n=6) in Cl_{pICG} was -68.5 ± 37.9 ml/min or 11.1% on day 1 and -107.4 ± 65.6 ml/min or 13.5% on day 14 as compared to the seated (s1) values. This suggests a 56.8% greater absolute decrease in Cl_{pICG} on day 14 as compared to day 1.

However, this apparently greater decrease in Cl_{pICG} after the terminal dose of captopril corresponds to the higher baseline value on day 14.

The decreases in Cl_{pICG} 1 hour after the administration of the initial and terminal dose of CA treatment (CA day 1 and day 14) as compared to seated (s1) values were consistent in 5 of the 6 patients studied (Figure 9). In the 5 patients who responded similarly the range was found to be from 9.5% and 22.1% on day 1 and 11.4% and 25.9% on day 14 (Table 5).

3.3.2.3. Short-Term Effects of Captopril on AUC and Cl_{pICG}

The effects of two-weeks captopril treatment on AUC and Cl_{pICG} were estimated by comparing the AUC and Cl_{pICG} values obtained during the four phases of day 14 (seated, upright, reseated and post-CA) with the appropriate phases of day 1. Table 7 shows the comparisons of mean (± SEM, n=6) AUC, Cl_{pICG}, expressed in absolute terms and normalized to body weight and body surface area of day 14 with the same data of day 1. As compared to day 1, AUC decreased by 13.9 ± 3.7 min.μg/ml (20.2%) in seated (s1), 15.5 ± 8.1 min·μg/ml (16.8%) in upright (up), 11.1 ± 7.4 min·μg/ml (15.1%) in reseated (s2) positions and 14.9 ± 6.7 min·μg/ml (19.1%) 1 hour after captopril administration (CA). Similarly, Cl_{pICG} increased in all four phases of day 14, as compared to day 1. The absolute increases (± SEM, n=6) in Cl_{pICG} from day 1 in seated (s1), upright (up), reseated (s2) positions and post-captopril (CA) were 176.8 ± 57.4 ml/min (28.7%), 118.2 ± 51.6 ml/min (25.5%), 145.9 ± 81.8 ml/min (25.2%) and 137.9 ± 57.1 ml/min (25.2%), respectively.

Figure 11 illustrates the changes in Cl_{pICG}, expressed in absolute terms and normalized to body weight and body surface area. The overall increase

Table 7. Comparison of the mean AUC_0^{∞} and Cl_{pICG} data (normalized to body weight and body surface area) obtained in the four study phases of day 14 with those of day 1.

	DAY 14 DIFFERENCE FROM DAY 1			
	Mean ± SEM, n = 6			
	s1	up	s2	CA
AUC_0^{∞}[1] min.μg/ml	-13.9 ±3.7	-15.5 ±8.1	-11.0 ±7.4	-14.9 ±6.7
% change in AUC_0^{∞}	-20.2	-16.8	-15.1	-19.1
Cl_p* ml/min	176.8 ±57.4	118.2 ±51.6	145.9 ±81.8	137.9 ±57.1
% change in Cl_p	28.7	25.5	25.2	25.2
Cl_p/BW* ml/min/kg	1.8 ±0.6	1.2 ±0.5	1.5 ±0.9	1.5 ±0.7
% change in Cl_p/BW	27.8	23.7	24.6	26.3
Cl_p/BSA* $ml/min/m^2$	80.9 ±26.4	52.3 ±23.2	66.0 ±39.2	64.8 ±27.6
% change in Cl_p/BSA	28.2	24.2	24.5	25.6

1 no statistical comparisons were made for AUC data
s1 data obtained in first seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after captopril dosing
SEM standard error of the mean
BW body weight
BSA body surface area

* statistically significant ($p \leq 0.05$) compared to combined day 1, MANOVA

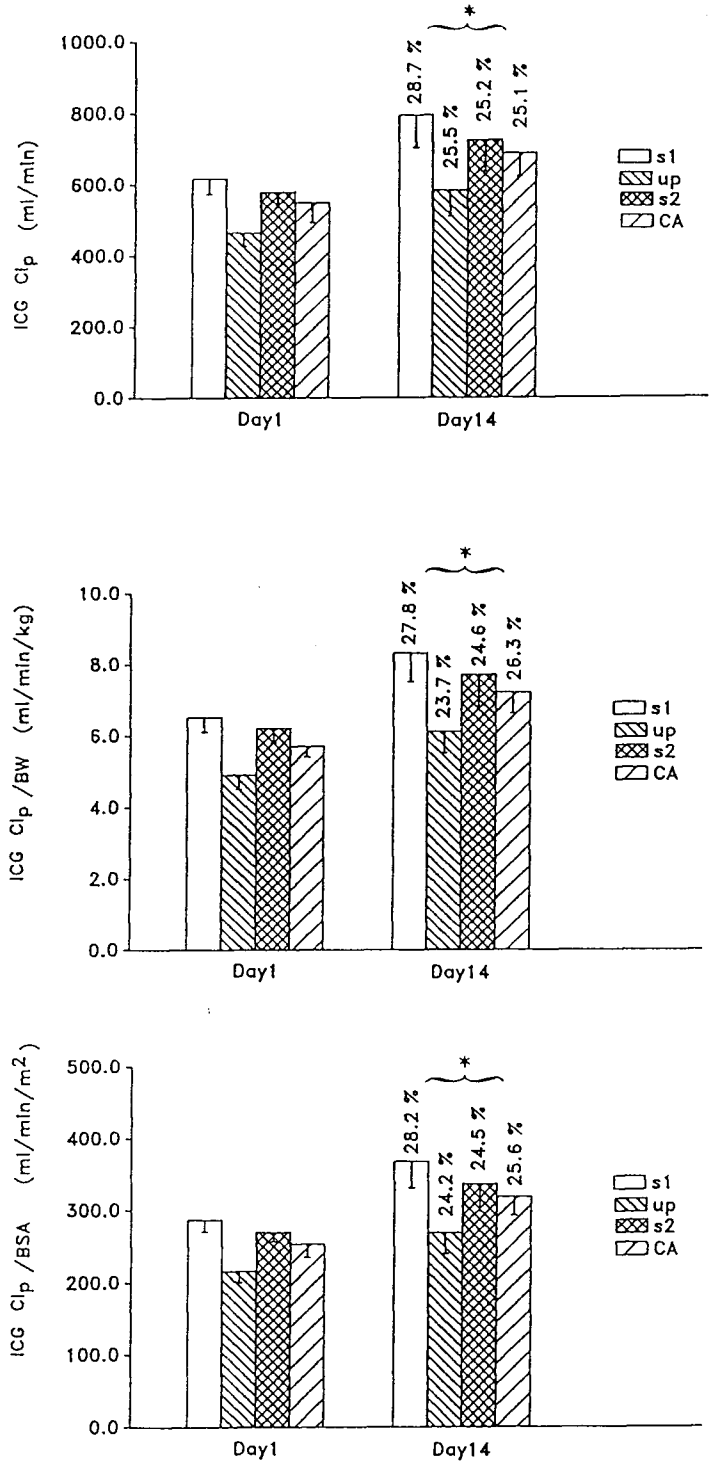


Figure 11. Comparison of Cl_{pICG} (normalized to body weight and body surface area)(mean ± SEM, n=6) before and after two-weeks captopril. * indicates significantly different from day 1, MANOVA, $p \leq 0.05$.

in Cl_{pICG} after two-weeks captopril treatment was statistically significant, whether expressed in absolute terms or per unit body weight and body surface area (p=0.039, p=0.048, p=0.044, MANOVA).

In examining the individual data of the six patients the increase in Cl_{pICG} was consistent in five of the six patients studied (Table 5, Figure 9). The magnitude of increase, however, showed large interindividual variability. The percent increase in Cl_{pICG} after two-weeks captopril treatment was in the range from 10.3% to 62.6% (6.1 fold difference) in seated (s1), from 14.2% to 54.6% (3.8 fold difference) in upright (up), from 5.9% to 61.6% (10.4 fold difference) in reseated (s2) and from 4.7% to 75.0% (15.9 fold difference) after CA administration (CA). One patient (C.B.) exhibited a decrease rather than increase in Cl_{pICG} in 3 of the four phases of the study (upright, reseated and post-CA), as compared to the day 1 values.

3.4. Changes in Liver Blood Flow (Q_H)

3.4.1. Hematocrit Data

As it is shown by equation (8) in Section 2.8.1., in order to estimate Q_H from Cl_{pICG} data the hepatic extraction ratio (E_H) of ICG and values of the hematocrit are required. In the present study, E_H was considered to be 1 assuming 100% hepatic extraction for ICG. Thus, - unless large changes in patients' hematocrit occur during the study - the changes in Cl_{pICG} reflects the changes also in Q_H.

In the present study, the hematocrit was measured in seated position before the first ICG injection (s1), during the sampling periods of upright

positions (up) and captopril dosing (CA). Table 8 shows the hematocrit values of the six patients obtained during the two study days. Postural shift from sitting to upright resulted in an increase in hematocrit by 3.8% and 5.2% day 1 and 14, respectively. Although this increase in hematocrit upon standing is relatively small it was statistically significant on both study days, day 1 and 14 ($p \leq 0.005$ and $p \leq 0.05$, respectively, paired sample *t*-test). There was no significant change in hematocrit (mean ± SD, n=6) during the post-captopril (CA) period, as compared to the baseline value (s1) either on day 1 or day 14. However, a statistically significant decrease in mean hematocrit was observed after two-weeks treatment with captopril when baseline (s1) and upright (up) values of day 14 were compared to that of day 1 (- 5.4%, $p \leq 0.05$ and - 4.3%, $p \leq 0.01$, respectively, paired sample *t*-test). Although, hematocrit values were significantly higher during standing and lower on day 14 as compared to that of day 1, they apparently did not affect the estimated changes in Q_H.

3.4.2. Q_H Data

Appendix 3 presents the individual Q_H data, expressed in absolute terms and normalized to body weight and body surface area for six patients during the seated (s1), upright (up), reseated (s2) and post-captopril periods of the study on day 1 and 14. Based on these data, the changes in Q_H and Q_H normalized to body weight and body surface area during the four study phases (seated (s1), upright (up), reseated (s2) and post-captopril(CA)) of day 1 and day 14 are shown in Figure 12.

Table 8. Changes in hematocrit in six patients with mild/moderate hypertension during postural change and before and after captopril treatment.

	DAY 1			DAY 14		
Patient	sl	up	CA	sl	up	CA
OW	0.430	0.453	0.452	0.400	0.430	0.400
MO	0.436	0.450	0.428	0.420	0.430	0.430
MS	0.440	0.460	0.430	0.440	0.458	0.440
BH	0.500	0.510	0.490	0.440	0.470	0.443
DA	0.465	0.480	0.455	0.449	0.458	0.420
CB	0.405	0.425	0.395	0.380	0.410	0.380
Mean ± SD:	0.446 ±0.03	0.463 ±0.03	0.442 ±0.03	0.421 ±0.03	0.443 ±0.02	0.419 ±0.03
% change upright[a]		3.81**			5.0**	
% change post-CA[a]			-0.9[NS]			-0.6[NS]
% change day 14[b]				-5.5*	-4.4*	-5.1[NS]

sl — data obtained in first seated position
up — data obtained in upright position
s2 — data obtained in reseated position
CA — data obtained 1 hour after captopril dosing
SEM — Standard error of the mean

a — % change during upright position, as compared to the seated (sl) values of day 1 and 14

b — % change after two-weeks captopril treatment, as compared to day 1

** — statistically significant ($p \leq 0.005$) compared to sl, paired sample *t*-test

* — statistically significant ($p \leq 0.05$) compared to sl, paired sample *t*-test

NS — statistically not significant ($p > 0.05$), compared to sl, paired sample *t*-test

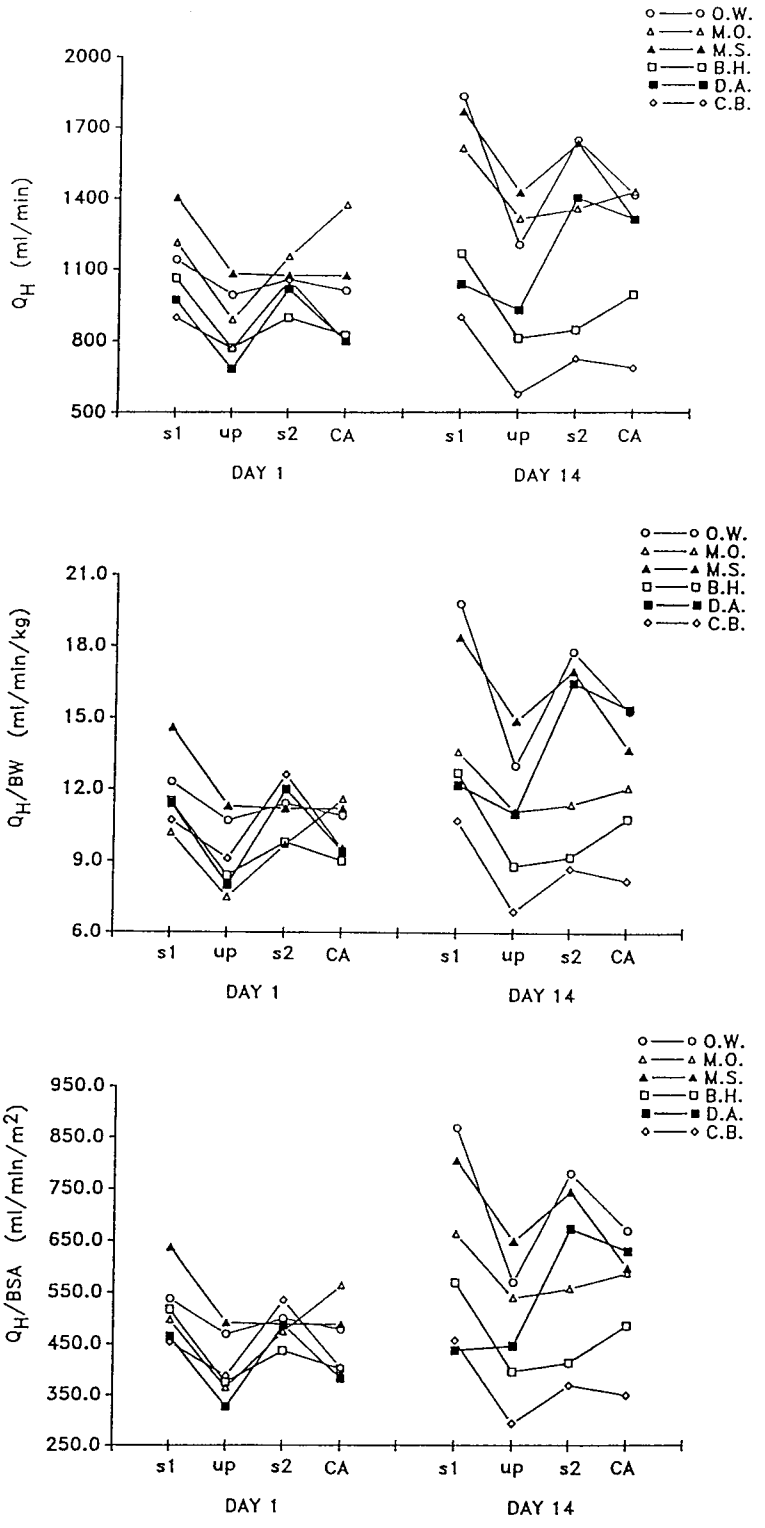


Figure 12. Q_H (normalized to body weight and body surface area) in seated (s1), upright (up), reseated (s2) and post-captopril (CA) phases obtained in six patients on day 1 and day 14.

3.4.2.1. Effects of Postural Change on Q_H Before and After Captopril Treatment

A summary of changes in Q_H and Q_H, normalized to body weight and body surface area (mean ± SEM, n=6) during the four study phases on day 1 and day 14 are shown in Table 9. Mean Q_H (± SEM, n=6) decreased from 1114 ± 74 to 863 ± 62 ml/min (22.5%) and from 1389 ± 164 to 1045 ± 132 ml/min (24.7%), respectively, upon standing on day 1 and 14, as compared to the seated (s1) values. When Q_H data were normalized to body weight and body surface area the magnitude of decreases in upright position were similar to those of the absolute values (22.3% and 25.2% on day 1 and 22.3% and 23.9% on day 14, respectively). The observed decrease in Q_H and in Q_H normalized to body weight and body surface area during postural change from sitting to upright was statistically significant, as determined by MANOVA ($p = 0.008$, $p = 0.004$ $p = 0.005$, respectively, on day 1 and $p = 0.003$, $p = 0.005$ and $p = 0.004$, respectively, on day 14). Further, mean Q_H (± SEM, n=6) increased from 863 ± 62 to 1043 ± 34 ml/min on day 1 and from 1045 ± 132 to 1271 ± 160 ml/min on day 14 when patients reassumed seated (s2) position, as compared to those of the upright (up) values. When reseated (s2) Q_H values were compared to those of seated (s1) values, the reseated (s2) values (mean ± SEM, n=6) were lower than seated (s1) values by -71 ± 69 (6.3%) and -118 ± 100 ml/min (8.5%) on day 1 and day 14, respectively (Figure 13, wide left diagonal bars). These differences from s1 values, whether Q_H was expressed in absolute terms or per unit body weight or body surface area, were statistically not significant ($p = 0.949$, $p = 0.910$, and $p = 0.968$, respectively, on day 1 and $p = 0.860$, $p = 0.862$ and $p = 0.921$, respectively,

Table 9. Changes in Q_H and Q_H normalized to body weight and body surface area (mean ± SEM, n=6) before and after captopril treatment.

	Mean ± SEM, n=6				Difference from s1			Difference from s2
	s1	up	s2	CA	up	s2	CA	CA
DAY 1								
Q_H	1114	863	1043	980	-250*	-71	-134	-63
ml/min	±74	±62	±34	±92	±36	±69	±68	±70
					-22.5%	-6.3%	-12.0%	-6.0%
DAY 14								
Q_H	1389	1045	1271	1193	-344**	-118	-196	-78
ml/min	±164	±132	±160	±119	±68	±100	±107	±73
					-24.7%	-8.5%	-14.1%	-6.1%
DAY 1								
Q_H/BW	11.8	9.2	11.1	10.3	-2.6**	-0.7	-1.5	-0.8
ml/min/kg	±0.6	±0.6	±0.5	±0.4	±0.3	±0.8	±0.7	±0.7
					-22.3%	-5.8%	-13.0%	-7.6%
DAY 14								
Q_H/BW	14.6	10.9	13.4	12.6	-3.7**	-1.1	-2.0	-0.9
ml/min/kg	±1.5	±1.2	±1.7	±1.1	±0.8	±1.1	±1.2	±0.8
					-25.2%	-7.8%	-13.8%	-6.5%
DAY 1								
Q_H/BSA	517.8	402.1	486.4	452.7	-115.7**	-31.3	-65.1	-33.7
ml/min/m^2	±27.2	±26.1	±13.0	±28.4	±15.3	±32.6	±30.2	±32.2
					-22.3%	-6.0%	-12.6%	-6.9%
DAY 14								
Q_H/BSA	632.8	481.9	588.7	552.2	-150.9**	-44.1	-80.5	-36.5
ml/min/m^2	±72.7	±52.6	±70.1	±47.7	±40.2	±57.2	±59.1	±33.8
					-23.9%	-7.0%	-12.7%	-6.2%

s1 data obtained in first seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after captopril dosing
SEM standard error of the mean
BW body weight
BSA body surface area

* statistically significant ($p \leq 0.05$), compared to s1, MANOVA.

** statistically significant ($p \leq 0.005$), compared to s1, MANOVA.

on day 14, MANOVA). There was, however, a tendency for reduction in Q_H during the reseated (s2) position, as compared to the seated (s1) measurements.

Figure 12 shows that the decrease in Q_H during postural change from sitting to standing (up) was consistent in the six patient, whether Q_H was expressed in absolute terms or per unit body weight or body surface area. The percent decrease in Q_H upon standing was in the range from 12.9% to 29.7% (2.3 fold difference) on day 1 and 10.4% to 35.8% (3.4 fold difference) on day 14. As contrast, large interindividual variability in Q_H were observed on day 1 and 14, when differences (expressed in percentage) between the reseated (s2) and seated (s1) values were calculated. Reseated (s2) Q_H values were lower in four patients in the range from -4.8% to 23.4% (4.9 fold difference) and were higher in two patients by 4.8% and 17.9% (3.7 fold difference) on day 1, as compared to the first seated (s1) measurement. After two-weeks captopril treatment the Q_H values were smaller in five of the six patients studied by 7.6% to 27.4 % and Q_H was higher in one patient by 35.1%, as compared to seated (s1) values.

Figure 13 shows that the absolute decrease (mean ± SEM, n = 6) in Q_H upon standing was -250 ± 36 ml/min or on day 1 and -344 ± 68 ml/min on day 14, as compared to the seated value. This suggests a 37.3% greater absolute decrease in Q_H after two-weeks captopril treatment in upright position as compared to day 1. However, the apparently greater response to upright posture is due to the higher control value (seated s1) value on day 14. When the decrease in Q_H from control value upon standing was expressed in percentage, the decrease in Q_H were similar on both study days (22.5% *vs* 24.7%).

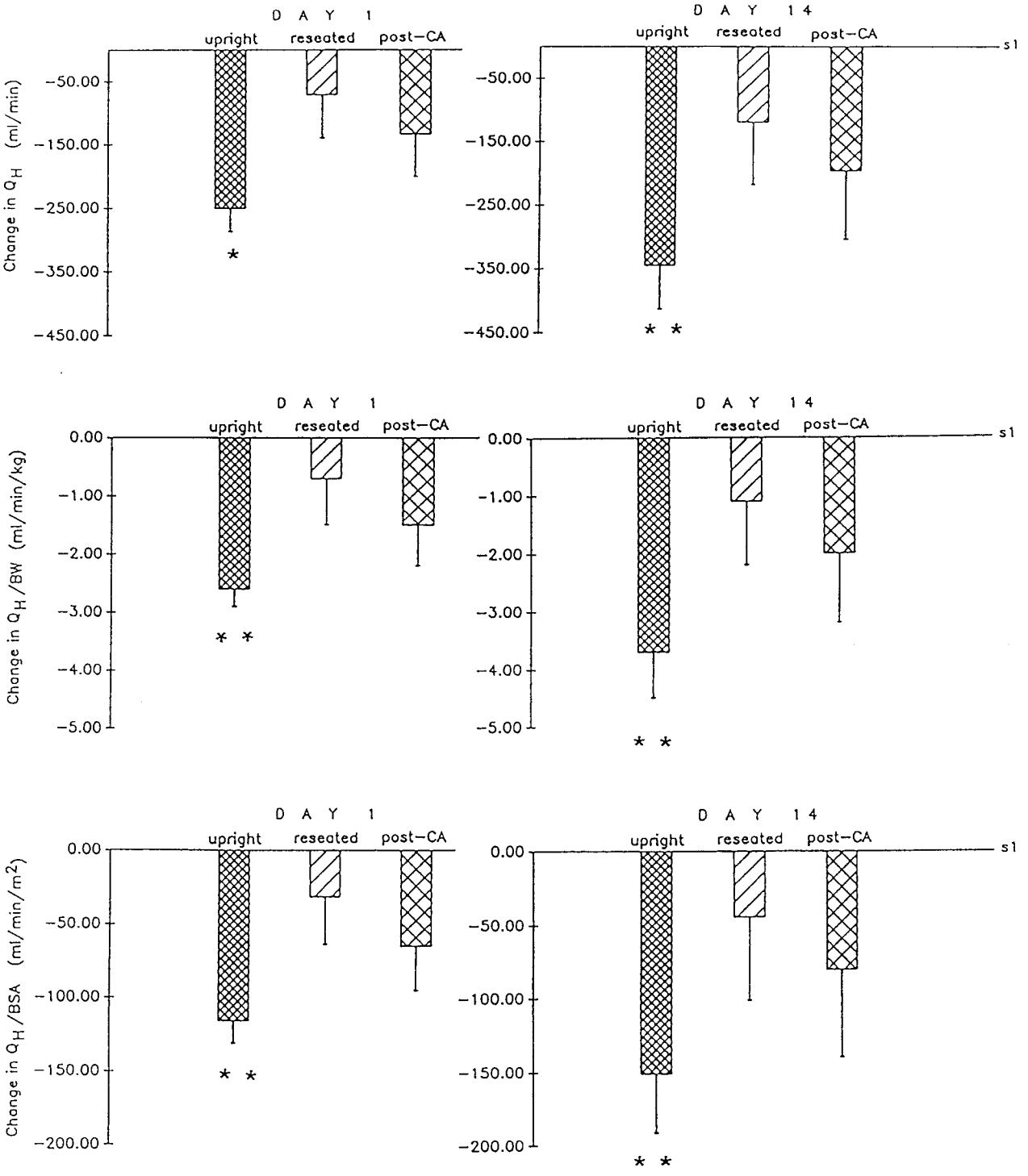


Figure 13. Effects of postural change and the initial (day 1) and terminal (day 14) dose of captopril on Q_H (mean ± SEM, n=6). * and * * indicates significantly different from seated (s1), MANOVA, $p \leq 0.05$ and $p \leq 0.005$, respectively.

3.4.2.2. Acute Effects of Captopril on Q_H

The purpose of including the reseated (s2) measurement in the study protocol was to stabilize Q_H after standing and before the administration of captopril. Therefore, the acute effects of captopril on Q_H were estimated by comparing the changes 1 hour after the initial and terminal dose of captopril to those obtained in the reseated (s2) position. Table 9 (last column, CA) depicts the changes in Q_H and Q_H normalized to body weight and body surface area (mean ± SEM, n=6) when Q_H values, obtained 1 hour after the administration of the initial captopril dose (day 1, CA) and the terminal captopril dose (day 14, CA), were compared to those of the reseated (s2) measurements. Mean (± SEM, n=6) Q_H slightly decreased from 1043 ± 34 to 980 ± 92 ml/min (6.0%) and from 1271 ± 160 to 1193 ± 119 ml/min (6.1%) after the initial and terminal dose of captopril. The absolute decrease in Q_H (mean ± SEM, n=6) after the initial and terminal dose of captopril were found to be -63 ± 70 (6.0%) and -78 ± 73 (6.1%) ml/min, respectively. This change in Q_H after the acute doses of captopril was statistically not significant. There was, however a tendency for decrease in Q_H, after the acute doses of captopril, as compared to the reseated (s2) values on both study days, whether Q_H data were expressed in absolute terms or per unit body weight and body surface area. The magnitude of decrease in Q_H was, however, similar to that observed when the two seated (s1 and s2) values were compared.

By comparing the individual responses of Q_H in the reseated (s2) periods to the initial and terminal dose of captopril (CA) it is apparent that in two patients there was no change (O.W.and M.S.), in one patient (M.O.) there was an increase and in two patients (D.A. and C.B.) there was a decrease in

Q_H (Figure 12). These results suggest large interpatient variability in the responses to the acute doses of captopril.

There was about a 5 hour time difference between the measurements of Q_H in the first seated (s1) position and after captopril administration. In order to evaluate how this time difference affected estimated Q_H, data obtained 1 hour after captopril dosing were compared to the Q_H data measured during the first seated (s1) phase of the study (Table 9, CA). As a result of this comparison, a reduction in Q_H (mean ± SEM, n=6) from 1114 ± 74 to 980 ± 92 ml/min and from 1389 ± 164 to 1193 ± 119 ml/min was noted on both study days, day 1 and 14, respectively.

Figure 13 shows that the absolute decrease in Q_H (mean ± SEM, n=6) 1 hour after captopril dosing was -134 ± 68 or 12.0% on day 1 and -196 ± 107 or 14.1% on day 14, as compared to the seated (s1) values (wide crosshatched bars). These data suggest a 46.6% greater absolute decrease in Q_H on day 14 than on day 1. However, the apparently greater decrease in Q_H after the terminal dose of captopril on day 14, as compared to the day 1 values is attributed to the higher baseline value (s1) of day 14. This is shown by the fact that the magnitude of reduction in Q_H expressed in percentage were almost similar on day 1 and 14 (12.0% *vs* 14.1%, respectively). The reduction in Q_H after the initial and terminal dose of captopril, as compared to the seated (s1) value was statistically not significant, whether Q_H was expressed in absolute terms or per unit body weight or body surface area ($p=0.107$, $p= 0.070$ and $p=0.084$, respectively on day 1 and $p=0.126$, $p=0.150$ and $p=0.231$, respectively on day 14, MANOVA).

3.4.2.3. Short-Term Effects of Captopril on Q_H

The results of the comparisons of mean Q_H and Q_H normalized to body weight and body surface area obtained during the four phases (s1, up, s2 and CA) of day 14 to that of the same phases of day 1 are presented in Table 10. The Q_H values after two-weeks therapy with captopril were greater in all four phases of day 14, as compared to the same phases of day 1. The absolute increase (± SEM, n=6) in Q_H was 275.0 ± 107.3 ml/min (24.7%) in seated (s1), 182.0 ± 90.6 ml/min (21.1%) in upright (up), 227.8 ± 147.8 ml/min (21.8%) in reseated (s2) positions and 212.8 ± 92.5 ml/min (21.7%) after captopril dosing (CA). Figure 14 shows the changes in mean Q_H and Q_H normalized to body weight and body surface area on day 1 and after short-term treatment with captopril, on day 14. The statistical test (MANOVA) just failed the level of significance, with p values of 0.062, 0.075 and 0.074 for the absolute values of Q_H and Q_H normalized to body weight and body surface area, respectively. The magnitude of the absolute increase in Q_H, expressed in percentage were similar in all phases of the study (range from 19.8% to 24.7%). This is illustrated on Figure 15 which shows that the increase in Q_H after two-weeks captopril treatment follows an almost parallel pattern with the day 1 data. The magnitude of the change in Q_H normalized to body weight and body surface area were slightly smaller, but similar to those of the absolute Q_H values.

In examining the data of individual subjects (Figure 12) it is apparent that the increase in Q_H after two-weeks captopril treatment was observed in five of the six patients studied. One patient (C.B.) exhibited a decrease in Q_H after prolonged administration of captopril. When the results of this

Table 10. Comparison of Q_H data (normalized to body weight and body surface area) obtained in the four study phases of day 14 with those of day 1.

	DAY 14 DIFFERENCE FROM DAY 1 Mean ± SEM, n = 6			
	s1	up	s2	CA
Q_H NS ml/min	275.0 ±107.3	182.0 ±90.6	227.8 ±147.8	212.8 ±92.5
% change in Q_H	24.7	21.1	21.8	21.7
Q_H/BW NS ml/min/kg	2.8 ±1.1	1.8 ±0.9	2.3 ±1.6	2.3 ±1.1
% change in Q_H/BW	23.8	19.4	21.1	22.7
Q_H/BSA NS $ml/min/m^2$	115.0 ±54.1	79.8 ±41.1	102.3 ±70.6	99.5 ±44.6
% change in Q_H/BSA	22.2	19.8	21.0	22.0

s1 data obtained in first seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after captopril dosing
SEM Standard error of the mean
BW body weight
BSA body surface area

NS statistically not significant ($0.05 < p < 0.1$), compared to combined day 1, MANOVA

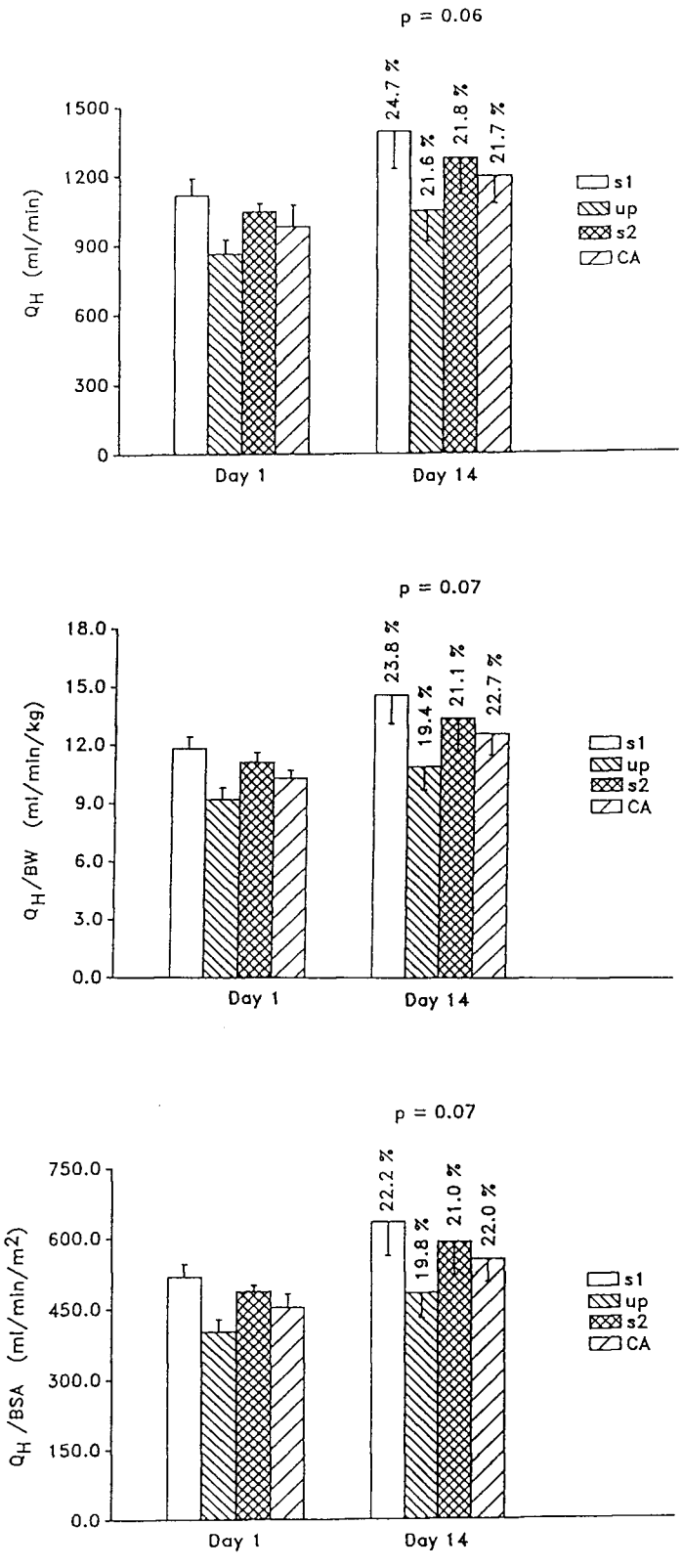


Figure 14. Comparison of Q_H (normalized to body weight and body surface area)(mean ± SEM, n=6) before and after two-weeks captopril.

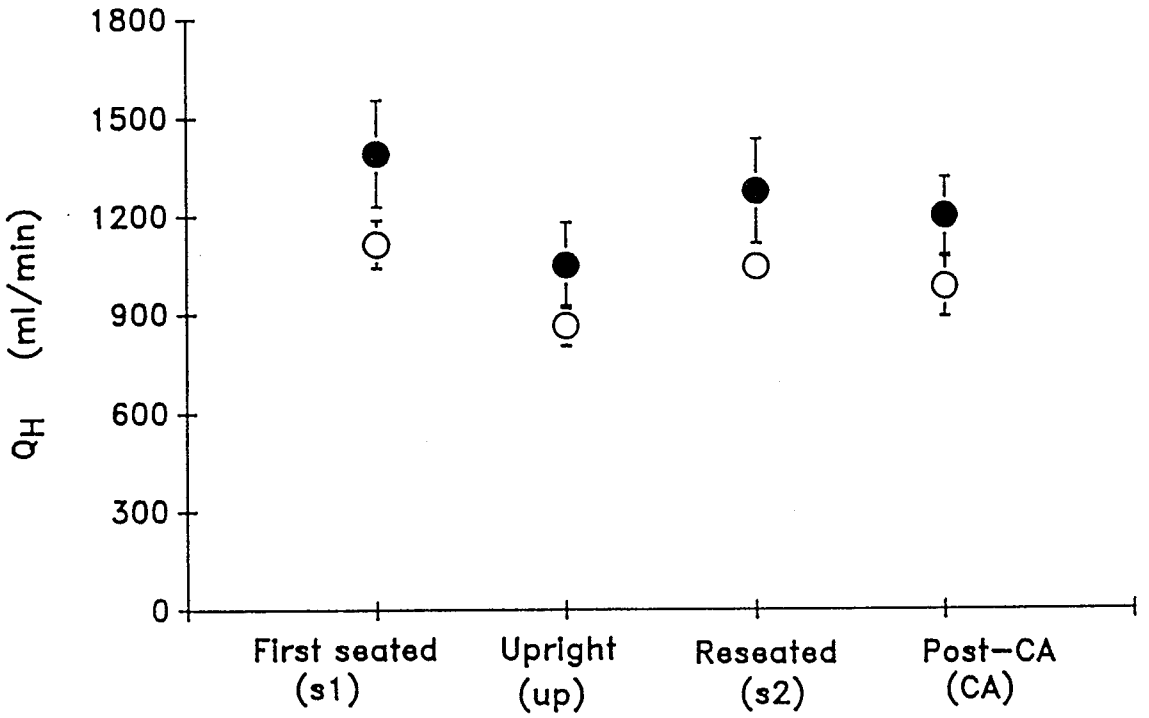


Figure 15. Increase in Q_H (mean ± SEM, n=6) from day 1 (open circles) to day 14 (closed circles).

patient were excluded from the statistical comparison, a statistically significant increase in Q_H (expressed in absolute terms and per unit body weight or body surface area) was obtained after two-weeks captopril therapy, as compared to the day 1 values ($p=0.011$, $p=0.014$ and $p=0.014$, respectively).

3.5. Changes in Blood Pressure and Heart Rate

Baseline (pretreatment) systolic and diastolic blood pressure, mean arterial pressure (MAP) and heart rate were recorded on both study days after the patient arrival to the hospital (average time ± SD, n=12 8:20 am ± 25 minutes) in seated position (mean time of sitting ± SD, n=12 66.9 ± 22.5 minutes) before any treatment was applied. The mean (± SEM, n=6) baseline systolic and diastolic blood pressure, MAP and heart rate obtained on day 1 were found to be 160.5 ± 5.3, 103.3 ± SEM, 118.0 ± 3.0 and 72.6 ± 4.1 mm Hg, respectively (Table 3). In order to evaluate the acute effects of the initial (day 1) and terminal dose (day 14) of two-weeks captopril therapy on blood pressure and heart rate comparisons were made between the baseline measurements (pretreatment,day 1 and treated, day 14) and those recorded 1 and 3 hours following the administration of captopril. The effects of short-term captopril therapy on blood pressure and heart rate were evaluated by comparing the treated baseline blood pressure and heart rate values obtained on day 14 to the pre-treatment baseline blood pressure and heart rate data of day 1.

3.5.1. Effects of Postural Change on Blood Pressure and Heart Rate

Figure 16 and Figure 17 show the changes in systolic and diastolic blood pressure, respectively, during day 1 and 14. There was no significant change in mean systolic blood pressure before captopril treatment. A slight but not significant increase in mean diastolic blood pressure (≈ 5-7 mm Hg) was observed during standing, as compared to the seated values.

Figure 18 shows that mean (± SEM, n=6) heart rate increased before and after captopril treatment from 73 ± 4 to 77 ± 5 beats/minutes and from 71 ± 4 to 77 ± 4 beats/minutes upon standing. The increase in heart rate during the upright position was statistically significant on both study days ($p \leq 0.025$ and $p \leq 0.025$ for day 1 and day 14, respectively, paired *t* test).

3.5.2. Acute Effects of Captopril on Blood Pressure and Heart Rate

The changes in systolic and diastolic blood pressure 1 and 3 hours after the initial captopril dose (day 1) of the six patients are shown in Table 11, Figure 19, respectively. In examining the individual blood pressure changes in the six patients it is apparent that systolic and diastolic blood pressure decreased in the range from -7.0 to -53 mm Hg and from -9.5 to -28.8 mm Hg, respectively, 1 hour after the administration of the initial captopril dose in five of the six patients studied. In one patient (B.H.) an extensive fall in systolic and diastolic blood pressures was noted 1 hour after the initial dose of captopril (53.0 and 28.8 mm Hg, respectively). Mean systolic, as well as diastolic blood pressures were further decreased 3 hours after captopril dosing, however, the absolute decrease from baseline

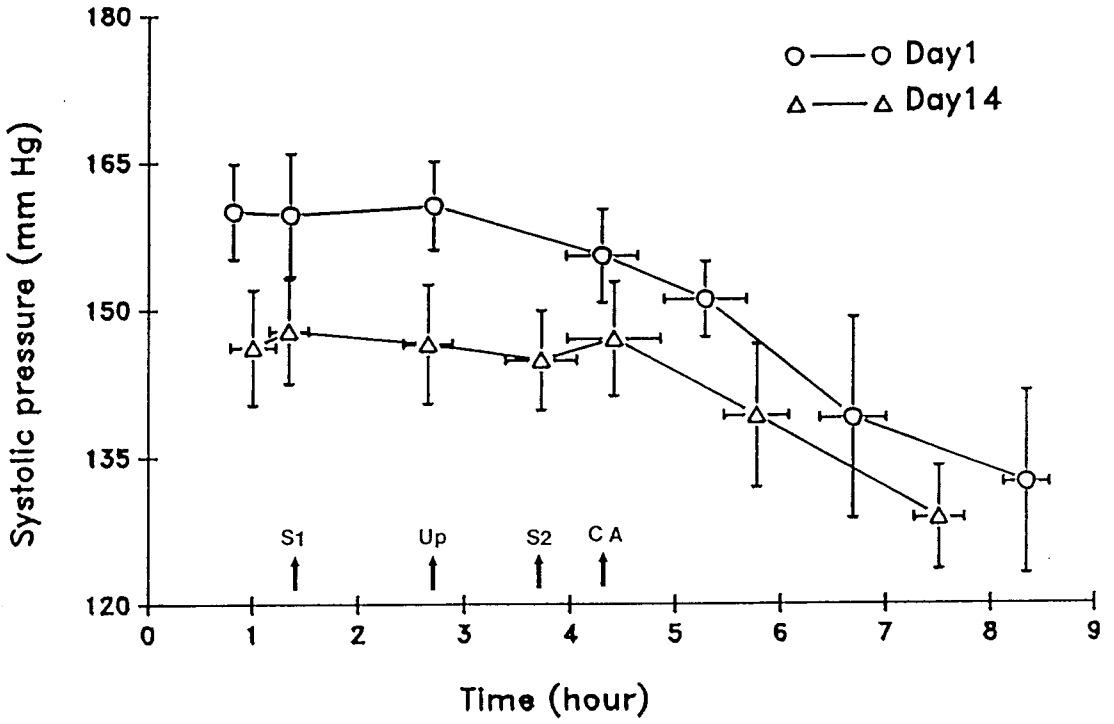


Figure 16. Systolic blood pressure (mean ± SEM, n=6) in seated (s1), upright (up), reseated (s2) position and post-captopril (CA) on day 1 and day 14.

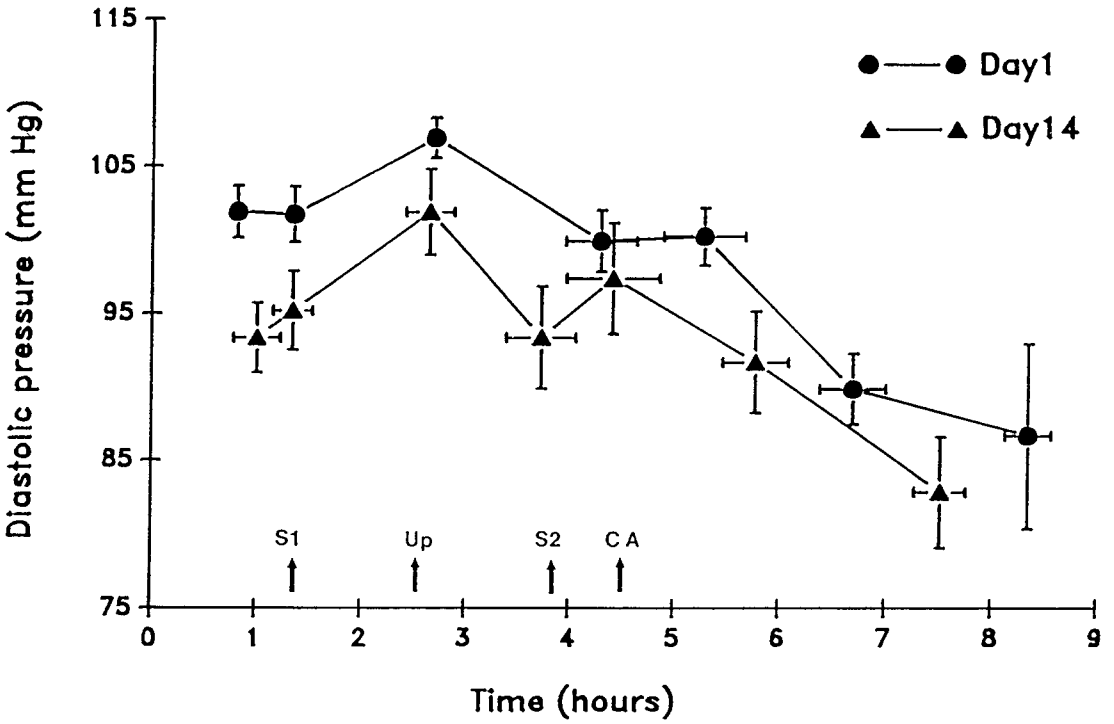


Figure 17. Diastolic blood pressure (mean ± SEM, n=6) in seated (s1), upright (up), reseated (s2) position and post-captopril (CA) on day 1 and day 14.

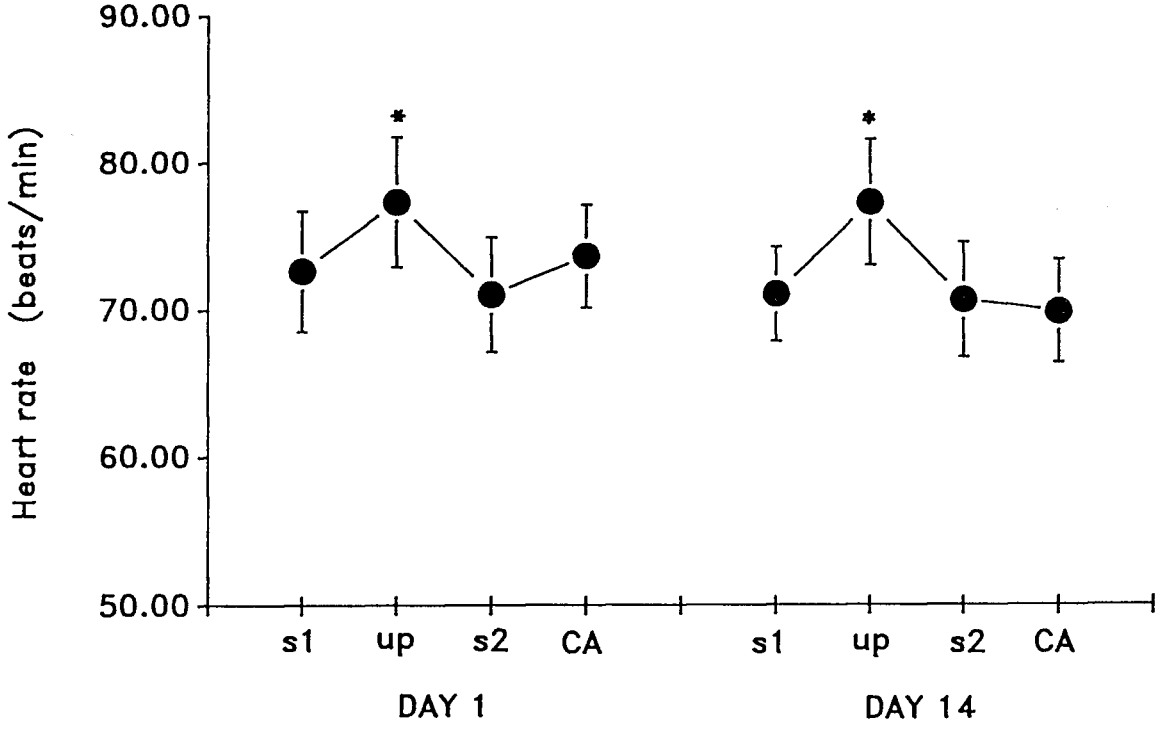


Figure 18. Heart rate (mean ± SEM, n=6) in seated (s1), upright (up), reseated (s2) position and post-captopril (CA) on day 1 and day 14. * indicates significantly different from seated (s1), paired sample *t*-test, $p \leq 0.05$.

Table 11. Change in systolic and diastolic blood pressure 1 and 3 hours after the initial dose of captopril in six mild/moderate hypertensive patients

Patient		INITIAL DOSE EFFECT (DAY 1)						
		Pre-treatment baseline	After CA		Difference from baseline		% change from baseline	
			1 Hr	3 Hr	1 Hr	3 Hr	1 Hr	3 Hr
		(mm Hg)						
MO	S	162.3	146.0	129.0	-16.3	-33.3	-10.1%	-20.5%
	D	102.3	82.0	78.0	-20.3	-24.3	-19.9%	-23.8%
OW	S	181.5	174.5	165.0	-7.0	-16.5	-3.9%	-9.1%
	D	103.0	105.5	107.5	2.5	4.5	2.4%	4.4%
MS	S	145.0	128.0	129.5	-17.0	-15.5	-11.7%	-10.7%
	D	104.0	90.5	85.0	-13.5	-19.0	-13.0%	-18.3%
BH	S	151.5	98.5	94.3	-53.0	-57.2	-35.0%	-37.7
	D	104.3	75.5	63.0	-28.8	-41.3	-27.6%	-39.6%
DA	S	154.6	139.0	140.0	-15.7	-14.7	-10.1%	-9.5%
	D	106.5	97.0	97.0	-9.5	-9.5	-8.9%	-8.9%
CB	S	167.8	147.5	136.3	-20.3	-31.4	-12.1%	-18.7%
	D	99.5	88.5	89.3	-11.0	-11.1	-11.1%	-10.2%

CA captopril
S systolic blood pressure
D diastolic blood pressure

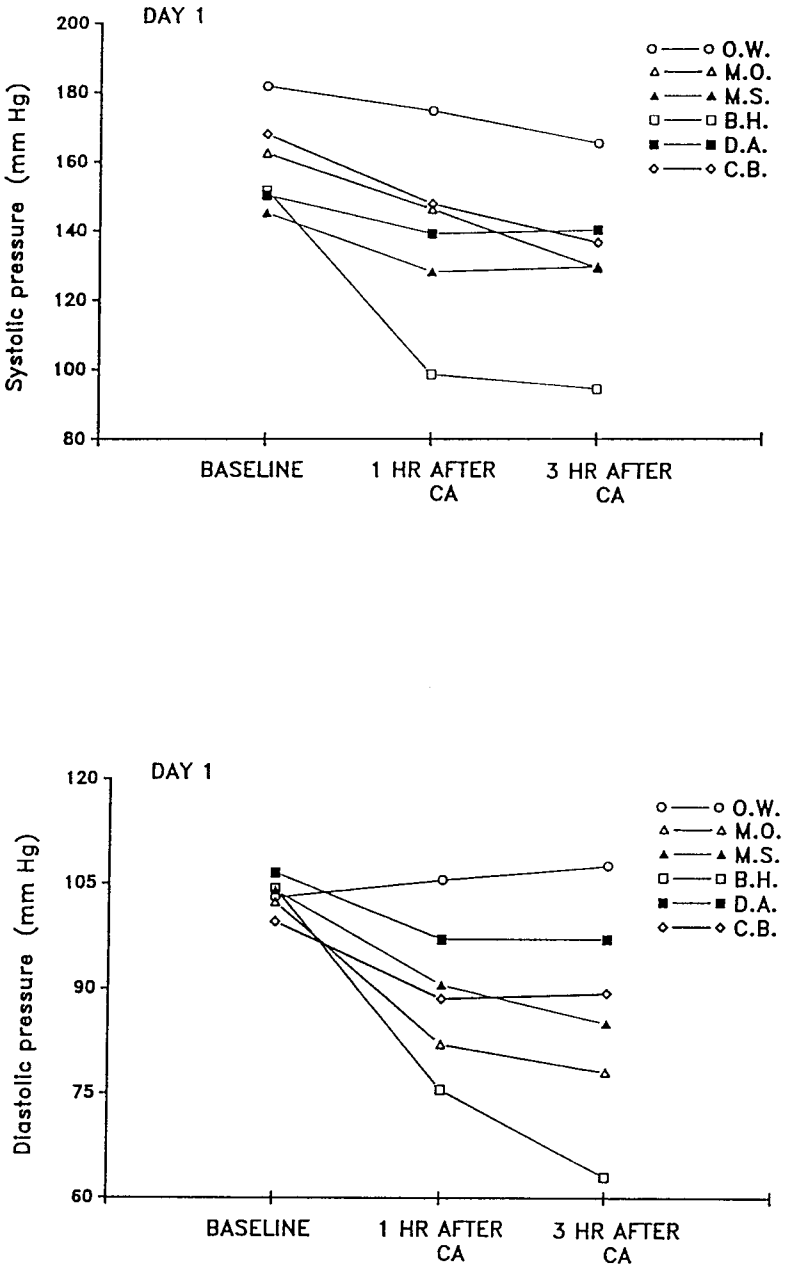


Figure 19. Changes in systolic and diastolic blood pressure 1 and 3 hours after the initial dose of captopril (day 1) in six mild/moderate hypertensive patients.

was not greater than after 1 hour of captopril dosing in two patients (D.A. and M.S.). The absolute decrease in systolic and diastolic blood pressures 3 hours after the initial captopril dose were in the range from 14.7 to 57.2 mm Hg and from 9.5 to 41.3 mm Hg, respectively, for the six patients. In the case of (O.W.) no decrease in diastolic blood pressure was noted 3 hours after the initial dose of captoril.

The results of the changes in mean (± SEM, n=6) systolic and diastolic blood pressures, mean arterial pressure and heart rate after the initial dose of captopril are summarized in Table 12. Systolic and diastolic blood pressures decreased from 160.5 ± 5.3 to 138.9 ± 10.2 mm Hg and from 103.3 ± 1.0 to 89.8 ± 6.3 mm Hg, respectively, 1 hour after the first dose of captopril. Systolic and diastolic blood pressures were further decreased with a nadir 3 hours after captopril (132.4 ± 9.3 and 86.6 ± 6.3 mm Hg, respectively).

Figure 20 shows that 1 and 3 hours after the initial captopril dose the absolute decrease in systolic and diastolic blood pressures (mean ± SEM, n=6) was -21.5 ± 6.5 mm Hg or 13.4% ($p \leq 0.025$, paired sample *t*-test) and -28.1 ± 6.7 mm Hg or 17.5% ($p \leq 0.005$, paired sample *t*-test) for systolic and -13.4 ± 4.3 mm Hg or 13.0% ($p \leq 0.025$, paired sample *t*-test) and -16.6 ± 6.4 mm Hg or 16.1% ($p \leq 0.025$, paired sample *t*-test, for diastolic blood pressure, respectively, as compared to baseline value.

The changes in systolic and diastolic blood pressures 1 and 3 hours after the terminal captopril dose (day 14) of the six patients are shown in Table 13, Figure 21, respectively. In one patient (O.W.) there was no decrease in systolic and diastolic blood pressures 1 hour after the terminal captopril dose. The absolute reduction in systolic and diastolic blood pressures for the six patient were in the range from -2.2 to -13.5 mm Hg

Table 12. Changes in mean (± SEM, n=6) systolic and diastolic blood pressure, mean arterial pressure and heart rate after the initial dose of captopril in patients with mild/moderate hypertension.

INITIAL DOSE EFFECT (DAY 1)

		After CA		Difference from baseline	
	Baseline[1]	1 Hr	3 Hr	1 Hr	3 Hr
Systolic BP (mm Hg)	160.5±5.3	138.9±10.2	132.4±9.3	-21.5±6.5* -13.4%	-28.1±6.7** -17.5%
Diastolic BP (mm Hg)	103.3±1.0	89.8±4.3	86.6±6.3	-13.4±4.3* -13.0%	-16.6±6.4* -16.1%
MAP[3] (mm Hg)	118.0±3.0	104.9±5.4	101.0±6.6	-13.1±4.3 -11.1%	-17.0±4.9 -14.4%
Heart rate (beats/min)	72.6±4.1	72.2±4.5	73.6±3.5	-0.4±1.0[NS] -0.5%	1.0±1.3[NS] 1.4%

CA captopril
SEM standard error of the mean

1 Untreated baseline blood pressure and heart rate were obtained in sitting position on day 1 after the patients' arrival at the hospital.

2 Treated baseline blood pressure and heart rate were obtained in sitting position on day 14 after the patients' return to the hospital.

3 no statistical comparisons were made for MAP data

** statistically significant ($p \leq 0.005$), compared to baseline, paired sample *t*-test

* statistically significant ($p \leq 0.05$), compared to baseline, paired sample *t*-test

NS statistically not significant ($p > 0.5$), compared to baseline, paired sample *t*-test

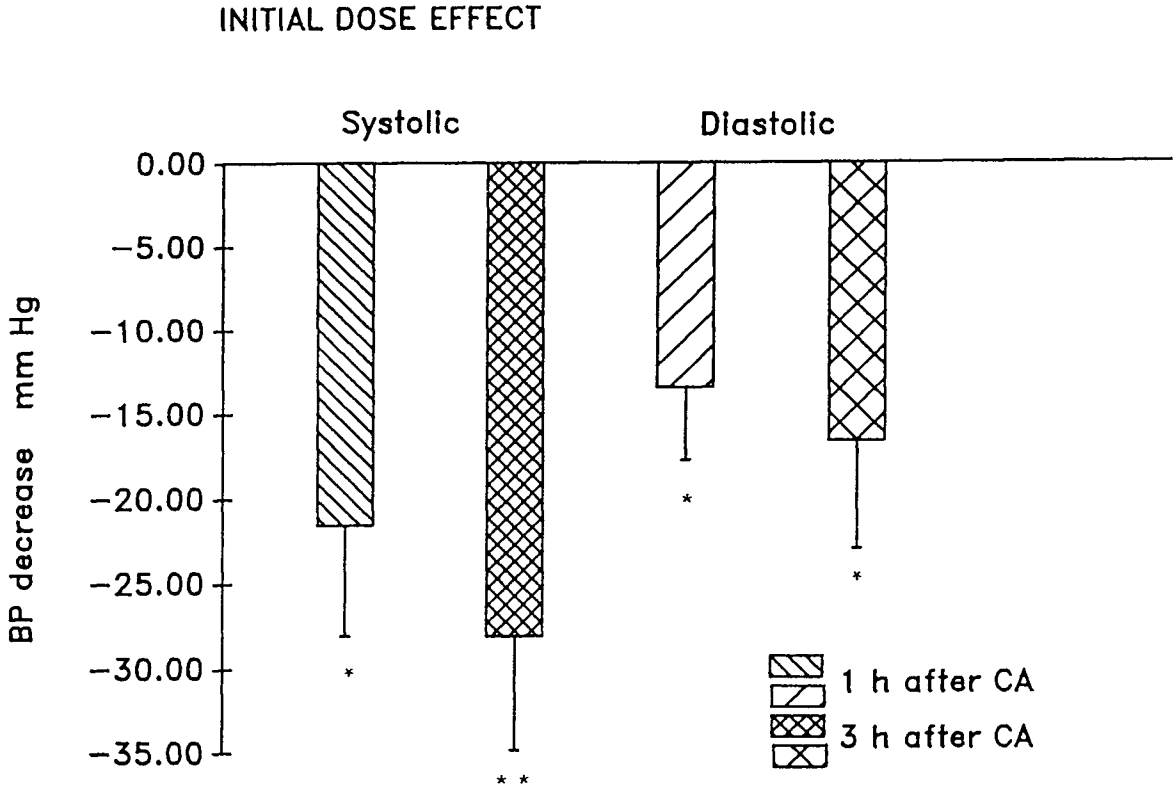


Figure 20. Decrease in systolic and diastolic blood pressure (mean ± SEM, n=6), as compared to the pretreatment values, 1 and 3 hours after the initial dose of captopril. * and * * indicates significantly different from baseline, paired sample *t*-test, $p \leq 0.05$ and $p \leq 0.005$, respectively.

Table 13. Change in systolic and diastolic blood pressure 1 and 3 hours after the terminal dose of captopril in six mild/moderate hypertensive patients

		TERMINAL DOSE EFFECT (DAY 14)						
			After CA		Difference from baseline		% change from baseline	
Patient		Treated Baseline	1 Hr	3 Hr	1 Hr	3 Hr	1 Hr	3 Hr
		(mm Hg)						
MO	S	161.0	147.7	138.7	-13.3	-22.3	-8.3%	-13.9%
	D	102.7	103.0	94.3	0.3	-8.3	0.3%	-8.1%
OW	S	161.7	169.3	149.0	7.6	-12.7	4.7%	-7.9%
	D	94.1	101.0	91.7	6.9	-2.5	7.3%	-2.6%
MS	S	130.8	123.5	112.5	-7.3	-18.3	-5.5%	-14.0%
	D	92.8	91.0	77.5	-1.8	-15.3	-1.9%	-16.4%
BH	S	134.2	125.0	122.5	-9.2	-11.7	-6.9%	-8.7%
	D	86.4	86.5	80.0	0.1	-6.4	0.1%	-7.4%
DA	S	128.7	126.5	127.0	-2.2	-1.7	-1.7%	-1.3%
	D	88.3	86.5	84.0	-1.8	-4.3	-2.1%	-4.9%
CB	S	156.8	143.3	123.0	-13.5	-33.8	-8.6%	-21.5%
	D	92.5	82.0	69.5	-10.5	-23.0	-11.4%	-24.9%

CA captopril
S systolic blood pressure
D diastolic blood pressure

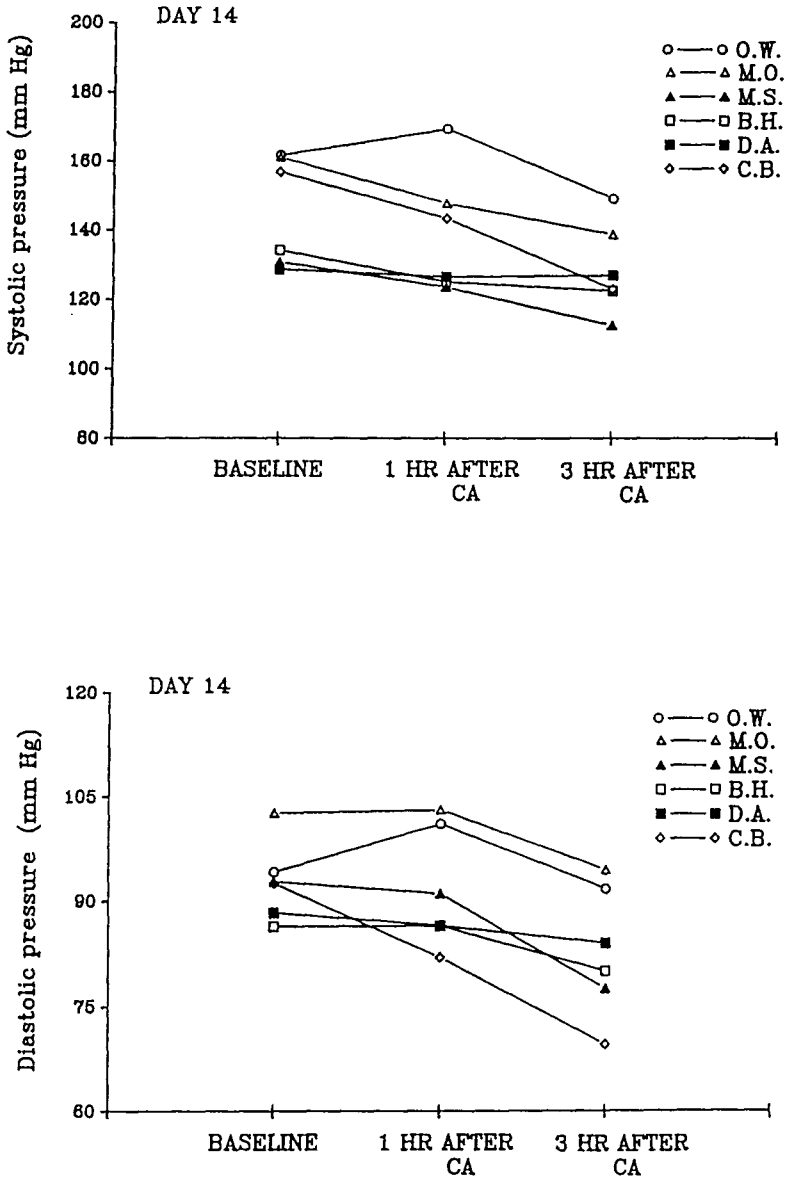


Figure 21. Changes in systolic and diastolic blood pressure 1 and 3 hours after the terminal dose of captopril (day 14) in six mild/moderate hypertensive patients.

(1.7-8.6%) and from -1.8 to -10.5 mm Hg (1.9-11.4%), respectively, after 1 hour and from -1.7 to 33.8 mm Hg (1.3-21.5%) and from -2.5 to -23.0 mm Hg (2.6-24.9%), respectively, after 3 hours of captopril administration, as compared to the baseline blood pressure of day 14.

Table 14 summarizes the changes in systolic and diastolic blood pressures, mean arterial pressure and heart rate (mean ± SEM, n=6) as compared to the treated baseline blood pressure and heart rate of day 14. The mean (± SEM, n=6) differences or relative changes in systolic and diastolic blood pressures 1 and 3 hours following the terminal captopril dose were -6.3 ± 7.3 mm Hg or 4.3% and -16.7 ± 9.9 mm Hg or 11.5% for systolic and -1.1 ± 2.3 mm Hg or 1.2% and -10.0 ± 3.2 mm Hg or 10.7% for diastolic blood pressure, respectively, as compared to the treated baseline of day 14. The decrease in blood pressure 1 hour after the terminal dose of captopril was statistically not significant, as compared to the treated baseline values, however, the decrease in blood pressure reached the level of significance 3 hours following captopril dosing ($p \leq 0.05$, paired sample *t*-test) (Figure 22).

The changes in mean arterial pressure 1 and 3 hours after the initial (day 1) and terminal dose (day 14) of captopril for six patients are presented in Figure 23.

Figure 24 shows that heart rate did not change or slightly increased 1 and 3 hours after the initial dose of captopril in the six patients studied, as compared to the control values (baseline, day 1). However, 3 hours after the terminal captopril dose heart rate decreased in four of the six patients studied (range from 3 to 6 beats/minutes) and increased in two patients by 2 and 8 beats/minutes. There was no significant change in heart rate (mean ± SEM, n=6) 1 and 3 hours following the initial and terminal dose of captopril

Table 14. Changes in mean (± SEM, n=6) systolic and diastolic blood pressure, mean arterial pressure and heart rate after the terminal dose of captopril in patients with mild/moderate hypertension

	TERMINAL DOSE EFFECT (DAY 14)				
		After CA		Difference from baseline	
	Treated Baseline[2]	1 Hr	3 Hr	1 Hr	3 Hr
Systolic BP (mm Hg)	145.5±14.5	139.2±16.4	128.8±11.9	-6.3±7.3[NS] -4.3%	-16.7±9.9* -11.5%
Diastolic BP (mm Hg)	92.8±2.3	91.7±3.5	82.8±3.8	-1.1±2.3[NS] -1.2%	-10.0±3.2* -10.7%
MAP[3] (mm Hg)	111.0±2.6	104.5±5.1	97.3±3.3	-6.4±3.1 -5.8%	-13.7±2.9 -12.3%
Heart rate (beats/min)	71.0±3.2	69.2±3.7	69.8±3.5	-1.8±1.3[NS] -2.5%	-1.2±2.1[NS] -1.7%

CA captopril
SEM standard error of the mean
MAP mean arterial pressure

1 Untreated baseline blood pressure and heart rate were obtained in sitting position on day 1 after the patients' arrival at the hospital.

2 Treated baseline blood pressure and heart rate were obtained in sitting position on day 14 after the patients' return to the hospital.

3 no statistical comparisons were made for MAP data

** statistically significant ($p \leq 0.005$), compared to baseline, paired sample *t*-test

* statistically significant ($p \leq 0.05$), compared to baseline, paired sample *t*-test

NS statistically not significant ($p > 0.5$), compared to baseline, paired sample *t*-test

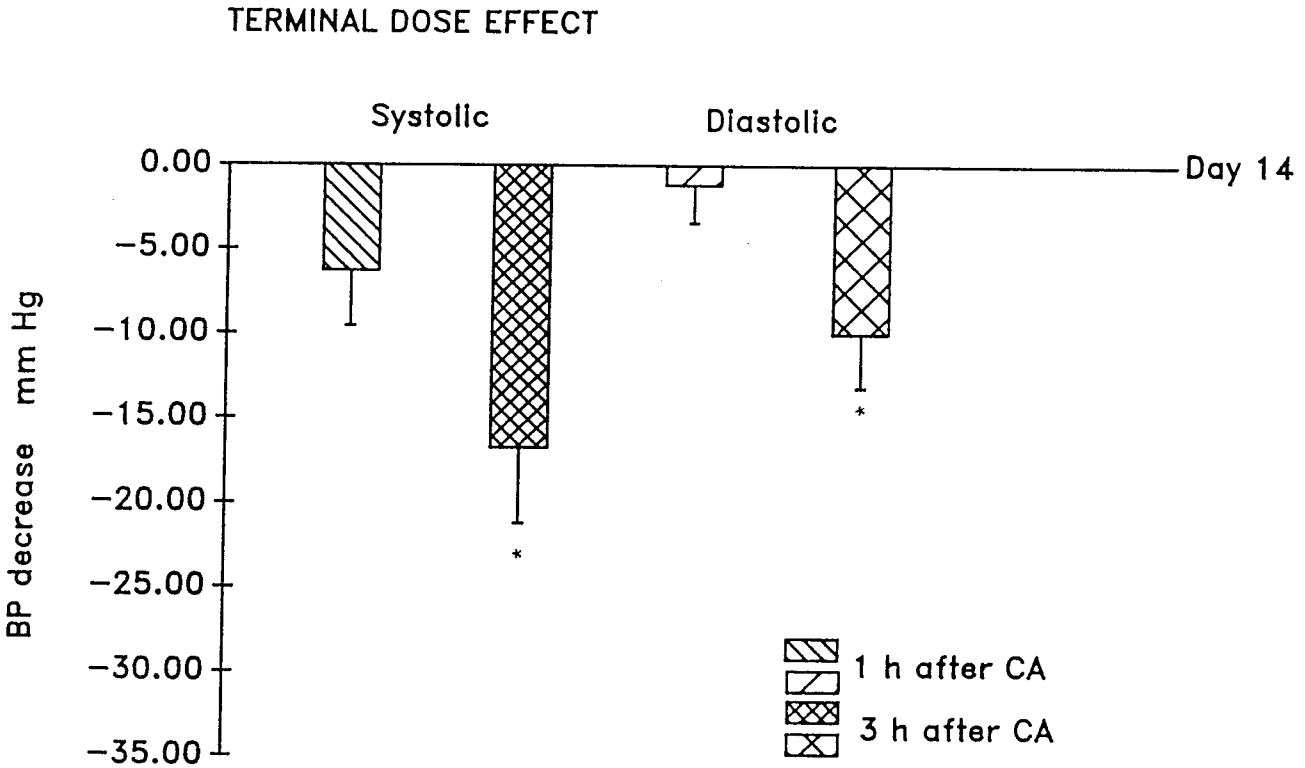


Figure 22. Decrease in systolic and diastolic blood pressure (mean ± SEM, n=6), as compared to the treated baseline of day 14, 1 and 3 hours after the terminal dose of captopril. * indicates significantly different from baseline , paired sample *t*-test, $p \leq 0.05$.

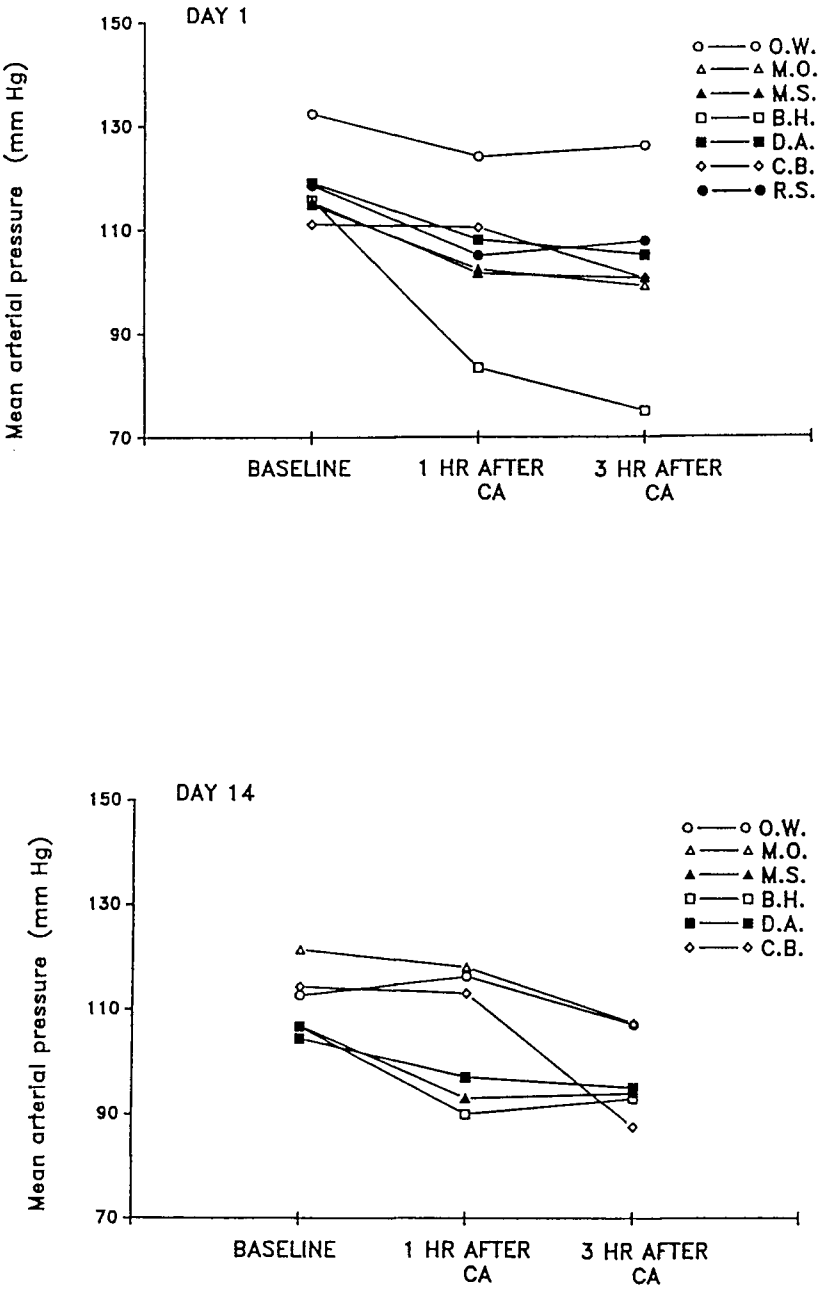


Figure 23. Changes in mean arterial pressure 1 and 3 hours after the initial (day 1) and terminal (day 14) dose of captopril in six mild/moderate hypertensive patients.

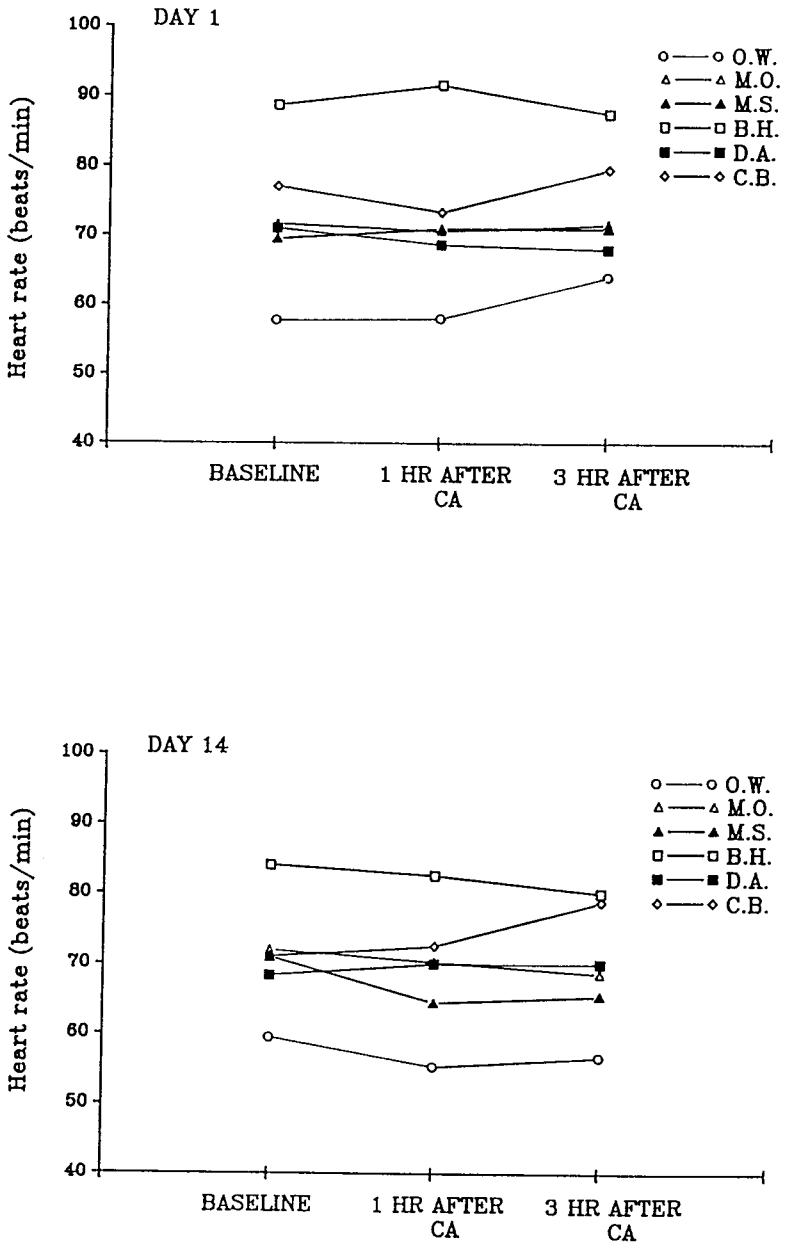


Figure 24. Changes in heart rate 1 and 3 hours after the initial (day 1) and terminal (day 14) dose captopril in six mild/moderate hypertensive patients.

(Table 12 and 14).

3.5.3. Short-Term Effects of Captopril on Blood Pressure and Heart Rate

The effects of short-term captopril therapy on blood pressure and heart rate were evaluated by comparing the treated baseline blood pressure and heart rate (day 14) to the pretreatment baseline blood pressure and heart rate of day 1.

Table 15 shows that systolic, as well as, diastolic blood pressures were lower in five of the six patients studied, as compared to pretreatment blood pressure, when they returned to the hospital after 14 days captopril therapy. The baseline blood pressure recordings on day 14 were performed about 20 hours after the last captopril dosing. The treated baseline systolic and diastolic blood pressures were lower than the pretreatment blood pressure, in the range from -1.3 to -26 mm Hg for systolic and from -7.0 to -18.2 mm Hg for diastolic blood pressure. In one patient (M.O.) the treated baseline blood pressure was similar to the pretreatment baseline blood pressure. The Black patient (O.W.) responded similarly to short-term captopril treatment as the Caucasian subjects. When individual systolic blood pressures, recorded after the terminal dose of captopril, were compared to the pretreatment baseline values the absolute decreases in systolic and diastolic blood pressures were in the range from -14.2 to -28.2 mm Hg and -2.0 to -20.0 mm Hg, respectively, after 1 hour and from -23.7 to -44.8 mm Hg and -8.0 to -30.0 mm Hg after 3 hours of captopril administration.

Table 16 shows that mean (± SEM, n=6) treated baseline systolic and diastolic blood pressures, recorded on day 14, were significantly lower than pretreatment values by -14.9 ± 7.7 mm Hg (9.3%, $p \leq 0.005$, paired sample *t*-

Table 15. Decrease in systolic and diastolic blood pressure after two-weeks captopril treatment, as compared to the pretreatment baseline in six mild/moderate hypertensive patients

		SHORT-TERM EFFECT				
		Difference from baseline (day 1)				
		Treated Baseline	After CA 1 Hr	After CA 3 Hr	% change from baseline 1 Hr	% change from baseline 3 Hr
Patient		(mm Hg)				
MO	S	-1.3	-14.7	-23.7	-9.0%	-14.6%
	D	0.3	0.7	-8.0	0.7%	-7.8%
OW	S	-19.7	-12.2	-32.5	-6.7%	-17.9%
	D	-8.9	-2.0	-11.3	-1.9%	-11.0%
MS	S	-14.2	-21.5	-32.5	-14.8%	-22.4%
	D	-11.3	-13.0	-26.5	-12.5%	-25.5%
BH	S	-17.3	-26.5	-29.0	-17.5%	-19.1%
	D	-17.8	-17.8	-24.3	-17.0%	-23.3%
DA	S	-26.0	-28.2	-27.7	-18.2%	-17.9%
	D	-18.2	-20.0	-22.5	-18.8%	-21.1%
CB	S	-11.0	-24.5	-44.8	-14.6%	-26.7%
	D	-7.0	-17.5	-30.0	-17.6%	-30.2%

CA captopril
S systolic blood pressure
D diastolic blood pressure

Table 16. Comparison of mean (± SEM, n=6) systolic and diastolic blood pressure, mean arterial pressure and heart rate obtained after two-weeks captopril treatment with the untreated value of day 1.

SHORT-TERM EFFECT

	Difference from baseline[1] (day 1)		
	Treated[2] Baseline	After CA 1 Hr	3 Hr
Systolic BP (mm Hg)	-14.9 ± 7.7[3**] - 9.3%	-21.3 ± 5.9** -13.2%	-31.7 ± 6.6** -19.7%
Diastolic BP (mm Hg)	-10.5 ± 6.4* -10.1%	-11.6 ± 8.0* -11.2%	-20.4 ± 8.0** -19.8%
MAP (mm Hg)	- 7.0 ± 4.1 -11.4%	-13.4 ± 5.2 -17.5%	-20.7 ± 2.7 - 5.9%
Heart rate (beats/min)	- 1.6 ± 1.3 - 4.7%	- 3.4 ± 0.9 - 3.9%	- 2.8 ± 1.6[NS] - 2.3%

CA captopril
SEM standard error of the mean
MAP mean arterial pressure

1 Untreated baseline blood pressure and heart rate were obtained in sitting position on day 1 after the patients' arrival at the hospital.

2 Treated baseline blood pressure and heart rate were obtained in sitting position on day 14 after the patients' return to the hospital.

3 All differences were calculated by subtracting mean baseline blood pressure and heart rate from the appropriate mean values of day 14.

** Statistically significant ($p \leq 0.005$), compared to pretreatment baseline, paired sample *t*-test.

* Statistically significant ($p \leq 0.05$), compared to pretreatment baseline, paired sample *t*-test.

test) and by -10.5 ± 6.4 mm Hg (10.1%, $p \leq 0.01$, paired sample *t*-test), respectively. Treated baseline MAP was lower than day 1 baseline by 11.4%. The absolute decrease (± SEM, n=6) in systolic and diastolic blood pressures after the terminal dose of two-weeks captopril therapy were -21.3 ± 5.9 mm Hg (13.2%) and -11.6 ± 8.0 mm Hg (11.2%), respectively, after 1 hour and -31.7 ± 6.6 mm Hg (19.7%) and -20.4 ± 8.0 mm Hg (19.8%) after 3 hours, respectively. The decrease in systolic and diastolic blood pressures 3 hours after the terminal dose of two-weeks captopril therapy were statistically significant ($p \leq 0.005$ for both blood pressures, paired sample *t*-test). The absolute decrease (± SEM, n=6) in heart rate was -2.8 ± 1.6 (2.3%) beats/minutes 3 hours after the terminal dose of captopril, as compared to the pretreatment values. This decrease in heart rate was statistically not significant, as determined by paired sample *t*-test.

Figures 25 and Figure 26 summarize the overall changes in systolic and diastolic blood pressures, respectively, during the two-weeks captopril therapy. There was a greater absolute decrease in systolic and diastolic blood pressures after 3 hours than after 1 hour of the administration of the initial or terminal dose of captopril, as compared to pretreatment values (-22 *vs* -28 mm Hg and -21 *vs* 32 mm Hg for systolic blood pressure and -13 *vs* -17 mm Hg and -12 *vs* -20 mm Hg for diastolic blood pressure, respectively). However, the absolute of decrease in systolic blood pressure from baseline, 1 and 3 hours after the terminal dose of captopril was similar to that observed 1 and 3 hours after the initial dose (-21 *vs* -22 mm Hg and -32 *vs* -28 mm Hg, respectively). Similarly, the extent of reduction in diastolic blood pressure 1 and 3 hours following the terminal and initial dose of captopril were similar, as compared to the pretreatment values (-12 *vs* -13 mm Hg and -20 vs -17 mm Hg, respectively).

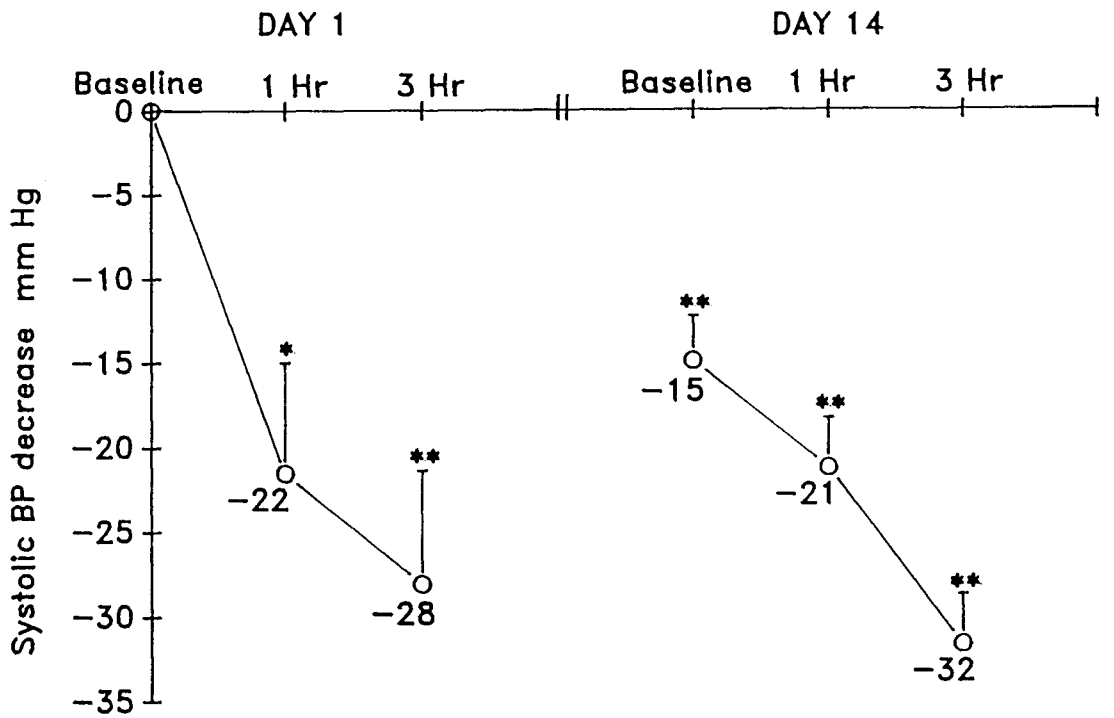


Figure 25. Decrease in systolic blood pressure (mean ± SEM, n=6) from pretreatment values after two-weeks captopril in mild/moderate hypertensive patients. * and * * indicates significantly different from baseline, paired sample *t*-test, $p \leq 0.05$ and $p \leq 0.005$, respectively.

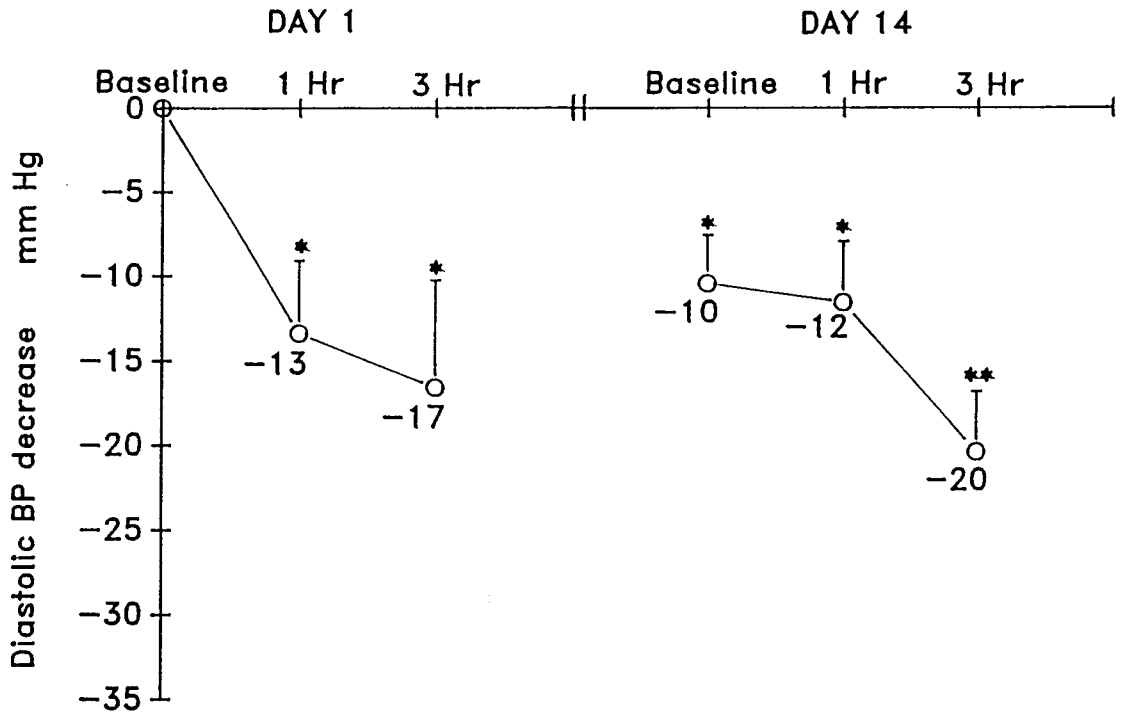


Figure 26. Decrease in diastolic blood pressure (mean ± SEM, n=6) from pretreatment values after two-weeks captopril in mild/moderate hypertensive patients. * and * * indicates significantly different from baseline, paired sample *t*-test, $p \leq 0.05$ and $p \leq 0.005$, respectively.

Figure 27 shows that there was a slight decrease in heart rate when treated baseline heart rate, measured on day 14 was compared to the pretreatment values. The mean difference (± SEM, n=6) from baseline was -1.6 ± 1.3 beats/minutes, (2.3%), $p \leq 0.25$, paired sample t-test). There was, also, a tendency for a decrease in heart rate 3 hours after the terminal dose of captopril, as compared to the pretreatment values (mean difference ± SEM, n=6 -2.8 ± 1.6 beats/minutes, 2.3%, $p \leq 0.1$) paired sample t-test).

3.6. Changes in Splanchnic Vascular Resistance

3.6.1. Effects of Upright Posture

Table 17 shows the changes in SVR during postural change from sitting to standing before and after captopril treatment. Mean SVR (± SEM, n=6) increased on day 1 and 14 upon standing from 6.58 ± 0.54 to 8.62 ± 0.66 mm Hg·sec/ml (31.0%) and from 5.34 ± 0.8 to 7.12 ± 1.09 mm Hg·sec/ml (33.3%), respectively. The increase in SVR observed on day 1 was statistically significant ($p \leq 0.05$, paired sample t-test). However, the increase on day 14 was slightly below the borderline significance ($0.05 < p < 0.1$, paired sample t-test). A decrease in SVR from 8.62 ± 0.66 to 6.65 ± 0.27 mm Hg.sec/ml on day 1 and from 7.12 ± 1.09 to 5.65 ± 0.98 mm Hg.sec/ml on day 14 occurred when patients reassumed seated position (s2) after standing. The values of SVR obtained in the first seated (s1) and reseated (s2) periods were similar (6.58 vs 6.65 mm Hg.sec/ml). Figure 28 illustrates the changes in SVR during postural change before and after captopril therapy.

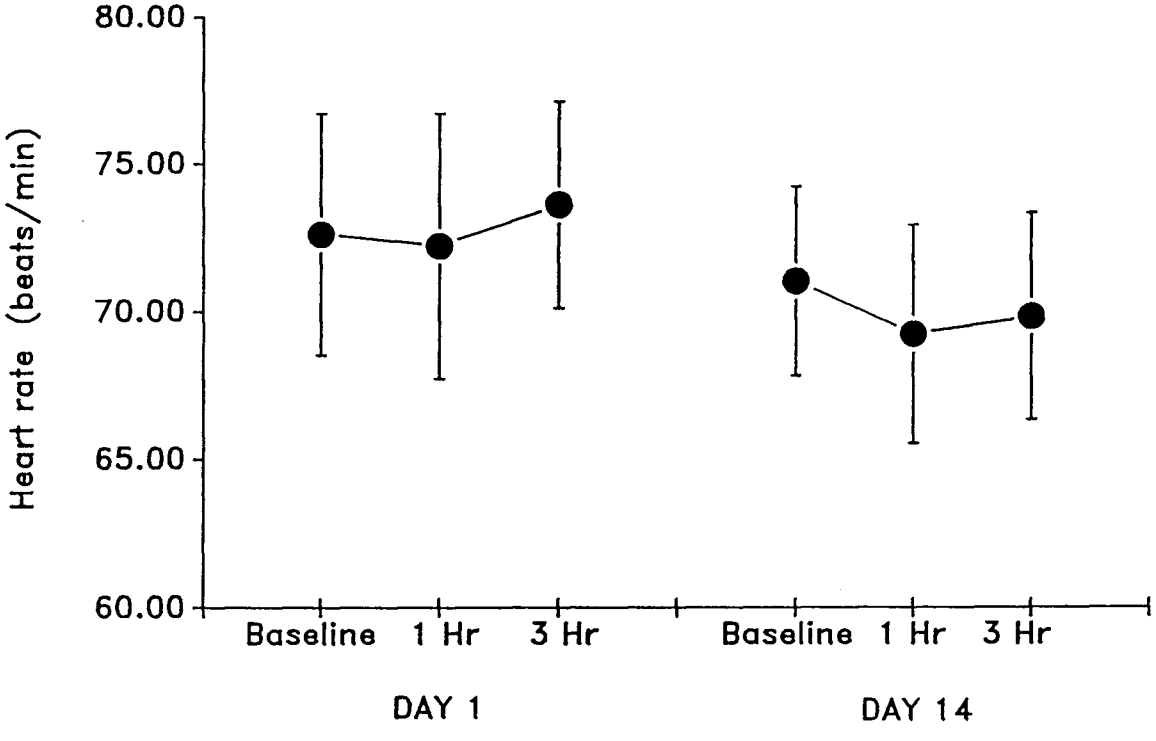


Figure 27. Acute effects of captopril on heart rate (mean ± SEM, n=6), 1 and 3 hours after captopril on day 1 and day 14.

Table 17. Changes in splanchnic vascular resistance from six patients with mild/moderate hypertension during postural change and before and after captopril treatment.

	Splanchnic Vascular Resistance (mm Hg sec/ml)							
	DAY 1				DAY 14			
Patient	s1	up	s2	CA	s1	up	s2	CA
OW	6.27	8.04	7.10	7.48	3.99	6.17	4.41	4.54
MO	6.23	8.24	5.93	4.45	4.49	5.71	5.35	4.95
MS	4.83	6.50	6.54	5.64	3.42	4.46	3.56	4.30
BH	6.33	8.96	7.08	5.45	5.64	8.26	7.42	5.72
DA	7.54	10.99	7.32	8.11	6.18	6.76	4.06	4.43
CB	8.29	9.01	5.96	7.59	8.31	11.38	9.14	7.57
Mean ± SEM:	6.58 ±0.54	8.62* ±0.66	6.65 ±0.27	6.46 ±0.66	5.34 ±0.80	7.12 ±1.09	5.65 ±0.98	5.25 ±0.56
% change upright[a]		31.0				33.3		
% change day 14[b]					-18.8	-17.4	-15.0	-23.0

s1 data obtained in first seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained in 1 hour after captopril dosing
SEM standard error of the mean

* statistically significant ($p \leq 0.05$) compared to s1, MANOVA

a % change during upright, as compared to s1

b % change after two-weeks captopril treatment, as compared to day 1 values

3.6.2. Acute Effects of Captopril on SVR

Figure 28 shows that SVR (mean (± SEM, n=6) after the initial (CA, day 1) and terminal (CA, day 14) dose of captopril was significantly not different from reseated (s2) values (6.46 ± 0.66 vs 6.65 ± 0.27 mm Hg.sec/ml and 5.25 ± 0.56 vs 5.65 ± 0.98 mm Hg.sec/ml, respectively).

In examining the individual data in Table 17 obtained on day 1 it is apparent, that SVR did not change after captopril administration in one patient (O.W.) increased and decreased in two (D.A. and C.B.) and three patients (M.O., M.S. and B.H.), respectively, as compared to the reseated (s2) values. Similarly, on day 14 the individual responses of SVR to the terminal dose of captopril were variable, as compared to the reseated (s2) values. Also, Figure 28 shows that there is no significant change in SVR (mean ± SEM, n=6) when data obtained after the initial and terminal dose of captopril were compared to the seated (s1) values (6.46 ± 0.66 vs 6.58 ± 0.54 mm Hg sec/ml and 5.25 ± 0.56 vs 5.34 ± 0.8 mm Hg.sec/ml, respectively). These data suggest no significant changes in the resistance sites of the hepatic vasculature after the acute doses of captopril, as compared to the seated (s1) or reseated (s2) values.

3.6.3. Short-term Effects of Captopril on SVR

Table 17 and Figure 28 show the changes in SVR after two-weeks treatment with captopril (day 14), as compared to the day 1 values. In examining the data of day 1 and 14 obtained in the four study periods (s1, up, s2 and CA) it is apparent that the values of the SVR (mean ± SEM, n=6) were lower during each measurements of day 14 than those of day 1. The magnitude of

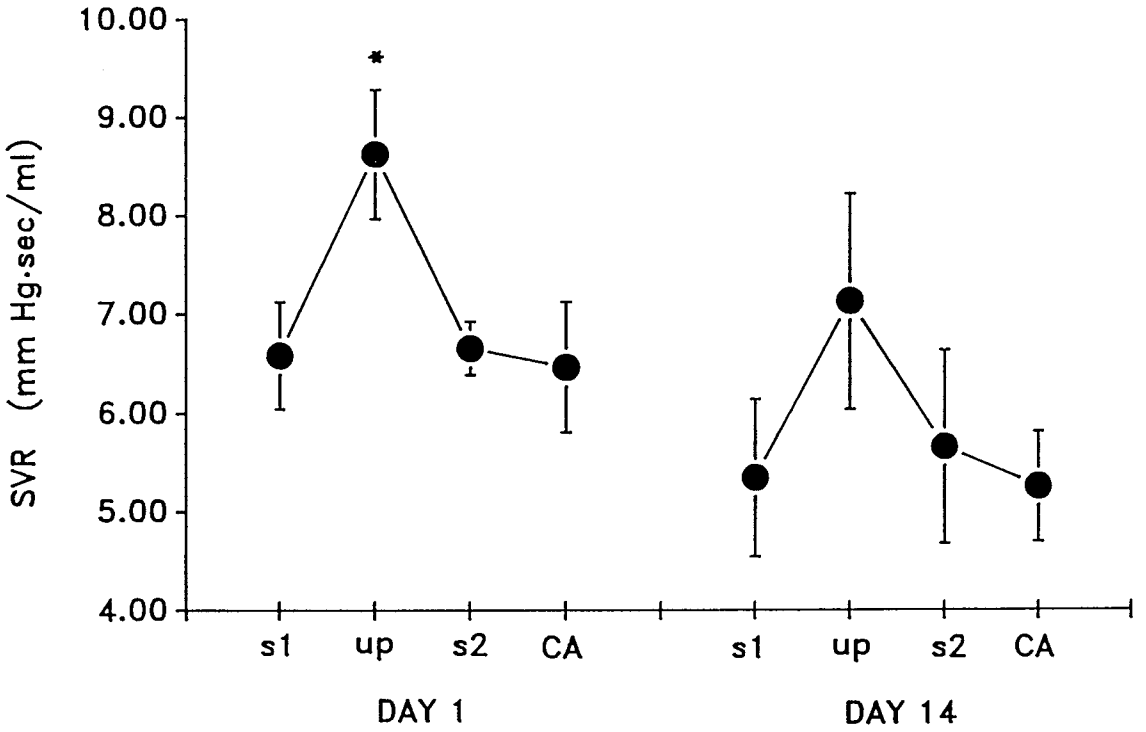


Figure 28. Splanchnic vascular resistance (SVR)(mean ± SEM, n=6) in seated (s1), upright (up), reseated (s2) positions and post-captopril (CA) on day 1 and day 14. * indicates significantly different from seated (s1), MANOVA, $p \leq 0.05$.

the decrease in SVR, in seated (s1), upright (up), reseated (s2) and post-CA (CA) periods was 18.8%, 17.4%, 15.03% and 23.04%, respectively (mean 18.6%), as compared to day 1. This decrease was consistent in five of the six patients studied. The overall decrease in SVR after two-weeks captopril treatment was, however, statistically not different from the values of day 1 ($p = 0.125$, MANOVA).

3.7. Serum Concentration Data of Captopril

Serum concentration *versus* time plots of unchanged captopril from the six patients for day 1 and day 14 are presented in Figure 29.

Table 18 presents the pharmacokinetic parameters of unchanged captopril such as the AUC_0^t, the peak serum concentrations (C_{max}), the time to reach peak serum concentration (t_{max}) and the serum concentrations of unchanged captopril at the end of blood sampling (C_{p3h}), from six patients on day 1 and day 14. The mean values (± SEM, n=6) and the changes in AUC_0^t and C_{max}, expressed in percentage, are also presented in Table 18. The mean serum concentration *versus* time profiles of unchanged captopril on day 1 and day 14 are shown in Figure 30. On day 1, mean (± SEM, n=6) C_{max} of 697.2 ± 192.8 ng/ml was reached between 30 and 180 minutes (mean ± SEM; 75.9 ± 22.2 minutes) after dosing. Thereafter, there was an exponential disappearance of captopril from serum, so that by the end of 180 minutes following administration of the initial dose of captopril, serum concentration of unchanged captopril (C_{p3h}) was in the range from 16.4 to 276.7 ng/ml. The AUC_0^t (mean ± SEM, n=6) for unchanged captopril was 802.73 ± 197.7 ng·h/ml after a single dose of captopril on day 1. There was large interindividual variability in AUC_0^t in the range from 190.4 to 1379.5 ng·h/ml (7 fold

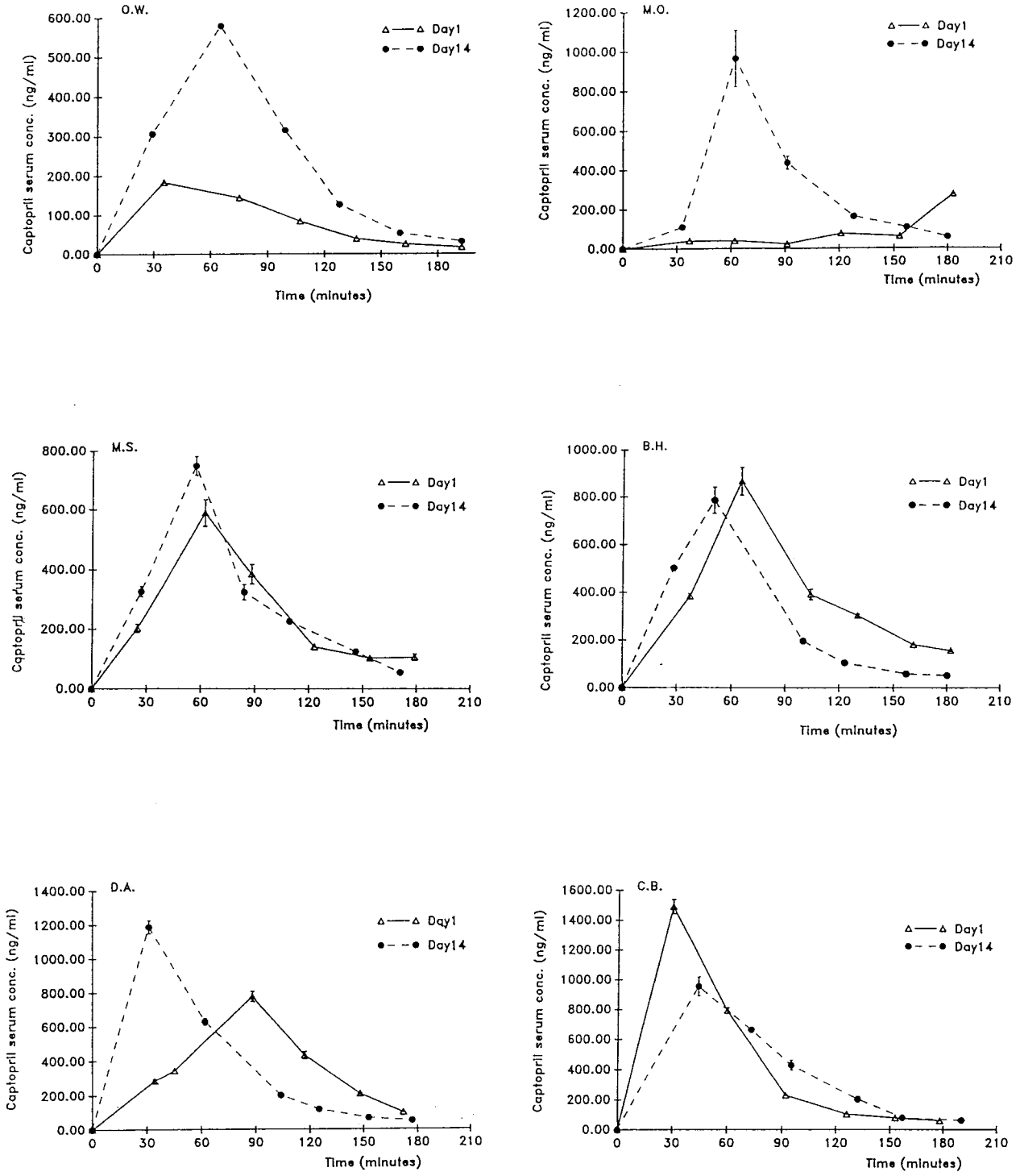


Figure 29. Serum concentration *versus* time profile of unchanged captopril from six patients with mild/moderate hypertension after the initial (dotted line) and terminal dose (solid line) of captopril.

Table 18. The data of AUC_0^t, C_{max}, t_{max} and serum concentration after 180 minutes of dosing (C_{p3h}) of unchanged captopril for six patients on day 1 and day 14

Patient		AUC_0^t ng·h/ml	C_{max} (ng/ml)	t_{max} (minutes)	C_{p3h} (ng/ml)
OW	Day 1	279.0	184.3	37.5	16.4
	Day 14	773.6	582.2	64.0	31.3
MO	Day 1	190.4	276.7	180.0	276.7
	Day 14	910.4	963.2	62.0	58.2
MS	Day 1	756.5	588.8	56.5	105.8
	Day 14	843.6	748.9	55.5	55.1
BH	Day 1	1153.3	866.7	70.5	156.5
	Day 14	887.7	785.8	64.0	52.4
DA	Day 1	1057.8	776.5	81.0	98.7
	Day 14	1187.5	1187.0	31.0	50.7
CB	Day 1	1379.5	1490.3	30.0	60.2
	Day 14	1236.1	955.7	36.5	62.3
Mean ± SEM:	Day 1	802.7 ±197.7	697.2 ±192.8	75.9 ±22.2	119.1 ±36.8
Mean ± SEM	Day 14	973.1[NS] ±78.1	870.5[NS] ±85.9	52.2 ±6.0	51.7 ±4.4
% increase in AUC_0^t		21.2			
% increase in C_{max}			24.9		

SEM standard error of the mean
NS statistically not significant ($p > 0.05$) compared to day 1, paired *t* test

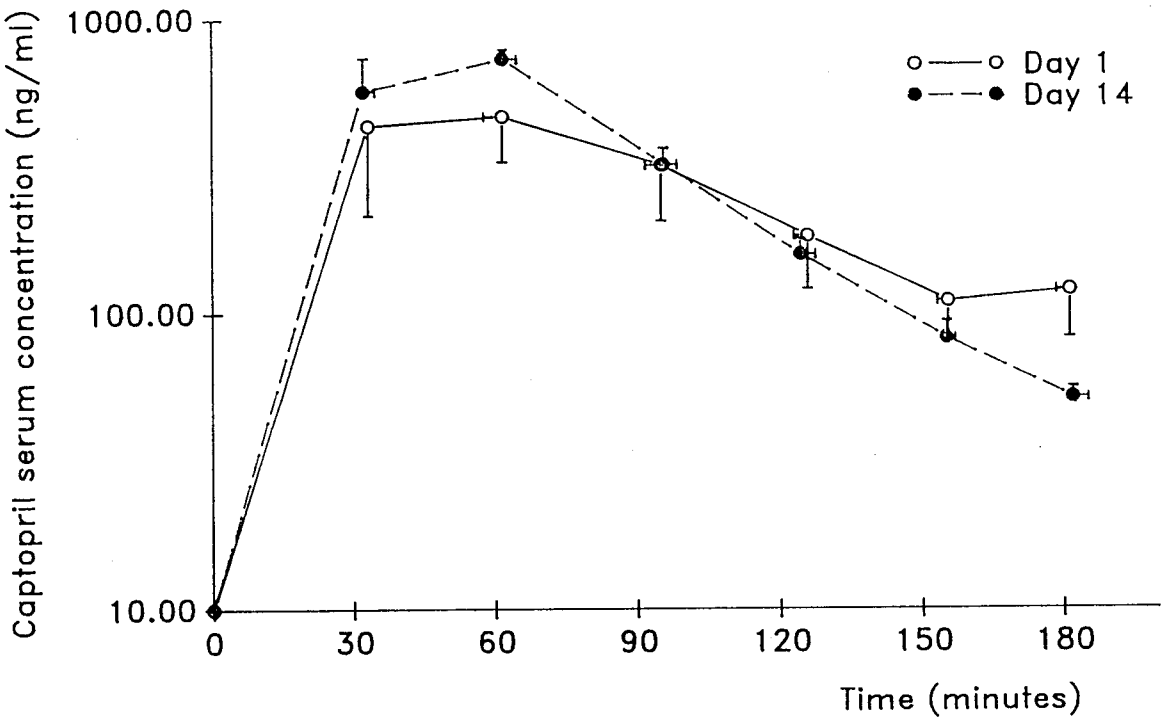


Figure 30. Serum concentration *versus* time profile of unchanged captopril (mean ± SEM, n=6) after a single dose (open circle) and the last dose (closed circle) of two-weeks captopril treatment.

difference). Similarly, C_{max} ranged from 184.3 to 1490.3 ng/ml (8 fold difference), which indicates large interindividual variation in this kinetic parameter. On day 14, after the terminal captopril dose, the time course of serum concentration for unchanged captopril was similar to day 1, except, that C_{max} of 870.5 ± 85.9 ng/ml (mean ± SEM, n=6) was observed at 52.2 ± 2.5 min and was 24.9% higher than on day 1. However, this higher C_{max} on day 14 was statistically not different from that of day 1. The individual values of C_{max} were less variable than on day 1, range from 582.2 to 1187.0 ng/ml (2 fold difference). Similarly, the AUC_0^ts of unchanged captopril for the six patients were in the range from 773.6 to 1236.1 ng·h/ml (1.6 fold difference), suggesting less individual variability in this kinetic parameter after two-weeks treatment, as compared to day 1. The AUC_0^t (mean ± SEM, n=6) on day 14 was 973.1 ± 78.1 ng·h/ml which is 21.2% larger than on day 1. A decrease, rather than an increase in AUC_0^t was observed in two patients (B.H. and C.B.). Due to this large variation in the responses of AUC_0^t, the observed increase in AUC_0^t, after 14 days captopril administration, as compared to the day 1 values, did not reach the level of significance, as determined by paired sample *t*-test ($p \leq 0.5$). About 180 minutes following the administration of the terminal dose of captopril (181.8 ± 3.4 minutes, mean ± SEM, n=6), unchanged captopril was in the range from 31.3 to 62.3 ng/ml).

4. DISCUSSION

4.1. Spectrophotometric Analysis of Indocyanine Green in Human Serum

Ultraviolet absorption spectrophotometric methods have been widely used to measure ICG in human plasma following intravenous injection (Soons *et al.*, 1990; Robson *et al.*, 1990; Rocci *et al.*, 1989; Grasela *et al.*, 1987; Svensson *et al.*, 1982) ICG plasma concentrations have traditionally been determined by measurements of absorbance of unextracted plasma samples at 800-805 nm (Grasela *et al.*, 1987; Hollins *et al.*, 1987; Grainger *et al.*, 1983). This method is particularly suitable for clinical studies since it is simple, rapid and relatively sensitive. Although adequate sensitivity of the assay methods have been shown, their specificity have been questioned, due to an interfering substance which absorbs at the same wavelength as the parent compound (Dorr and Pollack 1989, Grasela *et al.*, 1987; Heintz *et al.*, 1986). It has been demonstrated that the interfering compound(s) has longer elimination half-life than ICG, causing the "flattening" of the elimination phase of ICG plasma concentration *versus* time curve beyond 15 minutes (Meijer *et al.*, 1988). Recently, several high-performance liquid chromatographic (HPLC) methods were developed to improve assay sensitivity and specificity (Dorr and Pollack *et al.*, 1989; Rappaport and Thiessen, 1982, Svensson *et al.*, 1982). These HPLC methods have been demonstrated to be able to distinguish between the interfering substance and parent dye therefore were shown to be more specific than the spectrophotometric method. However, when the spectrophotometric and HPLC methods for measuring ICG in

animal species (Heintz *et al.*, 1986) and human plasma (Svensson *et al.*, 1982) were compared, it was confirmed, that the two analytical methods gave similar estimations of Cl_{pICG} and Q_H if blood sampling was limited to 15-20 minutes. Further, Grasela *et al.*, (1987) investigated the effects of posture and cimetidine on Q_H in human subjects and compared the changes in the pharmacokinetic parameters of ICG and Q_H by using both, spectrophotometric and HPLC assay methods. It was found that, although estimated Q_H values were consistently lower when ICG was assayed spectrophotometrically, the statistical inferences were similar regardless of the assay methodology (Grasela *et al.*, 1987). These findings were substantiated by the studies of Soons *et al.*, (1990) who found that despite the differences in the half-life and volume of distribution of ICG, the effects of nifedipine on ICG clearance and estimated Q_H were similar when ICG plasma concentrations were measured by spectrophotometric or HPLC methods. Based on these observations and the fact that the presently available, UV detector equipped HPLC system in our laboratory did not have the appropriate wavelength (700 nm) to permit the measurement of the low ICG concentrations in human plasma (Wang, 1991); therefore, in the present study, the modified spectrophotometric method (Caesar *et al.*, 1961) has been used to assess ICG concentrations in human plasma.

4.1.1. Validation of the Spectrophotometric Method

The stability of ICG is affected by various factors such as light, temperature and nature of the solvent (Gathje *et al.*, 1970). It has been shown in previous studies that the rate of decay of the spectral absorption of ICG in distilled water was rapid at ICG concentration of 1.25 mg/ml and

light exposure increased the rate of decay (Gathje *et al.*, 1970). Also, the rate of deterioration in aqueous solutions occurs more rapidly in dilute than in concentrated solutions (Fox, 1960). The results of the present study regarding the stability of ICG in deionized water confirms these findings. The stability of ICG in human plasma is greatly improved mainly due to the presence of albumin which forms complexes with the large molecules of ICG, thereby preventing further decomposition (Paumgartner, 1975; Gathje *et al.*, 1970). A shift in the absorption spectrum to higher wavelengths from 790 to 805 nm provides evidence that ICG binds to plasma proteins (mainly albumin) (Paumgartner, 1975). In the present study, the rate of decay of ICG in human serum was very low (Figure 3) at ICG concentrations 0.2 and 6.0 μg/ml during the observation period of 48 hours. This was indicated by the fact that the slopes of the two regression lines were close to zero. These results suggest that ICG is stable in human serum for 48 hours when stored at -20°C and protected from light. All patient serum samples in this study were analyzed within 48 hours.

The results of the assay validation procedures of the present study showed adequate accuracy in the quantitation of ICG in patient serum. The inter-day coefficients of variation ranged from 9.8% (0.2 μg/ml) and 1.6% (5.0 μg/ml) (Table 2). The intra-day coefficients of variation for concentrations 0.2, 1.0 and 5.0 μg/ml were 8.4%, 4.9% and 2.1%, respectively. Standard curves were reproducible from day to day with coefficients of variation of 1.6% for the slopes (Figure 4).

A linear relationship between absorbance and concentration of ICG in human bile and 0.5% human albumin was observed only up to 7.5 μg/ml and in human duodenal fluid up to 5 μg/ml (Björnsson *et al.*, 1982). In the present study, a linear relationship was observed between absorbance and ICG

concentration in the range from 0.2 to 5.0 μg/ml (Figure 6).

4.2. Estimation of Q_H by ICG Clearance

The estimation of Q_H using ICG clearance has been validated in both animals and humans (Grainger *et al.*, 1983). At low doses used in this study the hepatic clearance of ICG is limited by the rate of its delivery to the liver (Paumgartner, 1975). Estimation of Q_H by ICG clearance was based on certain assumptions (Robson *et al.*, 1990). First, it was assumed that no change in the hepatic extraction of ICG occurred during the study. Hence, the determined ICG clearance would be expected to reflect changes in Q_H and not an altered hepatic extraction ratio due to captopril therapy. To establish that captopril does not interfere with the hepatic extraction of ICG requires the catheterization of the hepatic vein. Since, this is a highly invasive technique, it could not be justified in this study (Soons *et al.*, 1990). However, direct measurements of the hepatic extraction of ICG have shown no changes after single oral doses of captopril in patients with essential hypertension (Crossley *et al.*, 1984), or liver cirrhosis (Eriksson *et al.*, 1984). Further, the assumption of 100% hepatic extraction of ICG has been frequently made, particularly in those studies which measured relative changes in Q_H (Soons *et al.*, 1990; Modi *et al.*, 1988). However, hepatic extraction ratio in healthy subjects has been found to be in the range from 0.70 to 0.78 (Wynne *et al.*, 1990; Ceasar *et al.*, 1961; Crossley *et al.*, 1984; Grainger *et al.*, 1983). Since, in the present study the ICG clearance method was used to estimate relative changes in Q_H, the hepatic extraction ratio was considered to be 1 (Soons *et al.*, 1990). Second, it was assumed that ICG rapidly distributed into the plasma compartment where

it completely bound to plasma proteins. This has been previously demonstrated by Cherrick *et al.*, (1960) in normal subjects.

In humans, the resting total liver blood flow is about 800-1200 ml/min (Richardson and Withrington, 1981). George, (1979) reported Q_H values in the range from 1090-1790 ml/min in healthy man. In essential hypertension and in patients with renal artery stenosis Q_H was significantly lower by ≈10% and ≈16%, respectively, than in control subjects (Messerli *et al.*, 1975). Daneshmend *et al.*, (1981) noted Q_H of 1214 ± 146 ml/min in healthy subjects assessed by ICG clearance. Crossley *et al.*, (1984) reported Q_H (± SEM) of 1127 ± 115 ml/min in patients with essential hypertension using the ICG clearance method. In the present study, pretreatment Q_H (± SEM, n = 6) was 1114 ± 74 ml/min which is compatible with the Q_H values reported in previous studies. When the absolute Q_H value was expressed per unit body weight or body surface area, resting baseline Q_H (± SEM) values were 11.8 ± 0.6 ml/min/kg and 517.8 ± 27.2 ml/min/m^2, respectively (Table 9).

4.3. Serum Concentration Data of ICG

Serum concentration *versus* time data of ICG were fit by the computer program AUTOAN (Sedman and Wagner, 1976) and the kinetic parameters and the pharmacokinetic model obtained. The disappearance of ICG from serum was fit to a single compartment model, but, often fit better to a two compartment model. Previous studies also reported biexponential decay for ICG, when blood sampling was extended after 15-20 minutes (Grainger *et al.*, 1983). However, in the present study, when ICG decay in serum was fit as a biexponential function, only 2 data points were used to calculate the kinetic parameters. Thus, parameter estimates based on the two-compartment

fit are less statistically reliable than those of the one-compartment model. Therefore, in this study for the estimation of kinetic parameters the single compartment model was used. This is consistent with previously reported studies (Wynne *et al.*, 1990 and 1989; Robson *et al.*, 1990). There was no change in the terminal elimination $t_{1/2}$ of ICG after two-weeks of captopril therapy (4.03 ± 0.68 *vs* 3.97 ± 0.52 minutes, mean ± SD, n=24). The values of $t_{1/2}$, in the present study, are in agreement with the findings of Shepherd *et al.*, (1985) who has reported 3.37 ± 0.51 and 3.89 ± 0.94 minutes for $t_{1/2}$ of ICG, before and after captopril treatment, in healthy subjects. Grasela *et al.*, (1987) has noted higher and Geneve *et al.*, (1990) lower $t_{1/2}$ values for ICG in healthy subjects (5.6 and 3.0 minutes, respectively).

In contrast to Svensson *et al.*, (1986) who reported no detectable levels of ICG in the plasma of two healthy subjects following five injections of 0.5 mg/kg i.v. bolus doses of ICG at ≈ 1 hour intervals, in the present study, trace amounts of ICG were detected in the serum prior to the second, third and fourth ICG injections. However, the amount of ICG was always less than 0.2 μg/ml, the lower limit of detection of the HPLC method used in the studies of Svensson *et al.*, (1986). In the present study, in one patient (M.O.) there was no detectable level of ICG prior to the next ICG injection on any of the study days. Since the residual ICG concentration was only ≈ 1.6% of the highest measured ICG concentration and the terminal $t_{1/2}$ ≈ 4.0 minutes, its contribution to the total AUC was not significant.

4.4. Changes in Cl_{pICG} and Q_H

4.4.1. Effects of Postural Change on Cl_{pICG} and Q_H Before and After Captopril

Numerous studies have demonstrated a decrease in Q_H due to postural change from supine (Culbertson *et al.*, 1951, Daneshmend *et al.*, 1981) or seated (Modi *et al.*, 1988) to standing. The decrease in Q_H is assumed to be mainly due to active vasoconstriction in the splanchnic circulation mediated through the activation of the sympathetic nervous system (Wilkins *et al.*, 1951).

In healthy subjects standing for 15 minutes after lying caused a decrease in ICG blood clearance (*i.e.* estimated liver blood flow) by 37% (Daneshmend *et al.*, 1981). Modi *et al.*, (1988) observed a significant (≈ 15 to 40%) decrease in Q_H, assessed by the ICG clearance method, after 2 hours standing in healthy subjects, as compared to seated. In an earlier report by Culbertstone *et al.*, (1951), Q_H decreased on the average ≈ 38% in normotensive and ≈ 29% in hypertensive subjects when body posture was changed from supine to the upright (Q_H was estimated by the BSP extraction method).

It is well established, that standing is a convenient physiological stimulus which causes a reproducible decrease in Q_H (Daneshmend *et al.*, 1981). Daneshmend *et al.*, (1981) used postural change as a physiological variable affecting Q_H and suggested its use to study the changes in kinetics of high clearance drugs. Modi *et al.*,(1988) applied postural shift from seated to upright to produce transient changes in Q_H and simulate the effect

of food on Q_H. The purpose for including measurements of Q_H in the upright posture in the present study was as follows:

1. To reproduce the well established decrease in Q_H, after postural shift from sitting to upright, in order to demonstrate, that the ICG clearance method is sensitive enough to detect changes in Q_H, in the magnitude of that induced by postural change in patients with mild to moderate hypertension.

2. To establish whether a two-week treatment schedule with captopril alters the magnitude of response to postural change.

3. To relate the magnitude of changes in Q_H upon standing to those observed after captopril treatment.

In the present study, in untreated patients a change in posture from seated to upright resulted in a significant decrease in Cl_{pICG} and Q_H. The absolute decrease (± SEM) in Cl_{pICG} and Q_H upon standing was 152.3 ± 18.3 ml/min (24.7%) and 250 ± 36 ml/min (22.5%), respectively, as compared to the seated values (Day 1, Figure 10 and Figure 13). Grasela *et al.*, (1987) reported a ≈ 23% decrease in the systemic clearance of ICG when healthy subjects moved from lying to standing position which is similar to the ≈ 25% reduction in Cl_{pICG} in upright posture found in the current study. The observed ≈23% decrease in Q_H, in the present study, before captopril treatment is lower than the decrease in Q_H on standing reported in healthy subjects (37% and 32% Daneshmend *et al.*, 1981 and Modi *et al.*, 1988, respectively), but compatible with the previously reported 29% decrease in Q_H in hypertensive subjects (Culbertson *et al.*, 1951). A tendency for slightly smaller decrease in Q_H in hypertensive subjects compared to the normotensives, in standing position, was also observed in the study by Culbertson *et al.*, (1951), in which Q_H decreased by 38% in normotensives and

29% in hypertensive subjects.

After two-weeks captopril treatment the absolute decrease (± SEM) in Cl_{pICG} and Q_H upon standing was 210.9 ± 38.8 ml/min (26.6%) and 344 ± 68 ml/min (24.7%), respectively, as compared to the seated value (Day 14, Figure 10 and Figure 13). Although the absolute decrease in Cl_{pICG} and Q_H when subjects move from seated to the upright position was greater by 38.5% and 37.3% after two-weeks captopril therapy, the decrease, expressed as a percentage of the seated value, was similar before and after captopril treatment (24.7% *vs* 26.6% and 22.5% *vs* 24.7%, respectively)(Table 6 and Table 9). The apparently greater decrease in Cl_{pICG} and Q_H upon standing after short-term captopril treatment is attributed to the higher seated Cl_{pICG} and Q_H value observed after two-weeks captopril treatment, as compared to the untreated values (616.9 *vs* 793.7 ml/min for Cl_{pICG} and 1389 *vs* 1114 ml/min for Q_H). Therefore, it can be concluded that the two-weeks treatment with captopril, in doses, which produced significant reduction in blood pressure did not alter the magnitude of homeostatic response in Q_H associated with postural change from seated to upright in mild/moderate hypertensive patients. When Cl_{pICG} and Q_H were normalized to body weight and body surface area the results were similar to those of absolute values (Table 6 and 9, Figure 10 and 13).

There is evidence to support the results of the present study. Vandenburg *et al.*, (1983) studied the effects of two and four-weeks captopril treatment on the responses of the sympathetic nervous system to postural changes from supine to standing and sitting in patients with essential hypertension. They found that while oral doses of captopril (25-150 mg *t.i.d.*) significantly decreased blood pressure, the acute changes in heart rate, blood pressure, plasma noradrenaline caused by postural change

were not altered by prolonged captopril therapy. Similarly, Muiesan *et al.*, (1982) reported no change in the acute responses of heart rate and plasma catecholamines to postural change after oral captopril doses of 300-600 mg/day for 8 weeks in subjects with essential hypertension. These results, supported by the present study, suggest that the activity of the sympathetic nervous system is not altered after prolonged therapy with captopril. The fact that the decrease in Q_H upon standing, as compared to the seated (s1) value was similar before and after two-weeks captopril therapy suggests that the sympathetic nervous system mediated response of Q_H, caused by postural change, is not altered by prolonged captopril.

Sixty minutes after reassuming the seated position (s2), the Cl_{pICG} and Q_H increased by ≈ 25% and ≈ 21%, respectively, before captopril treatment and by ≈ 24% and ≈22%, respectively, after two-weeks captopril therapy, as compared to the values obtained in the upright position. These results suggest that the magnitude of increase in Cl_{pICG} and Q_H during the reseated periods were similar before and after captopril therapy, as compared to the upright values. There was no significant difference between the mean Cl_{pICG} and Q_H values obtained in the seated (s1) and reseated (s2) positions (Table 6 and Table 9). However, individual data showed greater variability during the reseated period than in the upright posture when compared to the seated values (Figure 9 and Figure 12). Although no explanation for this variability in Q_H in the reseated position was found, it was also observed during the recovery period when normotensive patients reassumed lying position after being upright (Culbertson *et al.* 1951).

4.4.2. Acute Effects of Captopril on Cl_{pICG} and Q_H

Several studies have attempted to determine the effects of captopril on Q_H after single-dose administration in human subjects. Most of these studies have been carried out in either healthy subjects (Shepherd *et al.*, 1985), in patients with congestive heart failure (Faxon *et al.*, 1984; Levine *et al.*, 1984), in patients with liver cirrhosis (Eriksson *et al.*, 1984), or in patients with essential hypertension (Crossley *et al.*, 1984). There are conflicting reports regarding the acute effects of captopril on Q_H. This may partially be explained by either the different doses of captopril administered (Geneve *et al.*, 1990), the different techniques used to estimate Q_H (Bradley, 1974), and/or the different disease states of the patients (Gasic *et al.*, 1989).

Shepherd *et al.*, (1985) examined the effects of captopril, nitrates and propranolol on Q_H in healthy subjects. In this study a single 25 mg oral dose of captopril was used and Q_H was estimated by the ICG clearance method 100 min following captopril administration. The reason to estimate Q_H at this time point was that captopril exhibited maximal blood pressure lowering effect after single-dose administration in healthy subjects between 90 and 120 minutes (Shepherd *et al.*, 1985). It was noted, that while propranolol and glyceryl trinitrate significantly reduced Q_H, captopril caused no change, despite the significant fall in blood pressure. It has been noted (Gasic *et al.*, 1989), that under normal physiological conditions RAS may not play a major regulatory role within the splanchnic vascular bed, but may obtain importance in conditions with compromised haemodynamics, for example, congestive heart failure. Creager *et al.*, (1981) studied the acute effects

of 100 mg oral dose of captopril on regional blood flow in human subjects with congestive heart failure. In the 11 patients splanchnic blood flow (assessed by ICG clearance) decreased from 1.8 ± 0.4 to 1.5 ± 0.3 ml/min/kg (17%, p=0.051) and renal blood flow increased by 60 % between 60 to 120 minutes after a single oral dose of captopril. Also, total systemic vascular resistance decreased by 27%. It has been concluded (Craeger *et al.*, 1981), that selective peripheral vasodilation occurred after a single dose of captopril; that is, blood flow to the kidneys increased and to the liver and limbs decreased, in patients with congestive heart failure. Levine *et al.*, (1984) reported no statistically significant change in Q_H (estimated ICG blood clearance) and hepatic vascular resistance, 90 minutes following the administration of 25 to 100 mg oral dose of captopril in patients with congestive heart failure. However, in the 11 patients mean Q_H decreased from by ≈ 30%, assessed by the ICG clearance method and hepatic vascular resistance decreased by 11% (Levine *et al.*, 1984). Large interpatient variability was observed in this study, which resulted in no statistically significant change in Q_H. The large interpatient variation may be explained by the great differences regarding the age of the patients examined in this study (21-71 years). It is well recognized, that Q_H declines significantly with advancing age (Wynne *et al.*, 1989 and 1990). Wynne *et al.*, (1989) reported a significant negative correlation between age and Q_H (ICG clearance method) in 65 healthy subjects between 24 and 91 years of age. Therefore, the responses to captopril treatment may be more variable when patients with wide age range are selected to study changes in Q_H.

Eriksson *et al.*, (1984) investigated the acute effects of 12.5 and 25 mg oral dose of captopril on Q_H in seven patients with liver cirrhosis and

portal hypertension. Q_H was estimated by the constant infusion of ICG using hepatic venous catheterization. It was noted, that while mean arterial pressure significantly decreased 30, 60 and 90 minutes following captopril administration, Q_H remained unaltered. However, a decrease in mean arterial pressure and an unchanged Q_H suggest a decrease in SVR (*i.e.* SVR = MAP / Q_H). An important finding of this study was that the hepatic extraction ratio of ICG was unaffected by captopril therapy.

Although a large number of studies have investigated the pharmacological effects of captopril in patients with essential hypertension, very few studies have examined its influence on Q_H. In the studies of Crossley *et al.*, (1984) the acute effects of 50 to 100 mg oral dose of captopril on Q_H was examined in six patients with essential hypertension. In this study, Q_H was determined by the constant infusion of ICG and the catheterization of the hepatic vein. About 50 minutes following captopril administration, a uniform and significant reduction in Q_H from 1127 ± 115 to 841 ± 93 ml/min (25%, $p < 0.001$) was noted. Concomitantly, mean arterial pressure decreased significantly (8%) and total systemic vascular resistance decreased by 13% (non-significant). There was no significant change in cardiac output despite the large (27%) increase in one patient with congestive heart failure and a slight increase in two others.

In the present study, the acute effects of captopril were evaluated 1 hour after the initial captopril dose on day 1 and the terminal dose of two-weeks captopril therapy, on day 14. Captopril has been previously shown to exhibit its maximal blood pressure lowering effect within 0.5 to 1.5 hours in hypertensive patients (Tarazi *et al.*, 1980). There was no significant change in Cl_{pICG} and Q_H 1 hour after the administration of the initial dose of captopril in patients with mild to moderate hypertension, whether

expressed in absolute terms or per unit body weight or body surface area, as compared to the control values (reseated, s2) values (Day 1, Table 6 and Table 9). However, a slight ≈ 5.2% and ≈ 6.0% decrease in Cl_{pICG} and Q_H, respectively, was observed when data obtained 1 hour after captopril dosing were compared to the control values (Day 1, Table 6 and Table 9). However, this decrease in ICG clearance and Q_H after captopril administration is similar or even smaller in magnitude than the differences between the two seated positions before captopril treatment (6.3% *vs* 5.2% for Cl_{pICG} and 6.3% *vs* 6.0% for Q_H) (Table 6 and 9). Similarly, the terminal dose of two-weeks captopril therapy caused no significant changes in Cl_{pICG} and Q_H, as compared to the reseated values (Day 14, Table 6 and 9). There was a slight, 5.2% and 6.1% decrease in Cl_{pICG} and Q_H, respectively, 1 hour after captopril dosing, as compared to the reseated (s2) values, similar to that observed after the initial dose of captopril on day 1 (Day 14, Table 6 and 9). The fact that the response to the terminal dose of captopril was similar to that after the initial dose suggests that two-weeks captopril therapy does not interfere with the acute responses of Q_H to captopril administration in patients with mild-moderate hypertension.

These results agree with the findings of Shepherd *et al.*, (1985), Eriksson *et al.*, (1984) and Levine *et al.*, (1984), who observed no significant change in Q_H after a single captopril dose. However, the results of the present study are different from the studies of Crossley *et al.*, (1984) and Craeger *et al.*, (1981) who observed a significant decrease in Q_H in patients with hypertension and congestive heart failure, respectively.

The lack of response in Q_H after an acute dose of captopril observed in this study could not be caused by poor absorption of the drug, since the

serum concentrations of captopril increased rapidly after administration and were similar to the concentrations reported in other studies (Jarrott *et al.*, 1982; Kripalani *et al.*, 1980) (Figure 29). In addition, significant reductions in both, systolic and diastolic blood pressures were observed 1 hour after the first dose of captopril suggesting a pharmacologically active level of the drug. Differences from the studies of Crossley *et al.*, (1984) may arises from the different body positions of the subjects in which Q_H was estimated (lying *vs* sitting). Shepherd *et al.*, (1985), however, observed no significant changes in Q_H assessed by the ICG clearance method in normal subjects when patients were sitting or lying. Direct comparison between the present study and that performed by Crossley *et al.*, (1984) is difficult due to the different methods used for the estimation of Q_H. The catheterization of the hepatic vein used in the study of Crossley *et al.*, (1984) has the advantage that the hepatic extraction ratio of ICG can be determined, but is an invasive technique (George, 1979). The placement of the catheter in the hepatic vein may cause venous spasm (Bradley *et al.*, 1945) which may affect the results of the study. Another limitation of the method is that the concentrations of ICG in the blood samples obtained from the hepatic vein are influenced by the position of the catheter (Ohnhaus, 1979; George, 1979).

The lack of change in Q_H, despite a significant fall in arterial blood pressure may be explained by the "hydrodynamic" interaction between the hepatic artery and portal vein (Shepherd *et al.*, 1985). According to this theory simultaneous dilation of both, hepatic artery and portal vein produces an increase in one flow and a reduction in the other, thus, a constant blood flow maintained in the liver (Richardson and Withrington, 1981). It has been demonstrated, that captopril causes dilatation in the

veins (Simon *et al.*, 1985), as well as, in the arteries (Tarazi *et al.*, 1980).

Most recently, significant daily variation in estimated Q_H has been reported in healthy subjects when Q_H was estimated by the ICG clearance method four times a day (2:00 am, 8:00 am, 2:00 pm and 8:00 pm) (Lemmer and Nold, 1991). The plasma clearance of ICG and Q_H decreased by 24% and 26%, respectively, when values, estimated in the morning (8:00 am), were compared to those measured in the early afternoon (2:00 pm). Figure 31 shows the circadian rhythm in estimated Q_H obtained from the studies of Lemmer and Nold, (1991) together with the Q_H data obtained in the present study on day 1 and 14. The slight ≈ 5-6% and ≈ 11-14% decreases in Q_H observed when reseated (s2) and post-captopril (CA) data, respectively, were compared to the first seated (s1) values are similar to the decrease in Q_H when extrapolated data of Lemmer and Nold, (1991) are used for the comparison (17%). Since the four ICG injections for the estimation of Q_H in the current study were performed between ≈ 8:30 am and ≈ 2:00 pm (steep phase of the circadian variation curve), a decrease in Q_H values during the course of the study might be expected. Based on the reports of Lemmer and Nold, (1991) the apparent decrease in Q_H after the acute dose of captopril when compared to the seated (s1) values may be due to the circadian variation in Q_H, rather than a direct effect of captopril. Moreover, the decrease in estimated Q_H after postural change from sitting to upright were ≈ 23% and ≈ 25% before and after captopril therapy, respectively. This change in Q_H is much greater in magnitude than that after the acute doses of captopril whether compared to the reseated (s2) or seated (s1) values. Statistical comparisons between the effects of postural change and acute doses of captopril on Q_H suggest, that upright position has more pronounced effect on

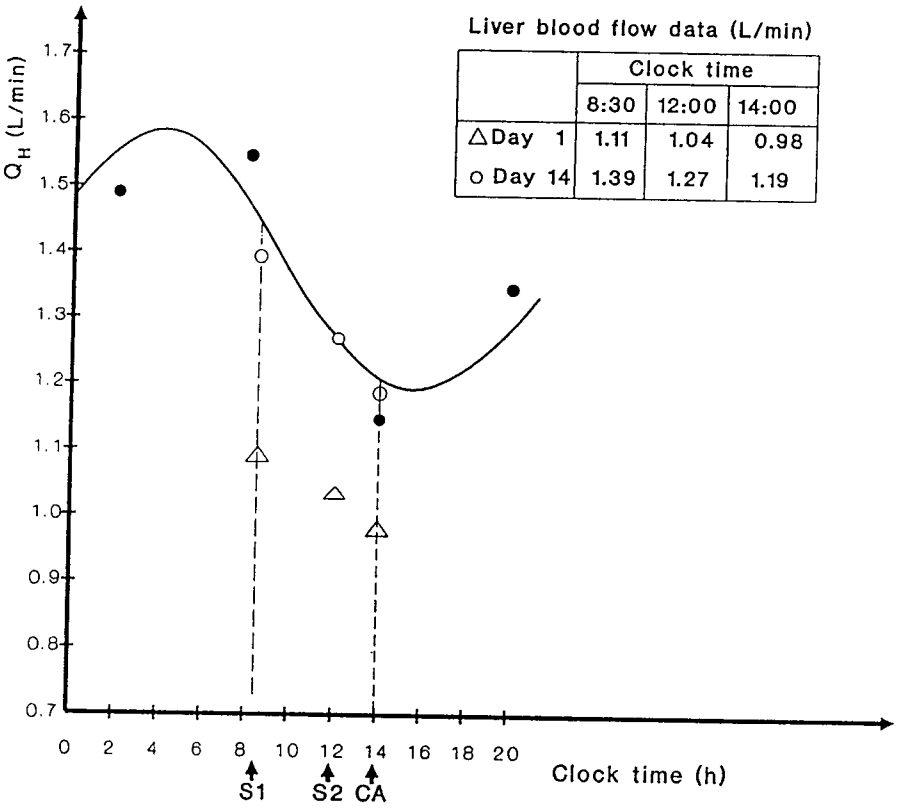


Figure 31. Changes in Q_H during the course of day 1 (open triangles) and day 14 (open circles), as compared to the changes in Q_H due to circadian variation (based on Lemmer and Nold, 1991).

Q_H than the acute administration of captopril ($p < 0.05$, paired sample *t*-test). Therefore, the biological significance of this small change in Q_H is uncertain.

In conclusion, there was no apparent acute change in Cl_{pICG} and Q_H after captopril administration. The decrease in Q_H during the course of day 1 and day 14 may be explained by the circadian variation in Q_H.

4.4.3. Short-term Effects of Captopril on Cl_{pICG} and Q_H

There is little information regarding the effects of captopril on Q_H after long-term administration either in human or animal species. Considering the complex haemodynamic changes associated with captopril therapy, the results of single-dose studies might be substantially different from those after prolonged captopril therapy (Brogden *et al.*, 1988; Crossley *et al.*, 1984)

Studies in anesthetized, normotensive and spontaneously hypertensive rats have demonstrated a significant ≈ 25% and ≈ 34% increase in Q_H, respectively, after oral treatments with 100 mg captopril for six weeks (Rozsa *et al.*, 1991). Similarly, Richer *et al.*, (1984) have reported a significant 2-fold increase in Q_H and 56% decrease in hepatic vascular resistance after 8 days treatment with 100 mg/kg oral dose of captopril in anesthetized, spontaneously hypertensive rats. In contrast, in a study involving human subjects with mild to moderate hypertension (Ventura *et al.*, 1985) no significant change in Q_H, estimated by the ICG clearance method, have been observed after 90 minutes and 12-weeks of treatment with 50 mg daily oral dose of captopril. There was, however, a slight (7%) decrease in SVR after 12 weeks treatment with captopril.

The results of the present study demonstrate a statistically significant increase in Cl_{pICG} by ≈ 25-29% ($p = 0.039$) after two-weeks captopril treatment, in all four phases of the study, as compared to the day 1 values (Table 7). Similarly, Q_H increased almost uniformly by ≈ 21-25% after two-weeks captopril therapy, in the four phases of the study, as compared to day 1 (Figure 14). The magnitude of increase in Q_H was similar when data were normalized to body weight and body surface area; however, the percent increase relative to day 1 values were slightly lower than those of the absolute values (≈ 19-24%, and ≈ 20-22%, respectively)(Figure 14). These results confirm the findings of Rozsa *et al.*, (1991) who observed a ≈ 34% increase in Q_H after six weeks treatment with captopril in spontaneously hypertensive rats, but different from the findings of Ventura *et al.*, (1985) in patients with essential hypertension. The lack of change in Q_H after 12-weeks treatment with captopril may be due to the lower captopril dose used in this study (25 mg twice daily to a maximum of 75 mg).

Figure 15 shows that the differences between the two study days (before and after two-weeks captopril therapy) were the same regardless which study phases were compared. The statistical test, however, just failed to achieve the level of significance when the effects of short-term captopril treatment on Q_H were compared to those of day 1 ($p = 0.062$, $p = 0.075$ and $p = 0.074$ for absolute Q_H and per unit body weight and body surface area, respectively). The lack of statistical significance is attributed to the one patient (C.B.) who exhibited a decrease in Q_H after short-term captopril therapy. When the statistical test was repeated by excluding the data of this patient, the increases in Q_H after two-weeks captopril therapy, whether expressed in absolute values or per unit body weight or body surface area, were statistically significant, as compared to the day 1 values ($p = 0.011$,

p = 0.014 and p = 0.014, respectively). In patient C.B. baseline systolic and diastolic blood pressures on day 14 were below pretreatment values by 11 and 7 mm Hg, respectively, indicating good patient compliance (Table 15). To find explanation these divergent result is difficult without the measurements of changes in parameters such as cardiac output and total peripheral resistance. A significant reduction in total peripheral resistance and an increase in SVR, for example, would suggest that other vascular beds were preferentially perfused after captopril therapy in this patient (Crossley *et al.*, 1984). Further, a significant decrease in cardiac output would explain a decrease in Q_H in this patient (Levine *et al.*, 1984).

Greenway and Lautt, (1972) proposed that the angiotensin II induced vasoconstriction in the capacitance vessels of the splanchnic circulation results in the mobilization of splanchnic blood pool and a decrease in blood volume by a maximum of 20%. Conversely, a reduction in angiotensin II, and dilatation of the splanchnic capacitance vessels, as a result of captopril treatment may increase liver blood volume by pooling of blood to the splanchnic circulation. This has been suggested by Stadeager *et al.*, (1989) who observed an approximate 25-28% increase in Q_H after acute enalapril administration. A significant correlation between liver blood volume and Q_H has been reported by Wynne *et al.*, (1989), suggesting that enhanced liver blood volume results in an increase in Q_H.

There is experimental and clinical evidence that the long-term blood pressure reducing effects of ACE inhibitors correlate better with the inhibition of ACE in certain tissues then the inhibition of ACE in plasma (Dzau, 1988; Waeber *et al.*, 1989). Since ACE is identical to the kininase II which degrades the vasodilatory bradykinin, ACE inhibition may lead to the accumulation of the potent vasodilator bradykinin in different tissues

(Antonaccio *et al.*, 1981) which may contribute to the long-term effects of these drugs. Captopril has been shown to increase the levels of vasodilator prostaglandins either directly or through the increase of bradykinin (Swartz and Williams, 1982). Further, some of the disulfide metabolites of captopril have been shown to be more potent inhibitors of ACE than the parent drug (Drummer *et al.*, 1985). Also, these disulfide metabolites may serve as a "depot" form of captopril from which captopril may be regenerated (Ondetti 1988). It has been suggested, that this interconversion between disulfide metabolites contributes to the long-term hypertensive effects of captopril. Whether these vasodilatory effects of captopril contribute to the observed increase in Q_H and decreased SVR after two-weeks captopril treatment remains to be determined.

4.5. Changes in Splanchnic Vascular Resistance

4.5.1. Calculation of Splanchnic Vascular Resistance

SVR is determined by the difference between mean arterial pressure and hepatic venous pressure divided by total Q_H (Gasic *et al.*, 1989). In the present study, however, because of the difficulties of measuring hepatic venous pressure and, since hepatic venous pressure is small compared to the mean arterial pressure (≈ 10%), for the calculation of total hepatic and portal resistance, the hepatic venous pressure has been ignored (Echtenkamp *et al.*, 1983; Messerli *et al.*, 1975; Culbertson *et al.* 1951; Wilkins *et al.*, 1951). Further, previous clinical studies reported no significant change in hepatic venous pressure after captopril administration in patients with liver cirrhosis (Eriksson *et al.*, 1984). Since ICG clearance method

determines total liver blood flow, which is the sum of hepatic arterial and portal venous flow, the term SVR refers to the total hepatic vascular resistance plus the resistance of the vessels in the splanchnic region (*i.e.* spleen, pancreas, intestine and stomach) (Echtenkamp *et al.*, (1983).

4.5.2. Effects of Postural Change on SVR Before and After Captopril Treatment

It has been reported by Culbertson *et al.*, (1951) that the significant reduction in estimated Q_H observed in their study when subjects were tilted from lying to standing was not associated with a significant change in mean arterial pressure. Since SVR, in the studies of Culbertson *et al.* (1951), was calculated as the ratio of mean arterial pressure and estimated liver blood flow, a significant ≈ 39% and ≈ 45% increase in SVR was noted in the hypertensive and normotensive patients, respectively. The results of the present study reaffirm these findings. Thus, a significant increase in SVR (± SEM) from 6.6 ± 0.54 to 8.6 ± 0.66 mm Hg·sec/ml (31.0%) was observed upon standing in the untreated patients on day 1, as a result of the significant increase in Q_H and small change in mean arterial pressure (Table 17, Figure 28). This is ≈ 8% less than the increase in SVR reported by Culbertson *et al.*, (1951) in hypertensive patients. The smaller increase in SVR upon standing, as compared to the values reported by Culbertson *et al.*, (1951) may be due to the different postures used in these studies (supine to upright *versus* seated to supine). The increase in SVR on standing was consistent among the six patients studied.

Following two-weeks of captopril therapy, average SVR (± SEM) increased from 5.3 ± 0.8 to 7.1 ± 1.1 mm Hg·sec/ml (33.3%) in upright position, as

compared to the seated values (Table 17, Figure 28). The increase in SVR was consistent among the six patients studied, however, the overall increase during standing was not statistically significant after two-weeks of captopril therapy. Although, SVRs after two-weeks of captopril treatment were lower by ≈ 19% and ≈ 17% in seated and upright positions, respectively, as compared to those before captopril treatment (Figure 28), the magnitude of increase in SVR upon standing was similar before and after captopril therapy (33.3% vs 31.0%). These results suggest that two-weeks of captopril therapy did not alter the reflex responses of SVR to postural change in mild to moderate hypertensive patients.

4.5.3. Acute Effects of Captopril on SVR

Despite the significant reduction in systolic and diastolic blood pressures (13.4% and 13.0%, respectively, $p < 0.05$) 1 hour after the administration of the initial captopril dose (Table 12) there was no significant change in SVR (Day 1, Table 17). In contrast to the initial captopril dose, the terminal dose of captopril did not change significantly the systolic and diastolic blood pressures 1 hour after administration (Figure 22) and SVR remained unaltered. The absence of a reduction in SVR is similar to the findings of Levine *et al.*, (1984) who observed significant reduction in total systemic vascular resistance 90 minutes after captopril dosing without a change in SVR in patients with congestive heart failure.

4.5.4. Short-Term Effects of Captopril on SVR

It is well established, that the hypertensive effects of captopril after

acute, as well as prolonged administration are associated with a decrease in total peripheral resistance (Tarazi *et al.*, 1980; Wenting *et al.*, 1982). The ≈ 15-23% reduction in SVR after two-weeks captopril therapy, observed in the present study suggests that vasodilation occur in the arterioles of the splanchnic area (Figure 28). This decrease in SVR may reflect a reduction in total peripheral resistance after continuous therapy with captopril. Previous clinical studies reported 11% and 18% decrease in total peripheral resistance after 4-weeks and 1-week captopril treatment in patients with essential hypertension (Wenting *et al.*, 1982; Tarazi *et al.*, 1980), which are similar in magnitude to that observed in the present study for SVR. However, larger decreases of up to 30% in total peripheral resistance have also been reported after acute or chronic treatment with captopril (Brogden *et al.*, 1988). Whether the observed increase in Q_H and decrease in SVR are consequences of either a direct interaction of captopril with the renin-angiotensin system in the splanchnic vascular system, an enhancement of a sympathetic modulating effect (Stadeager *et al.*, 1989) or a result of complex haemodynamic changes (Levine *et al.*, 1984) is difficult to establish. Statistical significance, at the 0.05 probability level, was not observed when the SVR data of six patients were calculated (MANOVA, $p = 0.12$). These data, however, suggest that a substantial reduction in SVR occurred after 14 days captopril therapy, in contrast to the effects of a single-dose of captopril, where no change in SVR was noted. Assuming that the changes in SVR reflect changes in total peripheral resistance, the results of the present study are in agreement with the studies of Simon *et al.*, (1985) who observed a more pronounced decrease in total peripheral resistance after 5 days treatment with captopril than after single dose administration in patients with essential hypertension. It has been

suggested (Simon *et al.*, 1985) that the effects of ACE inhibition appeared earlier in the large arteries than in the arterioles. Since the arterioles provide the largest resistance to blood flow (Shepherd and Vanhoutte, 1980) the lack of changes in Q_H after acute dosing with captopril and increased Q_H after two-weeks treatment may be explained by the findings of Simon *et al.*, (1985). An increase in QH in the presence of significant reduction in arterial blood pressure may occur when total peripheral resistance (including the resistance in the splanchnic region) decreases more than arterial pressure. In the present study, SVR decreased an average of ≈19% after two-weeks captopril therapy (Figure 28) and systolic and diastolic blood pressure declined by ≈13% and ≈11%, respectively, (Table 16) 1 hour after the terminal dose of captopril. Therefore, the relatively small decrease in blood pressure, as compared to the SVR, suggests an increase in QH under these described experimental conditions.

4.6. Changes in Blood Pressure

4.6.1. Effects of Postural Change on Blood Pressure Before and After Captopril

The hydrostatic changes that occur during postural shift from supine to standing are well established (Shepherd and Vanhoutte, 1980). The redistribution of blood to the legs results in a decrease in cardiac output which is counteracted by the reflex constriction of the peripheral vessels in order to maintain arterial pressure (Shepherd and Vanhoutte, 1980). Hence, the observed decrease in Q_H during standing is a result of this compensatory mechanism to maintain systemic arterial blood pressure. It has

been reported earlier (Culbertson *et al.*, 1951) that when normotensive or hypertensive patients were tilted to upright position, the large reduction in Q_H was not associated with large changes in mean arterial pressure. In contrast, Vandenberg *et al.*, (1983), reported significant increase in diastolic and slight increase in systolic blood pressures upon standing. This suggest that mean arterial pressure can be misleading considering the changes in systolic and diastolic blood pressures. This is due to the fact that mean arterial pressure is not exactly equal to the average systolic and diastolic pressures, because pressure usually remains for a shorter period of time at the highest pressure during contraction of the ventricle (systolic), than on the lower pressure during refilling the ventricle (diastolic) (Guyton, 1984). The resulting mean pressure is closer to the diastolic than to the systolic pressure. However, mean arterial pressure is important since it determines the average blood flow rate through the systemic vessels (Guyton, 1984). In the present study, diastolic blood pressure increased slightly by ≈ 7 and ≈ 5 mm Hg when subjects were in standing position, before and after 14 days captopril therapy, respectively. Systolic blood pressure, however, remained unaltered (Figure 16 and 17). The observed increase in diastolic blood pressure in standing position is in agreement with the studies of Vandenberg *et al.*, (1983).

4.6.2. Acute Effects of Captopril on Blood Pressure

The efficacy of captopril in decreasing blood pressure has been amply demonstrated in patients with hypertension (Brogden *et al.*, 1988; Tarazi *et al.*, 1980; McAreavey and Robertson 1990; Wenting *et al.*, 1982).

A reduction in supine or standing systolic and diastolic blood pressures

by about 10 to 20% after a single dose of captopril in patients with hypertension was reported by Brogden *et al.*, (1988). Mean arterial pressure decreased from 141.9 ± 6.9 to 130.2 ± 6.7 mm Hg, 1 hour after the administration of 50-100 mg oral dose of captopril in hypertensive patients (Crossley *et al.*, 1984). In another study, when a 50 mg captopril dose was administered to patients with essential hypertension mean arterial pressure decreased from 141 ± 6 to 119 ± 7 mm Hg (15.6%) 2 hours after drug administration (Wenting *et al.*, 1982). Tarazi *et al.*, (1980) reported a decrease in mean arterial pressure from 139.0 ± 4.7 to 120.0 ± 5.9 mm Hg, 30 minutes after captopril treatment in patients with essential hypertension.

In the present study, mean arterial pressure decreased from 118.0 ± 3.0 to 104.9 ± 5.4 mm Hg (11.1%) 1 hour after a single dose of captopril (Table 12). This ≈ 11% reduction in mean arterial pressure is in agreement with previous studies of Crossley *et al.*, (1984) and Tarazi *et al.*, (1980) who reported a ≈ 8% and ≈ 14% decrease in mean arterial pressure, respectively, after captopril administration. Systolic and diastolic blood pressures were significantly reduced from 160.5 ± 5.3 to 138.9 ± 10.2 mm Hg (13.4%) and from 103.3 ± 1.0 to 89.8 ± 4.3 mm Hg (13.0%), respectively 1 hour after the initial dose of captopril (Table 12 and Figure 20). The mean difference from pretreatment values 1 hour after the initial captopril dose was ≈ 22 mm Hg for systolic and ≈ 13 mm Hg for diastolic blood pressure. In contrast to the reports of Brogden *et al.*, (1988) maximal blood pressure reduction was observed after 3 hours of captopril administration when systolic and diastolic blood pressures and mean arterial pressure (mean ± SEM) were found to be 132.4 ± 9.3 mm Hg, 86.6 ± 6.3 mm Hg, and 101.0 ± 6.6 mm Hg, respectively, (Table 12, Figure 20). These blood pressure values 3 hours after the initial dose of captopril were lower than the pretreatment values

by ≈ 18%, ≈ 16% and ≈ 14%, respectively. The absolute reductions in systolic and diastolic blood pressures 3 hours after the initial dose of captopril were ≈ 28 mm Hg and ≈ 17 mm Hg, respectively, as compared to the pretreatment blood pressures (Figure 20). Parati *et al.*, (1989) found that the time required for maximal reduction in systolic and diastolic blood pressure was between 2 and 4 hours after the administration of a single 100 mg oral dose of captopril. The results of the present study substantiate these findings.

All patients responded well to the first dose of captopril 3 hours after administration, including the one black patient (O.W.). A lesser response to captopril in black patients, as compared to the white subjects, has been reported previously (Veterans Administration Cooperative Study Group, 1984). However, this reduced response to captopril could not be substantiated in the one black patient in the present study. The highest pretreatment systolic and diastolic blood pressures were recorded in patient O.W. (181.5 mm Hg) and patient D.A. (106.5 mm Hg), respectively (Table 3). The largest absolute reduction in blood pressure 1 hour and 3 hour after the administration of a single dose of captopril was observed in patient B.H.: 53 mm Hg (35%) and 57 mm Hg (38%) for systolic, 29 mm Hg (28%) and 41 mm Hg (40%) for diastolic blood pressure, respectively (Table 11). This profound blood pressure reduction may be a mild case of first dose hypotension as reported by several investigators (Hodsman *et al.*, 1983; Brogden *et al.*, 1988; Lees *et al.*, 1990). First dose hypotension (a decrease in supine systolic blood pressure > 50 mm Hg) (Hodsman *et al.*, 1983) most often developed in patients with either high pretreatment plasma renin and angiotensin II concentrations or secondary hypertension. In addition, it may occur in those patients who were previously treated with an ACE

inhibitor (Brogden *et al.*, 1988). Lees *et al.*, (1990) suggested that first-dose hypotension may be related to excess converting enzyme capacity. Patient B.H. also developed mild symptoms of non-productive cough after repeated treatment with captopril, however, discontinuation of the captopril therapy was not necessary. No other side effects were observed during captopril therapy.

Systolic and diastolic blood pressures were reduced slightly 1 hour after the terminal dose of the two-weeks captopril therapy by 6.3 ± 7.3 mm Hg (4.3%) and 1.1 ± 2.3 mm Hg (1.2%), respectively, as compared to the treated baseline blood pressure of day 14; however, this decrease was not statistically significant. In contrast, 3 hours after the terminal dose systolic and diastolic pressures were reduced significantly by ≈ 17 mm Hg (11.5%) and ≈ 10 mm Hg (10.7%), respectively (Table 14, Figure 22). The magnitude of decrease in systolic and diastolic blood pressures 1 and 3 hours after the terminal dose was much smaller than that after the initial dose of captopril, as compared to the baseline values of day 1 and day 14 (Figure 22). The smaller absolute reduction in systolic and diastolic blood pressures, as compared to those observed after the initial dose reflects the significantly lower treated baseline blood pressure of day 14. This is in agreement with the significant correlation between the initial blood pressure and the reduction in blood pressure, reported by Sumner *et al.*, (1988). Thus, the higher the initial blood pressure the greater the reduction in blood pressure due to antihypertensive therapy (Sumner *et al.*, 1988).

4.6.3. Short-Term Effects of Captopril on Blood Pressure

In the present study, systolic and diastolic blood pressures were maintained below the baseline value of day 1, prior to the terminal captopril dose on day 14. Clearly, systolic and diastolic blood pressures after readmission to the hospital on day 14 were significantly lower by 14.9 ± 7.7 mm Hg (9.3%, $p < 0.005$) and 10.5 ± 6.4 mm Hg (10.1%, $p < 0.05$), respectively, compared to the pretreatment blood pressure of day 1 (Table 16, Figure 25 and 26). Baseline blood pressure measurements on day 14 were performed approximately 20 hours after the intake of the captopril dose on the previous day, before patients returned to the hospital. These results are in agreement with the findings of Parati *et al.*, (1989) and Mancia *et al.*, (1987) who reported 22-24 hour blood pressure reduction following once daily treatment with 100 mg captopril. The 24-hour ambulatory blood pressure monitoring used in these studies provided automatic readings of blood pressure and heart rate at 10 or 20 minutes intervals and were performed 1 month after starting treatment with captopril. Parati *et al.*, (1989) also reported positive correlation between the early reductions in systolic and diastolic blood pressures (2-4 hour after the administration of 100 mg oral dose of captopril) and the changes in blood pressure after 24 hour in those essential hypertensive patients, who responded well to captopril therapy. This suggests that blood pressure reduction after 22-24 hours can be predicted by the early, peak reduction in blood pressure in patients with essential hypertension. In the present study, however, mean diastolic blood pressure (± SEM) was 92.8 ± 2.3 mm Hg which is slightly greater than 90 mm Hg, the generally accepted level of "normal" diastolic

blood pressure (Shepherd and Vanhoutte, 1980)

At the end of the two-week captopril treatment, systolic and diastolic blood pressures were lower than pretreatment values by ≈ 21 mm Hg (13%) and ≈ 12 mm Hg (11%), respectively, after 1 hour and by ≈ 32 (20%) and ≈ 20 mm Hg (20%), respectively, after 3 hours of the administration of the terminal dose of captopril (Figure 25 and 26). The absolute reduction in systolic and diastolic blood pressures, 1 and 3 hours after the terminal captopril dose were similar to the absolute reduction observed 1 and 3 hours after the initial captopril dose (-21 vs -22 mm Hg and -32 vs - 28 mm Hg, respectively, for systolic and -12 vs 13 mm Hg and -20 and 17 mm Hg, respectively, for diastolic blood pressure) (Figure 25 and 26). This suggests that once daily treatment with 100 mg captopril maintains blood pressure below the pretreatment values and provides balanced blood pressure control, which is in agreement with the studies of Parati *et al.*, (1989).

It is well established, that the decrease in arterial blood pressure after captopril administration is mediated through a fall in total peripheral resistance (Tarazi et al., 1980; Wenting *et al.*, 1982). A reduction in total peripheral resistance was reported 30 to 90 minutes after oral administration of captopril (Fouad et al., 1980). In the present study, we could not estimate total peripheral resistance since cardiac output was not measured. Based on previously reported results, the acute or short-term effects of captopril on cardiac output usually demonstrate no change in patients with essential hypertension (Tarazi et al., 1980). Therefore, the significant reduction in blood pressure observed in this study with no change in cardiac output would imply a decrease in total peripheral resistance. The fact that SVR did not change in the present study, approximately 1 hour after acute administrations of captopril,

suggests that other regional beds may contribute to the reduction of total peripheral resistance. Indeed, a 107% increase in forearm blood flow has been reported in normal subjects after a single-dose of captopril and renal blood flow increased by 60% in patients with congestive heart failure (Faxon et al., 1984).

In contrast, after two-weeks treatment with captopril SVR decreased on average by ≈ 19% in the four phases of the study, as compared to the day 1 values. Although, the overall decrease in SVR does not achieve the level of significance ($p = 0.125$), it may suggest that after prolonged treatment with captopril the reduction in SVR contributes to the overall reduction in total peripheral resistance.

4.7. Changes in Heart Rate

4.7.1. Effects of Postural Change on Heart Rate Before and After Captopril Treatment

It is well established that heart rate generally increases when healthy subjects change position from supine to upright due to the decrease in venous return (Shepherd and Vanhoutte, 1980). Muiesan *et al.*, (1982) reported an increase in heart rate in standing position by 5 beats/min which remained unaltered after eight weeks of treatment with captopril, in patients with essential hypertension. Vandenburg *et al.*, (1983) noted a much greater increase in heart rate (10 beats/min) when patients with essential hypertension changed position from lying to standing. In agreement with the studies of Muiesan *et al.*, (1982) the magnitude of change in heart rate in standing position was unaffected by two and four weeks

captopril therapy. The results of the present study substantiate the findings of Muiesan *et al.*, (1982) that heart rate increased significantly upon standing by 4 and 5 beats/min before and after two-weeks captopril therapy, respectively (Figure 18). Also, it is in agreement with the studies of Vandenburg *et al.*, (1983) that two-weeks captopril therapy does not interfere with the acute changes in heart rate caused by postural change in patients with essential hypertension.

4.7.2. Effects of Acute and Short-Term Captopril Treatment on Heart Rate

It has been reported previously, that inspite of the significant lowering of arterial pressure and peripheral resistance, heart rate does not usually show a significant increase in hypertensive patients after acute or short-term captopril treatment (Tarazi *et al.*, 1980; Crossley *et al.*, 1984). This has also been demonstrated, by Parati *et al.*, (1989) in patients with hypertension, where it was found that no change in heart rate occurred during the 24 hour period following 100 mg captopril dose once daily for 1 month, as compared to the placebo treated group (Parati et al., 1989). Wenting et al., (1982) reported no acute changes in heart rate in hypertensive patients following a single captopril dose. They noted, however, a significant decrease in heart rate from 76 ± 3 to 69 ± 2 beats/min, after 4 weeks of captopril therapy. In contrast, Fagard et al., (1982) reported a slight increase in heart rate 75 minutes after captopril treatment in patients with hypertension. The results of the present study are in agreement with the findings of Tarazi *et al.*, (1980), Crossley *et al.*, (1984) and Parati *et al.*, (1989). There was no change in heart rate 3 hours following the administration of the initial dose of captopril (72.6 ±

4.1 *vs* 73.6 ± 3.5 beats/min)(Table 12). In three patients (C.B., O.W., M.S.), however, heart rate increased in the range of 2-7 beats/min (Figure 24). Following two-weeks treatment with captopril, prior to the terminal dose, heart rate was not significantly different from control value (71.0 ± 3.2 *vs* 72.6 ± 4.1)(Figure 18). The terminal dose of captopril did not change the heart rate, however a slight (non-significant) decrease was observed when heart rate values at the end of the study were compared to the untreated values (72.6 ± 4.1 vs 69.8 ± 3.5) (Figure 27). Systemic vasodilation usually causes a reflex increase in heart rate (Shepherd and Vanhoutte, 1980) which was not observed in this study. The lack of increase in heart rate after captopril treatment could be explained by (1) an interference with the sympathetic nervous system and consequently, an alteration in baroreceptor reflexes (Muiesan et al., 1982) or (2) a concomitant arteriolar and venous vasodilatation (Tarazi et al., 1980).

4.8. Changes in Hematocrit

4.8.1. Effects of Postural Change on Hematocrit Before and After Captopril Treatment

Postural change from supine to standing has been shown to produce a reduction in the plasma volume of healthy subjects (Hagan *et al.*, 1978; Dixon and Paterson, 1978). This has been shown to occur as a consequence of increased capillary pressure and plasma efflux into the interstitial space (Hagan *et al.*, 1978). In the studies of Hagan *et al.*, (1978) the reduction in the plasma volume after 35 minutes standing was associated with a rise in hematocrit, hemoglobin and plasma proteins by 10.3%, 10.8% and 20.8%,

respectively. It has been suggested, that a minimum of 20 minutes required to stabilize the plasma volume in a new posture, however, maximal stability was achieved only after 40 and 60 minutes (Hagan *et al.*, 1978). These observations are important in relation to the present study because of the following reasons:

1. ICG highly bounds to plasma proteins after intravenous administration (Paumgartner, 1975). Since the concentrations of plasma proteins progressively change upon postural shift from sitting to standing (Hagan *et al.*, 1978) blood sampling before stabilization of plasma volume (40-60 minutes) could produce erroneous determinations of ICG serum concentrations. Hence, in the present study patients remained in the assumed position for a minimum of 60 minutes and posture was maintained during blood sampling for ICG in order to minimize the changes in plasma protein concentrations.

2. The hematocrit is an important factor to convert Cl_{pICG} to Q_H. Since its value is influenced by postural change, estimation of the hematocrit should be performed after the stabilization of the plasma volume following positional change. In the present study, blood sampling for hematocrit was performed during the blood sampling for ICG ($\approx$ 8 min after ICG injection) and 60 minutes after the patients assumed a new position, thereby minimizing the transient changes in hematocrit due to the postural change.

The observed $\approx$4% and $\approx$5% significant increase in hematocrit in the upright position, before and after captopril therapy, respectively, is smaller than the reported $\approx$ 10% increase in healthy subjects (Hagan *et al.*, 1978). The results of the present study, however, confirms the reported increase in hematocrit in standing position in patients with mild to

moderate hypertension, as a consequence of the plasma volume changes upon standing. Further, these results suggest that a two-week captopril treatment does not appear to alter the acute changes in plasma volume due to postural change.

4.8.2. Acute Effects of Captopril on Hematocrit

There was no significant change in hematocrit after the initial and terminal dose of captopril. A slight decrease which was less than 1% was observed on both study days when hematocrit values obtained 1 hour after captopril administration were compared to control values (seated, s1) (Table 8).

4.8.3. Short-Term Effects of Captopril on Hematocrit

Hematocrit values decreased after 14 days captopril treatment by about 5%, when compared to day 1 (Table 8). One possible explanation of the decrease in hematocrit may be an increase in plasma volume (Hagan *et al.*, 1978). It has been noted by Tarazi *et al.*, (1980), that plasma volume increased slightly, but significantly (5.1% ± 2.11%, $p < 0.05$) in those hypertensive patients whose blood pressure was decreased after 5 days treatment with captopril. It has been suggested (Tarazi *et al.*, 1980), that this increase in plasma volume may indicate venodilation, with intravascular redistribution of extracellular fluid volume. Although the biological significance of this small plasma volume increase has been questioned (Tarazi *et al.*, 1980), it may partially explain the decreased hematocrits observed in the present study.

4.9. Serum Concentration Data of Captopril

The pharmacokinetic behavior of captopril in patients with hypertension is well established (Kubo and Cody 1985; Jarrott *et al.*, 1982; Richer *et al.*, 1984). In the study protocol blood sampling for unchanged captopril was designed with the purpose to show (1) that captopril was well absorbed after oral administration and (2) that the estimation of Q_H was performed around or after the peak captopril serum concentration was achieved.

On both study days the absorption of captopril from the gastrointestinal tract was rapid. Serum concentrations of unchanged captopril on day 1 and day 14 were in the range from 41 to 1490 ng/ml and 111 to 1187 ng/ml, respectively, between 20 and 40 minutes (Figure 29). One patient (M.O.) exhibited very low serum levels of unchanged captopril in the first 150 minutes on day 1 which increased to 276.7 ng/ml at 180 minutes. This may be explained by either the poor absorption of captopril in this patient or high protein and/or enzyme binding of captopril.

Peak serum levels of unchanged captopril (C_{max}) the time required to reach C_{max} (t_{max}) was 75.9 ± 22.2 minutes (mean ± SEM) after the first captopril dose and 52.2 ± 6.0 minutes after two-weeks captopril treatment (Table 18). The t_{max} values for unchanged captopril were higher than those reported by Jarrott *et al.*, (1982) who found that C_{max} were reached at 53 ± 22.8 minutes after 100 mg oral dose of captopril in hypertensive subjects. In another study, (Cody *et al.*, 1982) higher t_{max} value of 85.8 ± 11.4 minutes was reported after a single captopril dose, which is slightly higher than the t_{max} of unchanged captopril found in the current study. Previous studies (Cody *et al.*, 1982; Jarrott *et al.*, 1982) noted significantly lower

t_{max} for unchanged captopril following short-term or chronic therapy which was not observed in the present study. This may be explained by the slow increase in unchanged captopril serum concentration in patient M.O. after the initial captopril dose. In the present study, average C_{max} of unchanged captopril after a single-dose was 697 ng/ml, which is higher than the 361 ng/ml C_{max} value noted by Jarrott *et al.*, (1982), but similar to the 800 ng/ml value reported by Kripalani *et al.*, (1980). There was, however a large interindividual variation in C_{max} of unchanged captopril after the first dose of captopril, ranging from 184.3 to 1490.3 ng/ml (8.1 fold difference)(Table 18). On day 14, C_{max} was less variable than on day 1 (range from 582.2 to 1187.0 ng/ml (2 fold difference). There was rapid disappearance of unchanged captopril from the blood thereafter, so that the concentration of unchanged captopril was 119 ± 36.8 ng/ml on day 1 and 51.7 ± 4.4 ng/ml on day 14, 3 hours after dosing. The rate of elimination of unchanged captopril was higher after two-weeks captopril administration.

The average Cmax of intact drug was higher after two-weeks captopril therapy than after the first dose (871 ng/ml vs 697 ng/ml). Also, the AUC for unchanged captopril after two-weeks captopril therapy was greater by 33.7% than that after the first captopril dose (1038.8 ng.h/ml vs 776.9 ng.h/ml). Jarrott et al., (1982) noted a two-fold increase in the AUC after chronic administration of captopril which is similar to the findings of the present study. However, Cody et al., (1982) found no change in the AUC of unchanged captopril in patients with congestive heart failure after 5 days treatment with 25 mg captopril 3 times a day.

5. SUMMARY AND CONCLUSIONS

5.1. UV Spectrophotometric Analysis of ICG in Human Serum

ICG in serum was analyzed spectrophotometrically by the modified method of Caesar *et al.*, (1961). Modifications regarding the preparation of standard curve samples have been described previously by Dorr *et al.*, (1989) and Rappaport & Thiessen, (1982).

The preliminary experiments confirmed that ICG was stable in human serum for 48 hours when stored at -20°. The accuracy of the spectrophotometric assay has been established by determining the reproducibility of the assay. Inter- and intraday coefficients of variation were less than 10%. Standard curves were linear in the concentration range of 0.2 - 5.0 μg/ml with a correlation coefficient of $r^2 = 0.999$. The coefficient of variation was < 10 %.

5.2. Effects of Postural Change

5.2.1. Cl_{pICG} and Q_H

Upright position significantly decreased Cl_{pICG} and Q_H from 616.9 ± 43.9 to 464.6 ± 36.9 ml/min (mean ± SEM) and from 1114 ± 74 to 863 ± 62 ml/min (mean ± SEM), respectively, before captopril treatment, as compared to baseline value. Similarly, after 14 days treatment with 100 mg daily oral doses of captopril, Cl_{pICG} and Q_H were significantly decreased from 793.7 ± 91.0 to 582.8 ± 74.0 ml/min and from 1389 ± 164 to 1045 ± 132 ml/min,

respectively, in the upright position. When compared to sitting values, the absolute decrease in Cl_{pICG} and Q_H in standing position was 152.3 ± 18.3 ml/min or 24.7% and 250 ± 36 ml/min or 22.5%, respectively, before captopril treatment and 210.9 ± 38.8 ml/min or 26.6% and 344 ± 68 ml/min or 24.7%, respectively, after short-term treatment with the drug. There was no significant difference in the response to postural change before and after captopril therapy. There was no significant difference in Q_H between the two seated (s1 and s2) measurements.

5.2.2. Hematocrit, Systolic and Diastolic Blood Pressure, Heart Rate

The standing position before, as well as after, captopril therapy was associated with a statistically significant increase in hematocrit by 3.8% and 5.0%, respectively.

Diastolic blood pressure increased slightly by 5-7 mm Hg on both study days in upright position, but systolic blood pressure and mean arterial pressure were unaltered.

Heart rate increased significantly by 4 and 6 beats/min in standing position before and after captopril treatment.

5.2.3. Splanchnic Vascular Resistance

Splanchnic vascular resistance (mean ± SEM) increased significantly in standing position from 6.58 ± 0.5 to 8.62 ± 0.7 mm Hg·sec/ml (31.0%) on day 1 and from 5.34 ± 0.8 to 7.12 ± 1.1 mm Hg·sec/ml (33.3%), after 14 days captopril administration.

5.3. Acute Effects of Captopril

5.3.1. Cl_{pICG} and Q_H

The acute effects of captopril on Cl_{pICG} and Q_H has been investigated in two occasions, 1 hour after the administration of the initial and terminal dose of captopril. There was no significant change in Cl_{pICG} and Q_H after the initial and terminal dose of captopril, as compared to the control values. However, a slight, not significant, decrease in Cl_{pICG} (5.2%) and Q_H (6.0%) was observed after the initial and terminal dose of captopril, respectively. This decrease in Q_H, 1 hour after captopril administration were similar in magnitude to the difference seen in Q_H between the seated (s1) and reseated (s2) measurements on day 1 and 14 (6.3% and 8.5%, respectively). These results suggest, that the slight decrease in Q_H 1 hour after captopril dosing is, most probably, due to other factors rather than a direct pharmacological effect of the drug. When the Q_H values obtained 1 hour after the initial and terminal captopril dose were compared to those of seated (s1) values, a decrease in Q_H from 1114 ± 74 to 980 ± 92 ml/min (12.0%) and 1389 ± 1193 ± 119 (14.1%), respectively, has been noticed. Since the decrease in Q_H during the course of the two study days was similar to that caused by diurnal changes, circadian variation in Q_H has been suggested to explain the apparent decrease in Q_H.

5.3.2. Systolic and Diastolic Blood Pressure and Heart Rate

Systolic and diastolic blood pressures significantly decreased by 21.5 ± 6.5 mm Hg (13.4%) and 13.4 ± 4.3 mm Hg (13.0%), 1 hour after the initial

dose of captopril, as compared to control values. Both, systolic and diastolic blood pressure, were further decreased 3 hours after a single-dose of captopril, resulting in an absolute decrease from pretreatment values by 28.1 ± 6.7 mm Hg (17.5%) and 16.6 ± 6.4 mm Hg (16.1%), respectively. One hour after the terminal dose of captopril systolic and diastolic blood pressures were not significantly lower than the treated baseline blood pressures on day 14. However, 3 hours after taking the terminal captopril dose, systolic and diastolic blood pressures were significantly lower by 16.7 ± 9.9 mm Hg (11.5%) and 10.0 ± 3.2 mm Hg (10.7%), than the treated baseline values of day 14. The relatively smaller decrease in systolic and diastolic blood pressure, after the terminal dose of captopril, as compared to the changes after the initial dose is attributed to the lower baseline values after two-weeks treatment with the drug. The absolute decrease in systolic and diastolic blood pressures, 1 and 3 hours after the terminal dose of captopril, when compared to the pretreatment values, were similar to that observed after the initial captopril dose (22 *vs* 21 mm Hg and 28 *vs* 32 mm Hg, respectively, for systolic pressure, 13 *vs* 12 mm Hg and 17 *vs* 20 mm Hg, respectively for diastolic pressure).

When compared to control values, heart rate did not change significantly after the acute doses of captopril.

5.3.3. Splanchnic Vascular Resistance

Despite the significant decrease in arterial blood pressure, 1 hour after the initial dose of captopril, no significant change in splanchnic vascular resistance was noted. Similarly, splanchnic vascular resistance was unaffected by the terminal dose of captopril, estimated 1 hour after the

intake of captopril. These data suggest that no significant changes occur in the splanchnic vascular resistance 1 hour after acute dosing with captopril.

5.4. Short-term Effects of Captopril

5.4.1. Cl_{pICG} and Q_H

The short-term effects of captopril on Cl_{pICG} and Q_H were determined by comparing data obtained after two-weeks captopril treatment (day 14) to those of the pretreatment values (day 1).

Following two-weeks oral captopril treatment (100 mg/day) Cl_{pICG} significantly increased by 176.8 ± 57.4 (28.7%), 118.2 ± 51.6 (25.5%), 145.9 ± 81.8 (25.2%) and 137.9 ± 57.1 ml/min (25.2%) in all four phases of the study, seated (s1), upright (up), reseated (s2) and post-captopril (CA), as compared to the appropriate values of day 1. Similarly, Q_H has been found to increase by 275 ± 107.3 (24.7%), 182.0 ± 90.6 (21.1%), 227.8 ± 147.8 (21.8%) and 212.8 ± 92.5 ml/min (21.7%) measured in the seated (s1), upright (up), reseated (s2) and post-captopril (CA) study phases, respectively. The increase in Q_H after 14 days captopril treatment followed an almost parallel pattern with the day 1 data. However, the difference in Q_H after short-term treatment, as compared to the day 1 values, just failed to achieve the level of significance whether Q_H was expressed in absolute terms or per unit body weight or body surface area ($p = 0.06$, MANOVA). The lack of statistical significance has been attributed to the different response of one patient who exhibited a decrease rather than increase in Q_H after two-weeks therapy. Excluding this patient's data from the statistical comparison, the increase

in Q_H after 14 days captopril administration was statistically significant (MANOVA, $p < 0.02$).

5.4.2. Hematocrit, Systolic and Diastolic Blood Pressure and Heart Rate

Except for the post-captopril (CA) measurement there was a significant reduction in hematocrit by 5.5%, 4.4% and 5.1% during the seated, upright and post-captopril phases of the study, after two-weeks captopril treatment, as compared to the day 1 values. This reduction in hematocrit can partially be explained by an increase in plasma volume, as a result of captopril therapy. The clinical significance of this reduction in hematocrit is uncertain.

On day 14, treated baseline systolic and diastolic blood pressures (mean ± SEM) were 145.5 ± 14.5 mm Hg and 92.8 ± 2.3 mm Hg, respectively. These values were significantly lower by 14.9 ± 7.7 mm Hg (9.3%) and 10.5 ± 6.4 mm Hg (10.1%), respectively, than those of the pretreatment values. Since the baseline blood pressure recordings on day 14 were performed approximately 20 hours after the intake of the last captopril dose, these results suggest that during prolonged treatment with captopril, blood pressure is maintained below the pretreatment values, a minimum of 20 hours after captopril dosing. This prolonged antihypertensive effect of captopril has been observed in all of the six patients studied.

Heart rate decreased slightly, but not significantly (2.3%), 3 hours after the terminal dose of captopril, as compared to pretreatment values.

5.4.3. Splanchnic Vascular Resistance

When compared to the seated (s1), upright (up), reseated (s2) and post-captopril (CA) study phases of day 1, there was a decrease in splanchnic vascular resistance by 18.8%, 17.4%, 15.0% and 23.0%, respectively, (mean 18.6%), after 14 days captopril treatment. The difference from day 1 values, however, was statistically not significant ($p = 0.125$, MANOVA). However, the decrease in splanchnic vascular resistance after two-weeks captopril treatment suggests changes in total peripheral resistance after prolonged captopril therapy.

5.5. Serum Concentration Data of Unchanged Captopril

Serum concentration *vs* time data of unchanged captopril shows that captopril was rapidly absorbed after oral administration and reached maximum serum concentration at 75.9 ± 22.2 minutes (mean ± SEM) on day 1 and 52.2 ± 2.5 minutes on day 14. The C_{max} (mean ± SEM) of unchanged captopril was found in the range from 184.3 to 1490.3 ng/ml (mean 697.2 ± 192.8 ng/ml) on day 1 and from 582.2 to 1187.0 ng/ml (mean 870.5 ± 85.9 ng/ml). These data suggest that adequate amounts of the pharmacologically active drug reached the systemic circulation on both study days. Although the C_{max} (mean ± SEM) was 24.9% higher after two-weeks captopril therapy than that after a single-dose, the difference in C_{max} on day 14 was statistically not significant, as compared to the day 1 value. Similarly, AUC_0^t (mean ± SEM) for unchanged captopril was found to be 973.1 ± 78.1 ng·h/ml, after the initial captopril dose which is 21.2% higher than the observed 802.7 ± 197.7 ng·h/ml AUC_0^t

following two-weeks captopril treatment. However, the increase in AUC_0^t, after 14 days captopril administration was statistically not significant. A rapid decline in the serum concentration of unchanged captopril was observed, so that 3 hours after captopril administration the serum concentration of unchanged captopril (mean ± SEM), on day 1 and 14, was 119.1 ± 36.8 ng/ml on day 1 and 51.7 ± 4.4 ng/ml, respectively.

:5.6. Conclusions

The upright posture induced a statistically significant decrease in Q_H which response was not altered after short-term treatment with captopril. The applied spectrophotometric method has been proved to be sensitive enough to measure changes in Q_H, in the magnitude caused by postural change from sitting to upright. The observed changes in heart rate, blood pressure and splanchnic vascular resistance upon postural change are in agreement with previous studies. The fact that the acute changes in hepatic blood flow, splanchnic vascular resistance and heart rate induced by upright posture were not altered by prolonged treatment with captopril suggests that captopril does not interfere with the homeostatic responses to postural change in patients with mild/moderate hypertension.

Despite the significant reduction in systolic and diastolic blood pressures, captopril has no apparent acute effect on Q_H and splanchnic vascular resistance as measured 1 hour post-dose. This was demonstrated in two separate occasions in the same subject. The lack of changes in Q_H after the acute doses of captopril cannot be explained by poor absorption of the captopril since serum concentration of unchanged captopril increased rapidly after administration and reached pharmacologically active level. Once daily

administration of captopril provided sustained blood pressure lowering effect, in spite of the rapid disappearance of the pharmacologically active drug from the systemic circulation.

Despite the fact that captopril has no apparent acute effect on Q_H there is a significant increase in Q_H on continued administration. The substantial decrease in splanchnic vascular resistance suggest splanchnic vasodilation after prolonged captopril therapy. The captopril induced increase in liver blood flow on continued captopril administration should be considered when drugs, exhibiting high hepatic clearance are coadministered.

Further studies are necessary to establish the clinical significance and the extent of change in the kinetic parameters of coadministered high clearance drugs, where liver blood flow alterations would be expected to impact clearance.

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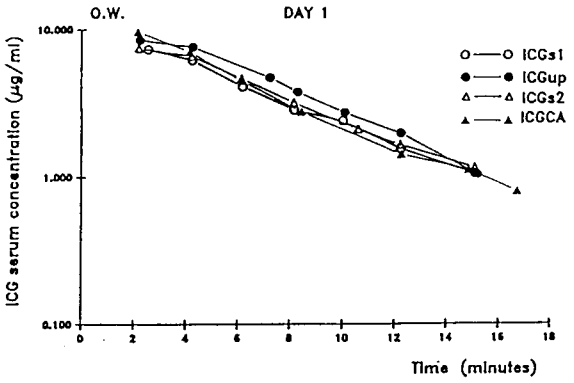
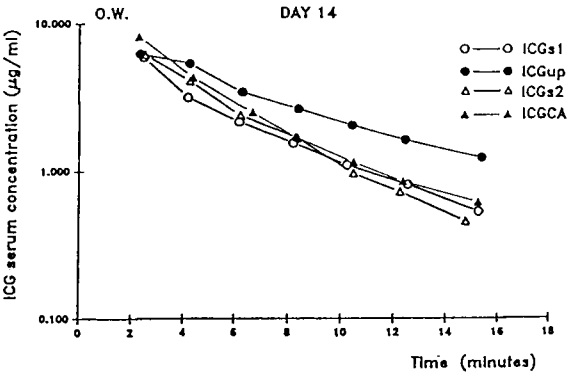
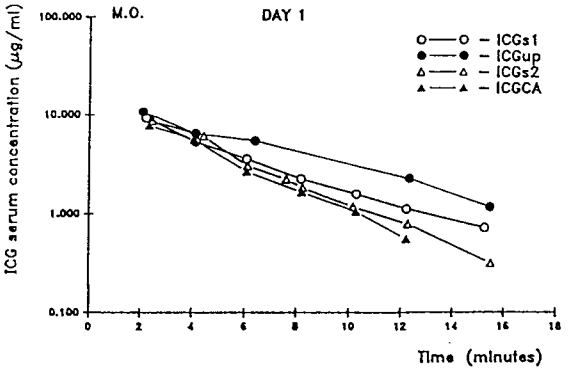
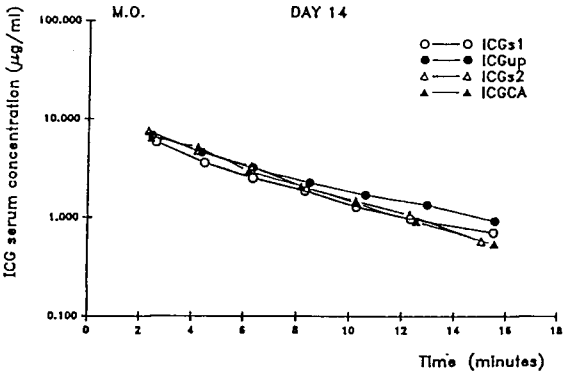
APPENDIX

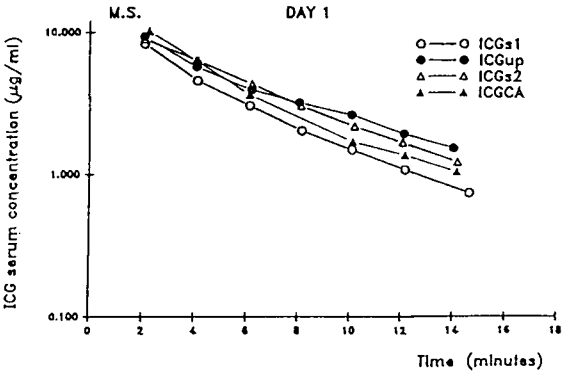
Appendix 1. Semi-logarithmic plots of ICG serum concentration *versus* time curves from six patients with mild/moderate hypertension obtained on day 1 and day 14 in seated (s1, open circles), upright (up, closed circles), reseated (s2, open triangles) and 1 hour after captopril dosing (CA, closed triangles).

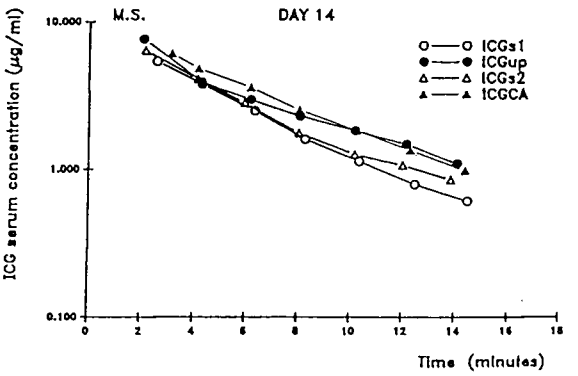












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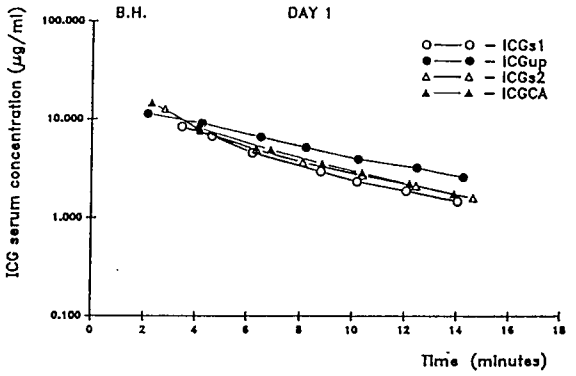

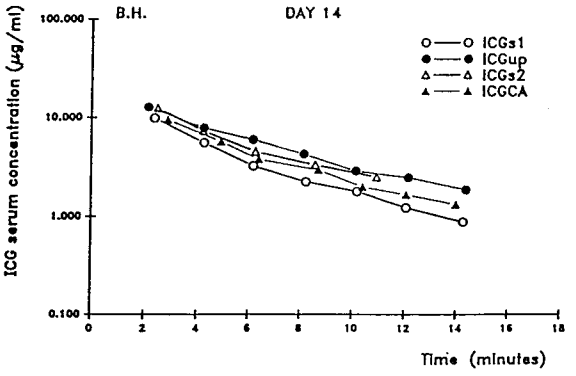

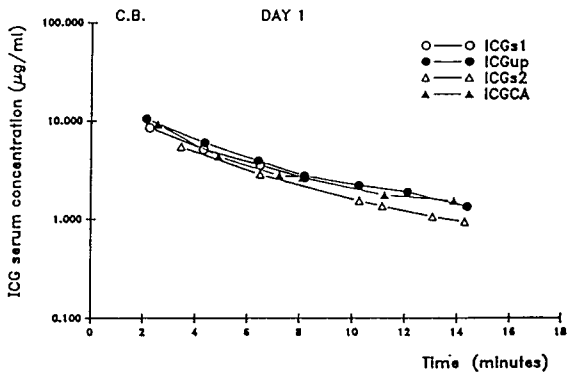

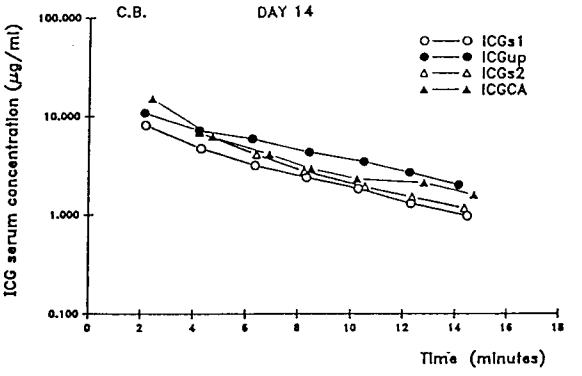

Appendix 2. Clp (normalized to body weight and body surface area) data of ICG before and after the initial dose (day 1) and terminal dose (day 14) of captopril

Clp / body weight of ICG (ml/min/kg)

Patient	Day 1				Day 14			
	s1	up	s2	CA	s1	up	s2	CA
MO	5.8	4.1	5.6	6.6	7.8	6.4	6.6	6.9
OW	7.0	5.9	6.3	6.0	11.4	7.5	10.2	8.8
MS	8.2	6.1	6.3	6.4	10.3	8.0	9.5	7.6
BH	5.8	4.1	4.9	4.6	7.1	4.7	5.2	6.1
DA	6.1	4.2	6.4	5.1	6.7	5.9	9.1	9.0
CB	6.4	5.2	7.5	5.7	6.7	4.1	5.4	5.1
Mean:	6.5	4.9	6.2	5.7	8.3	6.1	7.7	7.2
± SEM:	± 0.4	± 0.4	± 0.4	± 0.3	± 0.8	± 0.6	± 0.9	± 0.6

Clp / body surface area of ICG (ml/min/m²)

Patient	Day 1				Day 14			
	s1	up	s2	CA	s1	up	s2	CA
MO	280.5	200.7	271.0	322.5	381.2	310.3	320.5	337.7
OW	306.6	256.4	277.1	261.7	498.5	327.3	447.7	384.3
MS	356.9	265.0	273.3	278.1	450.5	349.8	416.2	333.7
BH	258.3	183.6	218.4	204.8	318.5	209.7	231.3	271.5
DA	248.4	169.8	260.3	208.3	274.1	241.7	370.2	364.4
CB	269.7	222.6	317.8	244.0	282.9	172.9	228.4	216.3
Mean:	286.7	216.3	269.7	253.2	367.6	268.6	335.7	318.0
± SEM:	± 16.3	± 15.8	13.0	± 18.2	± 37.7	± 29.0	± 37.8	± 25.6

s1 data obtained in seated position
up data obtained in upright position
s2 data obtained in the reseated position
CA data obtained 1 hour after the administration of captopril
SEM standard error of the mean

Appendix 3. Q_H data before and after the initial (day 1) and terminal dose (day 14) of captopril

	Q_H (ml/min)							
	Day 1				Day 14			
Patient	s1	up	s2	CA	s1	up	s2	CA
MO	1212	889	1154	1374	1615	1315	1358	1431
OW	1139	992	1057	1011	1835	1205	1648	1415
MS	1402	1080	1074	1074	1770	1426	1635	1311
BH	1062	770	898	825	1169	814	849	997
DA	970	683	1017	799	1040	932	1404	1313
CB	897	766	1057	798	902	580	729	690
Mean:	1114	863	1043	980	1389	1045	1271	1193
± SEM:	± 73.9	±61.9	± 34.3	± 92.1	± 163.5	± 132.4	± 160.4	± 119.1

	Q_H / body weight (ml/min/kg)							
	Day 1				Day 14			
Patient	s1	up	s2	CA	s1	up	s2	CA
MO	10.2	7.5	9.7	11.6	13.6	11.1	11.4	12.1
OW	12.3	10.7	11.4	10.9	19.8	13.0	17.8	15.3
MS	14.6	11.2	11.2	11.2	18.4	14.9	17.0	13.7
BH	11.5	8.4	9.8	9.0	12.7	8.8	9.2	10.8
DA	11.4	8.0	12.0	9.4	12.2	11.0	16.5	15.4
CB	10.7	9.1	12.6	9.5	10.7	6.9	8.7	8.2
Mean:	11.8	9.2	11.1	10.3	14.6	10.9	13.4	12.6
± SEM:	± 0.6	± 0.6	± 0.5	± 0.4	± 1.5	± 1.2	± 1.7	± 1.1

	Q_H / body surface area (ml/min/m²)							
Patient	s1	up	s2	CA	s1	up	s2	CA
MO	497.3	364.8	473.7	563.8	662.9	539.6	557.4	587.2
OW	537.9	468.8	499.3	477.5	867.0	569.3	778.6	668.3
MS	637.3	490.7	488.0	488.0	804.4	647.9	743.2	595.9
BH	516.6	374.7	436.8	401.5	568.8	395.7	413.0	484.9
DA	464.2	326.6	486.6	382.2	437.3	445.9	671.8	628.3
CB	453.3	387.1	534.2	403.3	456.2	293.0	368.3	348.8
Mean:	517.8	402.1	486.4	452.7	632.8	481.9	588.7	552.2
± SEM:	± 27.2	± 26.1	± 13.0	± 28.4	± 72.7	± 52.6	± 70.1	± 47.7

s1 data obtained in seated position
up data obtained in upright position
s2 data obtained in reseated position
CA data obtained 1 hour after the administration of captopril
SEM standard error of the mean