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**ERRATUM**

Page 16, paragraph 2, line 4: "sequelae" for "sequalae"

# CHANGES IN INTEGRATED CARDIOVASCULAR PHYSIOLOGY DURING INOTROPIC STIMULATION IN THE EARLY POSTNATAL PERIOD.

**A Thesis Submitted For The Degree of Doctor Of Philosophy,**
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## SUMMARY OF THESIS

***Background.*** Inotropic agents are widely used in the treatment of critical illness in the newborn. However, while it is recognised that inotropes can affect cardiac and vascular function, as well as systemic and myocardial metabolism, and that responses may be influenced by developmental processes, little information is available about the integrated effects of inotropes across these systems in the neonate, or how these effects change in the initial week after birth. Using an experimental lamb model, the aim of this thesis was to examine changes and underlying mechanisms in integrated cardiovascular physiology in the neonatal period during administration of dobutamine and dopamine, two inotropic agents which are commonly used in clinical practice.

***Methods.*** Seventy-six anaesthetised, open-chest lambs (50 aged 1-2 days and 26 aged 7-10 days) were instrumented with fluid-filled catheters in the external jugular vein, coronary sinus, pulmonary artery, aorta and left atrium, a micromanometer-tipped catheter placed in the left ventricle (LV) and ultrasonic flow-probes placed around the ascending aorta and circumflex coronary artery. A thermistor was passed into the pulmonary trunk to measure cardiac output by thermodilution.

Pulmonary arterial, aortic and left atrial pressures, ascending aortic and circumflex coronary artery blood flow and cardiac output were measured. LV external work, systemic and pulmonary vascular resistance were calculated and the maximal rate of rise of LV pressure ($dP/dt_{MAX}$) derived from the LV pressure signal. Blood samples were obtained from the aorta, pulmonary artery and coronary sinus to measure $O_2$ content and to calculate systemic and LV myocardial $O_2$ delivery and consumption.

The effects of stepwise, incremental infusions of dobutamine and dopamine were assessed on *1)* central haemodynamics and ventricular performance, *2)* systemic and pulmonary vascular responses, *3)* systemic oxygen delivery and consumption and *4)* left ventricular myocardial oxygen delivery and consumption. In subgroups of animals, the effects of selective adrenoreceptor blockade, systemic inhibition of nitric oxide (NO) synthesis or elevation of central arterial pressure by proximal arterial occlusion on responses to dobutamine were also examined.

***Results.*** Increases in heart rate and cardiac function produced by dobutamine were similar in both age groups, but the reduction in pulmonary vascular resistance was blunted in 1-2 day lambs. Increases in systemic $O_2$ delivery were similar in both groups, but the beneficial effects of these increases in 1-2 day lambs were largely offset by rises in systemic $O_2$ consumption that were associated with elevations in body temperature. Dobutamine-related increases in LV myocardial $O_2$ consumption were similar in both groups. However, as rises in LV myocardial blood flow and $O_2$ delivery were attenuated in 1-2 day animals, the increase in LV myocardial $O_2$ consumption was supported by elevations in the LV myocardial $O_2$ extraction ratio.

From adrenoreceptor blockade studies, it appeared that dobutamine-related increases in heart rate and $LVdP/dt_{MAX}$ were related mainly to a $\beta_1$ receptor action and that blunting of pulmonary vasodilator responses in 1-2 day lambs was not mediated by a developmental shift in the $\alpha/\beta$ adrenoceptor profile. In addition, increases in systemic $O_2$ consumption and body temperature were unaffected by individual adrenoceptor blockade, but were substantially blunted by combined $\alpha_1$, $\beta_1$ and $\beta_2$ blockade.

Pulmonary vascular and systemic $O_2$ consumption responses to dopamine were similar to those of dopamine. However, other physiological responses to dopamine infusion differed from those of dobutamine. Mean arterial pressure was increased by dopamine, but reduced by dobutamine. In addition, the peak increase in heart rate was greater during dobutamine, while LV external work increased more with dopamine.

Inhibition of NO synthesis did not alter increases in cardiac function during dobutamine infusion in either group, nor did it alter changes in pulmonary and systemic vascular resistance in 1-2 day animals. However, in the 7-10 day group, inhibition of NO synthesis enhanced dobutamine-induced reductions in systemic and pulmonary vascular resistance. Although increases in systemic $O_2$ delivery were unaltered, inhibition of NO synthesis prevented dobutamine-induced increases in systemic $O_2$ consumption and body temperature in 1-2 day animals. Inhibition of NO synthesis attenuated increases in LV myocardial $O_2$ delivery and consumption during dobutamine infusion, but did not alter the LV $O_2$ delivery-consumption relationship.

***Conclusions.*** These experimental studies indicate that substantial developmental changes in integrated cardiovascular responses to inotropic agents occur in the neonatal period, particularly in relation to the pulmonary circulation and the balance between systemic $O_2$ delivery and consumption.

## ACKNOWLEDGEMENTS

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I acknowledge that the work included in this thesis is my own. It has not been presented for any other degree to Monash University or any other tertiary institution.

[redacted]

Daniel James Penny. February 2004

[redacted]

## PRESENTATIONS TO SCIENTIFIC SOCIETIES BASED ON THIS WORK.

**American Heart Association Annual Meeting 1995**
Sano T, Penny DJ, Forster K, Smolich JJ. Nitric oxide modulates the effect of dobutamine in the postnatal pulmonary circulation.

**The Perinatal Society of Australia and New Zealand Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Improved systemic oxygen delivery during inotropic stimulation is offset by increased systemic oxygen consumption in neonatal lambs.

**The Perinatal Society of Australia and New Zealand Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Enhanced modulation of inotropic responses by nitric oxide during postnatal development.

**The Perinatal Society of Australia and New Zealand Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Pulmonary, but not systemic responses to dobutamine are reduced in the neonatal period.

**British Cardiac Society. Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Increased modulation by nitric oxide of dobutamine-induced pulmonary vasodilation during postnatal development in sheep.

**British Cardiac Society. Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Increases in systemic oxygen consumption offset improvements in systemic oxygen delivery during dobutamine infusion in newborn lambs.

**The Cardiac Society of Australia and New Zealand Annual Meeting 1996**
Penny DJ, Sano T, Forster KM, Smolich JJ. Differing postnatal time course of inotropic response to dobutamine and its modulation by nitric oxide.

## PUBLICATIONS BASED ON THIS WORK.

Penny DJ, Sano T, Smolich JJ. Increased systemic oxygen consumption offsets improved delivery during dobutamine infusion in newborn lambs.

***Intensive Care Med.*** 2001; 27: 1518-25

Smolich JJ, Sano T, Penny DJ. Blunting of pulmonary but not systemic vasodilator responses to dobutamine in newborn lambs.

***Pediatr Res.*** 2000; 47: 107-13

Perny DJ. The basics of ventricular function. ***Cardiol Young*** 1999; 9: 210-223

# CHAPTER 1. INTRODUCTION

"Within the infant rind of this weak flower
Poison hath residence and medicine power."

William Shakespeare, Romeo and Juliet (1594).

## *1.1 Development of Inotropic Treatments In Critical Illness*

In 1775 a patient suffering from severe dropsy, the clinical condition which we would today recognise as congestive cardiac failure, presented to Dr William Withering who was at that time the most fashionable physician outside London. According to Withering, since no effective treatment was available to treat this fatal condition, the patient went to a local gypsy for treatment with a secret, herbal remedy. To the surprise of all concerned, the patient rapidly improved and went on to live for some time. Intrigued, Withering located the gypsy in remote Shropshire, where he convinced her to reveal the ingredients of the herbal remedy. Withering also had an interest in botany and had at the same time had been working on a major treatise which attempted to classify all the plants in Great Britain.[1] He recognised that the active ingredient within the remedy was likely to be the purple foxglove *digitalis purpurea*. This supposition stimulated a decade of intense work by Withering in which he documented the medicinal properties of *digitalis*, and which culminated in his treatise "*An Account of The Foxglove and Some of its Medical Uses, with Practical Remarks on Dropsy and Other Diseases*".[2] This publication also marked the foundation of our knowledge of a class of agents which act by stimulating the function of the heart, agents now known as inotropes.

Digitalis-type agents remained the mainstay of treatment for cardiac failure for almost 200 years. Indeed as recently as 1968, Leon Goldberg wrote[3]: "*The basic treatment of congestive heart failure has remained the same for decades – digitalis preparations and diuretics...Although literally hundreds of cardiac glycosides of different chemical structure have been investigated, not one has been found which has a better therapeutic ratio that the original glycosides of Digitalis purpurea.*"

However, more than seven decades earlier, the foundations for a very different approach to the treatment of congestive heart failure were being laid. In 1897, John Jacob Abel, inaugural Director of the Department of Pharmacology at Johns Hopkins, together with Cornelius Crawford, isolated a substance from the adrenal gland of the sheep.[4] Abel observed that "*This substance was 'a sulfate (which) is very active physiologically. A small quantity suffices to raise the blood pressure*". He named this agent epinephrine, later known as 'adrenaline' in Europe. During the 20th century a number of related agents, classified as catecholamines because of their chemical structure and all with slightly different pharmacological effects were isolated. The heterogeneous effects of these "epinephrine-like" agents and their differing responses to "anti-epinephrine drugs" were described by Raymond Ahlquist who in 1948 proposed that the effects of epinephrine-like agents were mediated through their affinity for different receptor types.[5] This receptor theory initially gained little acceptance, and as Ahlquist himself commented[6]:

*"The original paper was rejected by the Journal of Pharmacology and Experimental Therapeutics, was a loser in the Abel Award competition, and finally was published in The American Journal of Physiology due to my personal friendship with the great physiologist, W.F. Hamilton*". However, work that followed confirmed the receptor theory of Ahlquist and provided detailed analyses of the effects of the different epinephrine-like agents on cardiovascular physiology.

Goldberg[3] proposed that it might be possible to develop a catecholamine for the treatment of congestive heart failure and indeed, the potential benefits of intravenous adrenaline in patients with low cardiac output after cardiac surgery had been demonstrated by Coffin et al.[7] in 1966 ***(Figure 1:1)***. However the earliest

catecholamines had undesirable actions which significantly limited their clinical utility.[3] These unwanted effects included a severe reduction in renal blood flow which accompanied adrenaline infusions and the excessive tachycardia with isoprenaline administration.

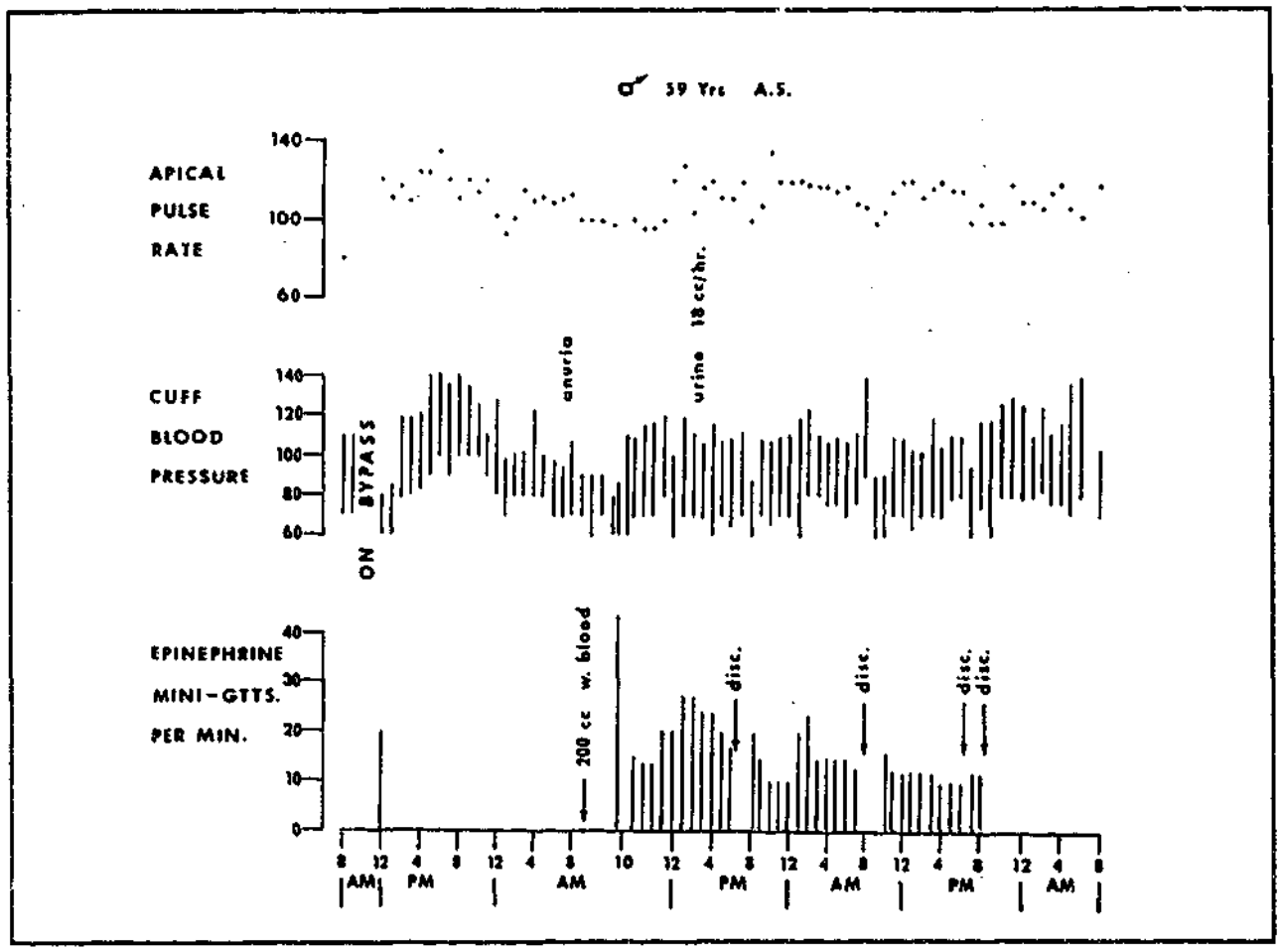


***Figure 1.1*** *Clinical course of a 59-year-old man following aortic valve replacement. Adrenaline (epinephrine) solution: 4mg/250ml glucose 5%. From Coffin et al.*[7]

Subsequently two other catecholamines with therapeutic profiles better suited for patients with cardiovascular disease were developed. The first of these was dopamine (3-hydroxy tyramine), an endogenous catecholamine, which has since been used more than any other cardiovascular agent to treat the low cardiac output state. Dopamine has a wide spectrum of cardiovascular effects when administered intravenously, and its actions are mediated by the stimulation of dopaminergic as

well as adrenergic receptors, and by release of endogenous norepinephrine.[8,9] The second was dobutamine, developed in 1975 by Tuttle and Mills[10] by modifying the chemical structure of isoprenaline. With inotropic properties equivalent to those of adrenaline, together with significantly fewer unwanted chronotropic and vascular effects than isoprenaline, dobutamine had a clear appeal over its predecessors. Not surprisingly, dobutamine was soon introduced into clinical practice to increase myocardial contractility, cardiac output and blood pressure in patients with hypotension and heart failure. For nearly three decades, dopamine and dobutamine have remained the mainstay of inotrope therapy in clinical practice.

## *1.2 Effects of Inotropes On Integrated Physiology*

In recent years, our understanding of the physiology and pharmacology of catecholamines has increased, in tandem with a greater appreciation of the pathophysiology of critical illness. The pharmacological and therapeutic profiles of catecholamines have extended beyond their effects on myocardial contractility, to include more diverse influences on integrated physiology. These include changes in systemic and pulmonary vascular tone, alterations in myocardial blood flow, $O_2$ consumption and metabolism, and modulation of substrate (particularly $O_2$) utilisation in peripheral tissues. Furthermore, more recent data suggest that the actions of adrenergic agents can be profoundly regulated by other intracellular and intercellular messengers, including the ubiquitous mediator nitric oxide (NO).

*1.2.1 Changes in Cardiac Contractility During Adrenergic Stimulation.* The cellular mechanisms underlying the increase in contractility during adrenergic

stimulation are now well-established. These effects are known to be mediated predominantly through interactions between the catecholamine and the β-adrenoreceptors, although myocardial α-receptors may also play a role.[11,12] Once a catecholamine interacts with the β-receptor, a conformational change is produced within it, resulting in activation of a stimulatory G protein. The G protein amplifies the stimulus by activating the enzyme adenylate cyclase, resulting in the production of the second messenger cyclic AMP (c-AMP). In turn, c-AMP activates protein kinase A, leading to the phosphorylation of a variety of c-AMP dependent proteins that govern excitation-contraction coupling, and result in enhanced contractility.[13] The profound effects of inotropic stimulation on myocardial activation can be demonstrated in vivo, by examining the changes in any of a number of indices of left ventricular myocardial contractility, which are described in more detail in Appendix H of this thesis.

*1.2.2 Systemic Blood Flow.* When an inotrope is prescribed, it is generally with the goal of optimising (or improving) cardiac output. However, it is known that an increase in myocardial contractility alone may not necessarily generate the desired increases in cardiac output during inotrope therapy[14], as changes in cardiac output reflect a complex interaction between the heart and the vascular system. Thus, for any given inotropic state, there is an optimal vascular load which will maximise ventricular performance and hence cardiac output.[15,16] Therefore an assessment of the clinical profiles of catecholamines should consider not only their direct myocardial actions, but also their effects on systemic vascular resistance and blood flow. These interactions during catecholamine infusion have been elegantly demonstrated in a canine model, in which dobutamine increased myocardial

contractility, while reducing systemic vascular resistance, suggesting a complex and desirable interaction between the myocardium and peripheral vascular system in response to this agent.[16]

*1.2.3 Myocardial $O_2$ Consumption, Blood Flow and $O_2$ Delivery.* In addition to increasing myocardial contractility and cardiac output, catecholamines also raise myocardial $O_2$ consumption. This increase in $O_2$ consumption appears to be at least partly related to a their effects on heart rate, wall tension and contractility.[17] However, catecholamines also appear to have an additional, less defined, so-called 'oxygen-wasting' effect, which has been attributed to changes in cardiac oxidative metabolism, and increased energy requirements for excitation-contraction coupling during inotropic stimulation.[18-20]

In the adult heart, the increase in $O_2$ consumption observed during inotropic stimulation is generally met by an equivalent increase in myocardial blood flow and $O_2$ delivery due to coronary vasodilatation[21] ***(Figure 1:2)***. This vasodilator response may be effected by a local metabolic feedback,[22,23] with a further contribution being made by direct stimulation of the coronary vascular β-adrenoceptors.[24] The ability of the adult myocardium to improve local $O_2$ delivery in response to changes in demand or consumption is an extremely important homeostatic mechanism, that may determine the degree of contractile reserve,[25] while also protecting the myocardium from the toxic (or anaerobic) effects of work-induced ischaemia.[26]

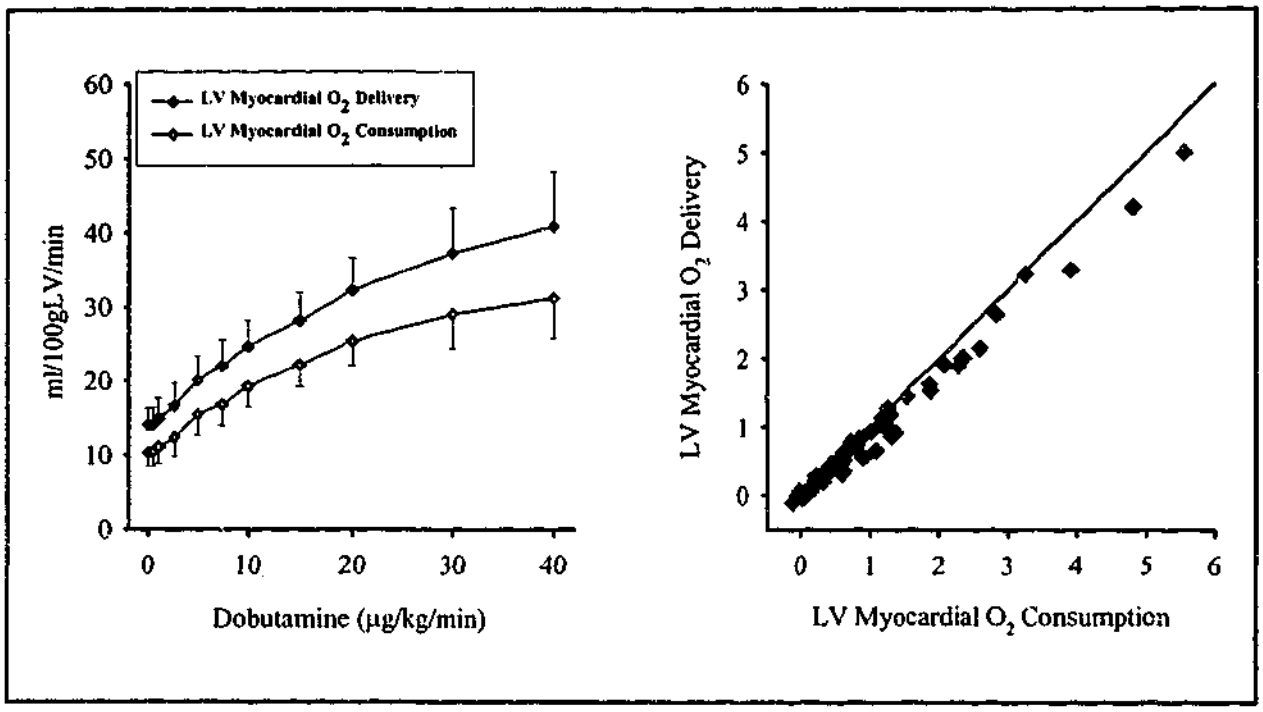


***Figure 1:2.*** *Changes in LV myocardial $O_2$ delivery and Consumption during dobutamine infusion up to 40µg/kg/min in sheep (left-hand panel). Changes in LV myocardial $O_2$ consumption were closely coupled to proportional changes in $O_2$ delivery (right-hand panel)(Adapted from Penny & Smolick)*[27].

*1.2.4* ***Systemic Delivery and Consumption of Metabolic Substrates.*** Having translated the elevated ventricular work into flow-work in the aorta, an important property of an inotropic agent, which is a key determinant of its clinical utility is its influence on tissue oxygenation. The balance between systemic $O_2$ delivery and consumption is often deranged in the critically ill,[28-31] as tissue $O_2$ demands (which are often increased) may not be met by an adequate supply of substrate. One of the goals of inotropic therapy must therefore be to address and re-set any disturbance of this balance. Oxygen delivery is a function of cardiac output and arterial $O_2$ content and it has been well-demonstrated in both humans[32-34] and experimental animals,[35] that agents such as dobutamine and dopamine augment systemic $O_2$ delivery by way of direct increases in cardiac output. In some species an additional augmentation in systemic $O_2$ delivery may be achieved through increases in arterial $O_2$ content resulting from an increase in haemoglobin level due to splenic contraction.[36]

Beta-adrenergic agonists also stimulate $O_2$ systemic $O_2$ consumption through their effects on myocardial work, enhanced energy requirements for the breakdown and cycling of stored fuels such as triglycerides and glycogen, and elevations in the rate of cellular metabolism.[37-39] In the healthy adult, this augmentation in systemic $O_2$ consumption is far exceeded by the concomitant increase in systemic $O_2$ delivery,[35,32,33] and as a result, infusion of β-adrenergic agonists reduce systemic $O_2$ extraction, that is, the $O_2$ consumed per unit $O_2$ delivered ***(Figure 1:3)***.

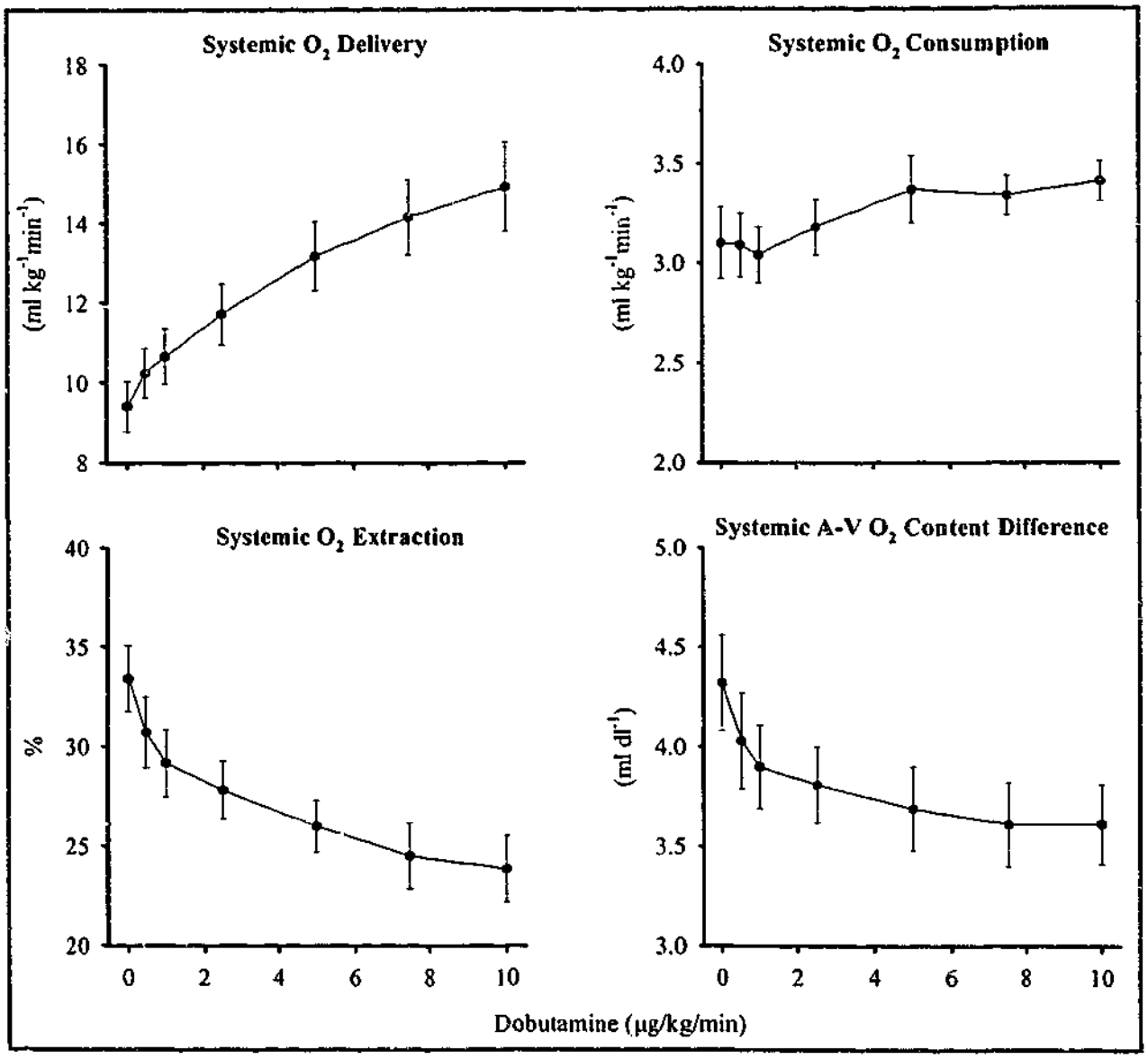


***Figure 1:3.*** *Systemic $O_2$ delivery and consumption during dobutamine infusion in anaesthetised, open-chested adult sheep. The increase in systemic $O_2$ delivery exceeded the increase in consumption (top panels), so that, as a result, the systemic $O_2$ extraction ratio and A-V $O_2$ content difference fell (bottom panels) (From Penny & Smolich.* [27]*)*

*1.2.5 Regulatory Mechanisms.* Any complex physiological effector system must be regulated by a wide variety of physiological processes. There has been considerable recent interest in the manner by which the actions of catecholamines are modulated by other intra- and intercellular messengers. Recent studies suggest that one such powerful modulator of the actions of catecholamines is nitric oxide (NO), a biologically labile compound which is synthesized from its precursor L-arginine in many cell types by a number of isoforms of the enzyme nitric oxide synthase (NOS).[40,41] NO acts primarily through stimulation of soluble guanylate cyclase with subsequent elevation of intracellular cyclic GMP levels.[42,43] The potential cellular interactions between NO and catecholamines, or their messengers, are of scientific and clinical interest. For example, this interaction may in part account for the blunted responses to adrenergic agents which can be seen in the setting of vasodilatory septic shock, in which there are well-described alterations in the endogenous production of NO.[44,45]

There are multiple potential mechanisms whereby NO may modulate the effects of catecholamines within the heart, including of the contractile apparatus, through effects on the coronary vasculature and through influences on myocardial $O_2$ consumption. NO is synthesised in the cardiac myocytes, a site where it may directly regulate myocardial contractility,[46,47] and may also potentially modulate the actions of adrenergic agonists. It was previously shown that exogenous NO decreased the contractile responses of isolated papillary muscle to norepinephrine.[48] Conversely, inhibition of NO synthesis using $N^{\omega}$-nitro-L-arginine (L-NNA), a stereospecific analogue of L-arginine, enhanced the inotropic action of isoproterenol in isolated rat ventricular myocytes[49] and intracoronary infusion of a NO synthase inhibitor

augmented the β-adrenergic responsiveness in the presence,[50] but not absence[51] of autonomic blockade. Taken together, these results imply that a nitric oxide-mediated mechanism may limit the usual inotropic responses to myocardial β-adrenergic stimulation.

NO may play a further modulatory role through its coronary vascular actions. Inhibition of NO synthesis reduced coronary blood flow by almost 50% in isolated, perfused rat hearts[52] and blunted the increase in myocardial blood flow in response to tachycardia in a canine model of pacing-induced heart failure.[53] A blunted myocardial blood flow response to adrenergic stimulation occurring in both the left[54] and right[55] ventricles has also been demonstrated after intracoronary administration of nitric oxide synthase inhibitors.

The role of NO in the regulation of myocardial $O_2$ consumption is more controversial. The current literature reports diverse effects of intracoronary NO synthase inhibitors, with some groups suggesting preservation of $O_2$ consumption,[56-60] and others, a reduction.[61]

In addition to important myocardial influences, NO is, of course, a potent regulator of systemic and pulmonary vascular tone. Interactions between adrenoceptor stimulation and the NO pathway in vascular smooth muscle have been well described. In isolated mesenteric arteries, isoprenaline-induced relaxation was attenuated by NOS inhibition,[62] while in porcine pial arteries in vivo, catecholamine-induced vasodilation was associated with an increase in cGMP levels and was inhibited by administration of a NOS antagonist.[63] In rings of rat aorta, the increase in cGMP release, induced by isoprenaline was attenuated by NOS inhibition.[64]

Moreover, our findings in adult sheep suggest that the rise in aortic blood pressure occurring after systemic NOS inhibition may also play an important role in the potentiation of the LV inotropic actions of β-adrenergic stimulation in the intact circulation[65] ***(Figure 1:4)***.

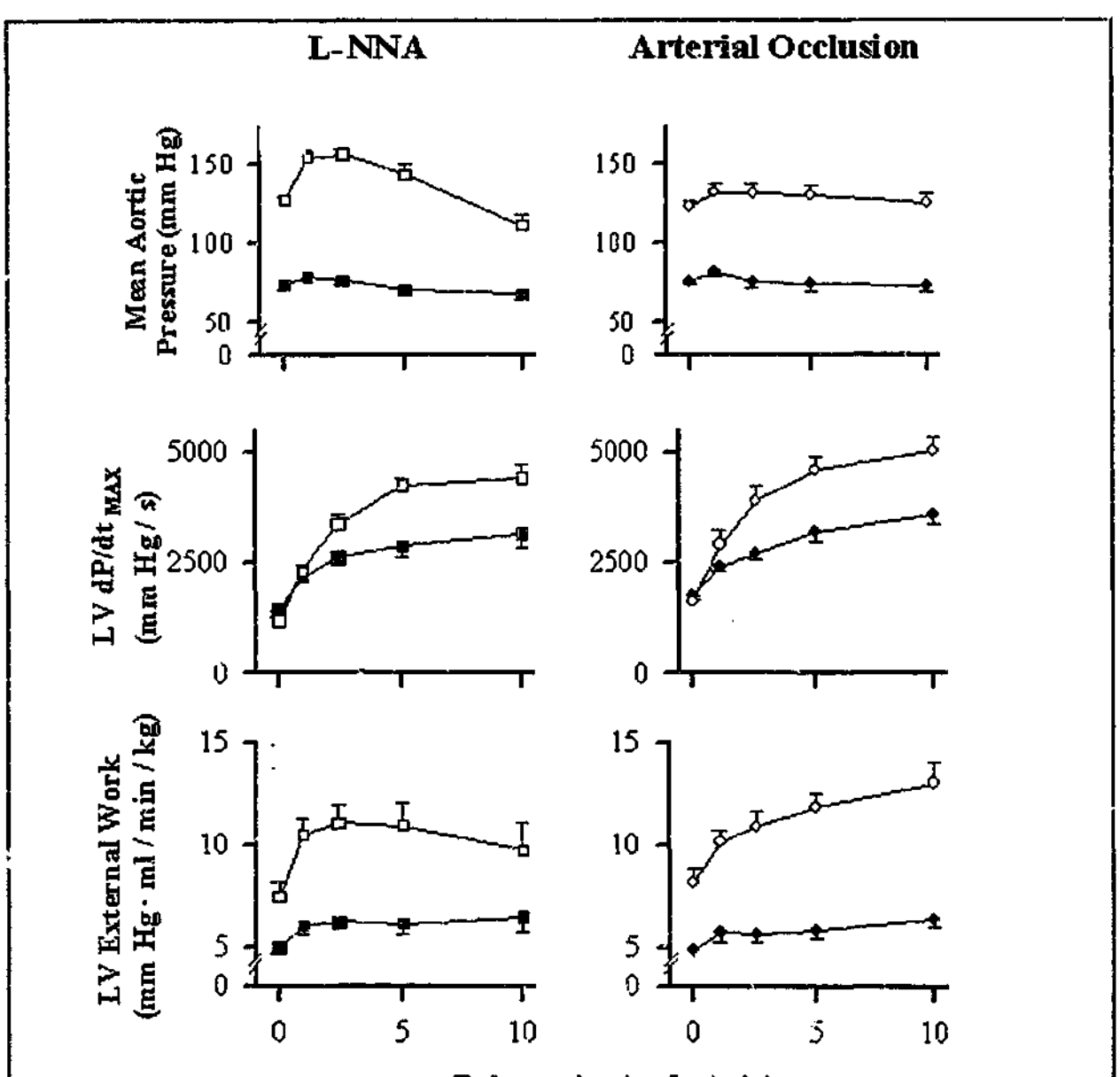


***Figure 1:4.*** *Arterial pressure, LV $dP/dt_{MAX}$ and LV external work in anaesthetised, open-chested sheep during dobutamine infusion before (closed symbols) and after (open-symbols) systemic NOS inhibition with intravenous L-NNA (left-hand panel) or partial aortic occlusion (right-hand panel). From Penny et al.*[65]

Finally, NO is a potent regulator of cellular metabolism.[66,67] NO synthase has been identified in the mitochondria of heart, skeletal muscle, and kidney where it is

thought to play a central role in the regulation of oxidative phosphorylation.[68,69] Nitric oxide has been shown to blunt respiration in isolated mitochondria through inhibition of cytochrome oxidase[70,71] and exposure of hepatocytes to NO led to inhibition of mitochondrial aconitase, NADH-ubiquinone oxidoreductase, and succinate-ubiquinone oxidoreductase (complexes I and II of the mitochondrial electron transport chain).[72] Conversely, inhibition of NO synthesis has been shown to increase whole body $O_2$ consumption[73] and $O_2$ consumption in skeletal muscle.[74] Nonetheless, at least in adult sheep, while systemic inhibition of NO synthesis increases resting systemic $O_2$ consumption, it does not alter the changes in systemic $O_2$ consumption or delivery during subsequent dobutamine infusion[27] ***(Figure 1:5).***

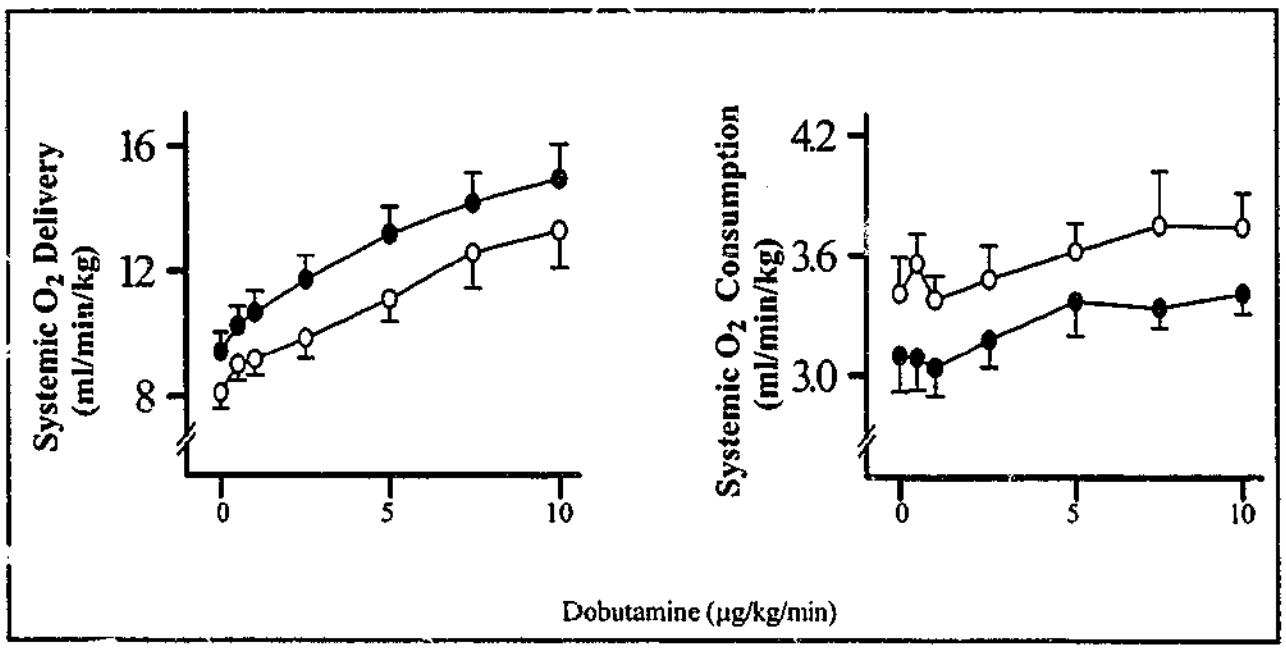


***Figure 1:5.*** *Systemic $O_2$ Delivery and Consumption in anaesthetised, open-chested sheep during dobutamine infusion before (closed symbols) and after (open-symbols) systemic NOS inhibition with intravenous L-NNA. While L-NNA altered both resting $O_2$ delivery and consumption, it did not alter the changes during subsequent dobutamine infusions. (From Penny et al.[27]).*

### *1.3. Clinical Use of Adrenergic Agents in The Neonate.*

The need to apply inotropic therapy to the neonate arose from two parallel, but interdependent milestones. First, the successful introduction of assisted ventilation in

the preterm infant stimulated the development of the sub-specialty of neonatology. Hypotension and acidosis affect approximately 40% of extremely premature neonates,[75] and are important determinants of morbidity and mortality in this fragile group. Inotropes are administered to approximately 50% of these patients.[75] In the term infant, transient myocardial ischaemia is a common consequence of perinatal asphyxia and sepsis. The resultant myocardial dysfunction and shock can produce profound circulatory disturbance and can present a difficult management challenge in terms of pharmacotherapy for the neonatologist.

The second development was the advent of paediatric cardiac surgery. Indeed, it was in this patient group that inotropes were first used in children. In 1978, Driscoll et al. first described the use of dopamine to treat circulatory shock in 24 children, ranging in age from 2 days to 18 years. Eighteen were studied after cardiac surgery, 2 had unoperated congenital heart disease and 4 had severe infection. Thirteen patients responded favourably, with an increase in arterial pressure and urine output, four did not respond and 7 had an equivocal response. Nine of the 20 "responders" survived.[76] As neonatal cardiac surgery and post-operative intensive care has advanced, with the introduction of increasing complex surgical procedures in even the tiniest of neonates, the use of inotropic agents has become almost ubiquitous.

Despite the widespread use of both dopamine and dobutamine in neonatal intensive care, there is little consensus as to the indications for their use, whether one agent has superior therapeutic properties over the other, optimal dosing regimens and end-points of therapy. Inotropes are widely used to treat systemic arterial hypotension in the preterm neonate, as in these fragile infants it is known that systemic hypotension is associated with periventricular haemorrhage and poor neurodevelopmental

outcome.[77-80] The rationale for aggressively treating systemic hypotension in this group has been to preserve adequate organ perfusion, in particular, cerebral blood flow. However, there is little consensus among neonatologists as to what is a 'normal blood pressure', or how (if at all) this reflects organ perfusion. Indeed, some studies have suggested that in fact, blood pressure is a poor correlate of cardiac output[81] and that in many preterm infants cerebral perfusion may be independent of systemic blood pressure.[82] Furthermore, our own data suggest that increases in systemic arterial pressure after dopamine administration may not be indicative of increases in either cardiac output or cerebral blood flow[83] ***(Figure 1:6)***.

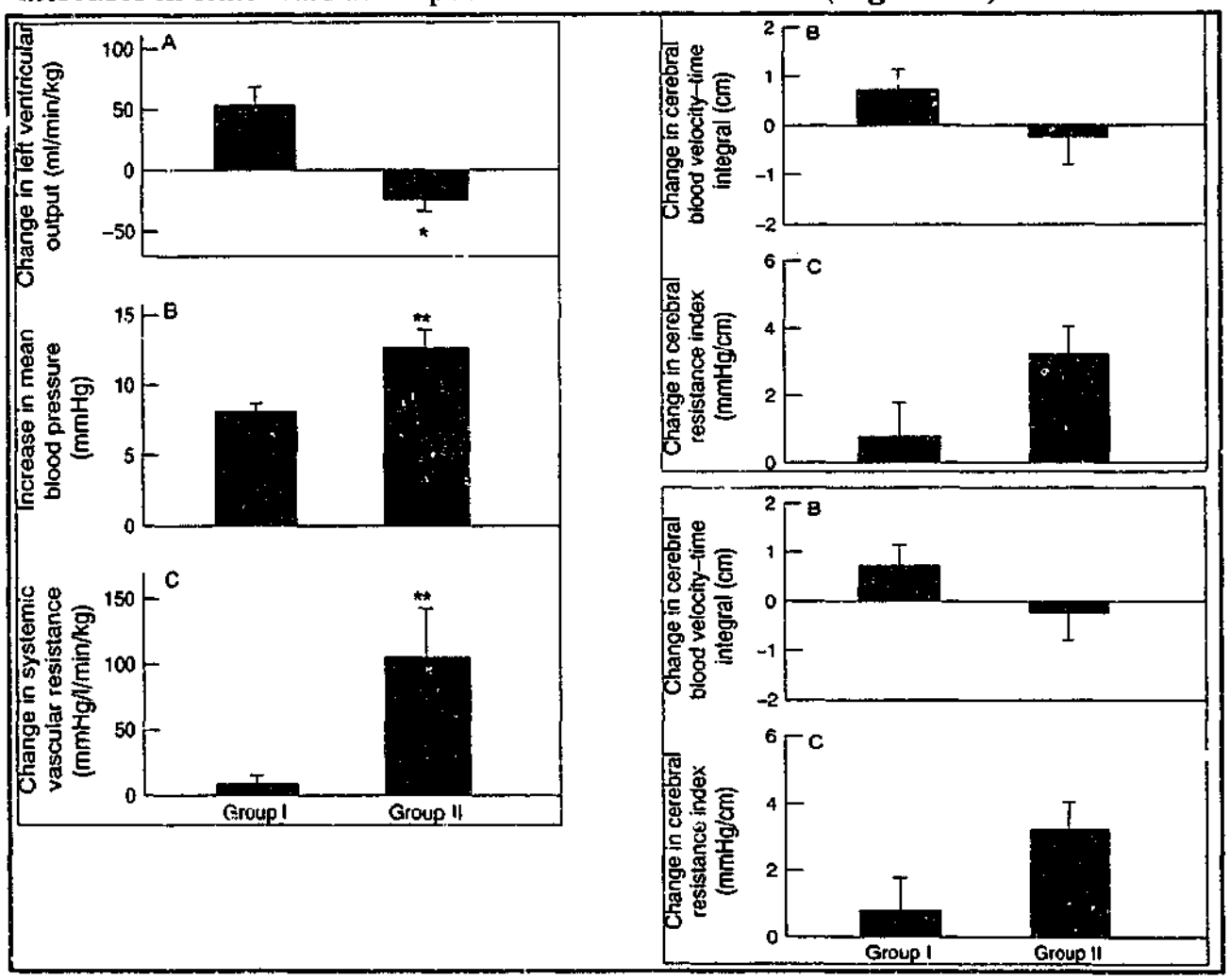


***Figure 1:6*** *Changes in left ventricular output, arterial pressure and systemic vascular resistance during dopamine infusion in preterm infants (left-hand panel). The dopamine-induced increase in arterial pressure was associated with an increase in ventricular output in Group I infants, and a reduction in Group II. In the former, dopamine increased mesenteric artery and cerebral artery velocity-time-integral, suggesting and increase in flow (right-hand panel), while in the latter, a reduction occurred. From Zhang et al.*[83]

Given these caveats, a recent systematic review comparing dopamine and dobutamine in the treatment of preterm infants with systemic hypotension concluded that there was no evidence of a significant difference between dopamine and dobutamine in terms of neonatal mortality, incidence of periventricular leukomalacia or severe periventricular haemorrhage. It was suggested that dopamine was more successful than dobutamine in treating systemic hypotension, with fewer infants having treatment failure. However, there was no evidence of a significant difference in change in left ventricular output when dopamine was compared with dobutamine.[84]

In the term infant, perinatal asphyxia is an common cause of hypotension and myocardial dysfunction,[85] and is associated with significant mortality and morbidity. Dopamine is widely used in this patient group with the goal of improving cardiac output and preventing death and longterm sequalae.[86,87] However, there is once again insufficient evidence from randomised trials to indicate whether inotropes improve outcome in these infants.[87]

In the infant after open-heart surgery, inotropes are used almost ubiquitously to aid separation from bypass and to optimise systemic haemodynamics in the early post-operative period. Bypass itself, along with hypothermia and aortic cross-clamping, can result in profound circulatory disturbances in the early hours after surgery, with the natural 'nadir' of cardiac output being reached between 6 and 12 hours after separation from bypass.[88] Again, there is very little consensus as to the optimal approach to inotropic therapy at this critical time, with local protocols dictating practice in most cases.

## *1.4 Potential Developmental Changes Influencing The Effects of Inotropic Agents on Neonatal Integrated Physiology*

Even during the very early experience of inotropes in the newborn, it was acknowledged that their actions might be considerably different in this patient group. Indeed in the correspondence which followed Driscoll's initial paper, Kliegman[89] advised caution in the use of inotropes in the newborn because of potential developmental considerations which could alter their clinical effects. However, despite extensive clinical experience, the importance of these developmental influences remains controversial. These developmental influences on the effects of inotropes include changes in cardiac structure and function, changes in vascular responses and structure, alterations in the matching between myocardial $O_2$ delivery and consumption, in systemic $O_2$ metabolism and in the regulation and action of NO.

While it is clear that considerable change occurs in cardiac structure and function during fetal development,[90] data relating to maturity of the heart after birth is quite inconsistent. It is likely that this diversity reflects, at least in part, the wide variety of species studied, all of which appear to demonstrate different maturational rates in the early postnatal period. Thus, studies of intact and isolated myocardium in cats, rats and rabbits indicate that force generation remains below adult levels for a number of weeks after birth, associated with reduced numbers of β-adrenoreceptors, as well as altered function of the sarcoplasmic reticulum and organisation of myofibrils.[91-94] However, while some studies in lambs, the most widely studied species for the examination of perinatal cardiovascular physiology have demonstrated a reduced mechanical performance of the left ventricle during the early postnatal period, that

appeared to correlate with immaturity of myosin ATPase activity,[95] others have demonstrated an elevated resting left ventricular contractility in the early neonatal period, which progressively decreased as postnatal development progressed.[96]

The effects of postnatal development on the contractile responses to inotropic stimulation are also controversial, and appear to be to an extent species-dependent. The observation of a blunted response of in vitro neonatal rabbit myocardium to isoprenaline administration, in contrast with a normal response to forskolin (a direct activator of adenyl-cyclase) may give some important insight into some of the observed maturational differences. These findings suggest that while myocardial adenyl-cyclase is well developed in the neonatal heart, its coupling with the β-adrenergic receptor may be functionally incomplete resulting in a reduced responsiveness to adrenergic stimulation.[97] Other studies demonstrated that inotropic responses to isoprenaline in isolated papillary muscle were fully developed in neonatal rats [98], but reduced in muscle from neonatal dogs.[99] In vivo studies are equally inconsistent, with one study demonstrating a reduced cardiac output response to isoprenaline in younger lambs,[100] while another observed a similar cardiac output response to the same agent in younger lambs, but a reduced contractile response.[96] A third study demonstrated no change in the contractile response to isoprenaline during postnatal development in piglets.[101]

A second mechanism for an altered overall response to dobutamine or dopamine in the young neonate may lie within the vasculature. For example, the systemic vasodilator response to adrenergic stimulation with isoprenaline was considerably less in neonatal lambs than adult sheep[102] and in rabbit aortic strips, the relaxation

response to isoprenaline was considerably blunted and increased progressively during the first month of life.[103] Important changes also occur in the pulmonary vasculature after birth. The density of pulmonary vascular α-adrenoceptors is high in the fetal and early newborn periods and declines during postnatal development, while the number of β-adrenoceptors rises progressively after birth.[104,105] The pulmonary arteries are highly muscularized at birth,[106] presumably as a legacy of the high *in utero* pulmonary arterial pressures,[107] but this muscularization recedes in the first few weeks of life.[106] Finally, the capillary bed in the lungs of newborn lambs is almost fully perfused at rest and has only a limited capacity for additional microvascular recruitment with rises in pulmonary blood flow.[108]

There are no data which assess the degree to which the ability to match increases in myocardial $O_2$ consumption and delivery during inotropic stimulation is developed in the very young neonate. However, work from this laboratory has indicated that the substantial elevation in left ventricular myocardial $O_2$ consumption apparent during the normal perinatal transition is dependent on an increase in myocardial $O_2$ extraction, as the concomitant increases in myocardial blood flow are only modest and can not alone meet this increase in demand.[109] This suggests that at this critical time, the balance between myocardial $O_2$ delivery and consumption could become easily jeopardised. Furthermore, it has been suggested that the coronary vasodilator response to adenosine, an important vasodilator metabolite which is thought to play an important role in the coronary vasodilator response to inotropes in the adult, may be blunted in the neonate.[110]

There is also considerable potential for developmental modulation of the changes in systemic $O_2$ metabolism during inotropic stimulation. This is of particular importance because, especially in precocial mammals (i.e. those which are well-developed at birth), neonatal survival is in part dependent on the maintenance of body temperature by non-shivering thermogenesis occurring in brown adipose tissue (BAT).[111-113] Such thermogenesis is normally activated by release of the catecholamine noradrenaline from sympathetic nerves within BAT[114,115] and is accompanied by substantial rises in $O_2$ consumption and body temperature.[116] The thermogenic properties of BAT relate to the presence of a unique 'uncoupling protein', which is located on the inner membrane of BAT mitochondria and which uncouples oxidative phosphorylation, thereby producing heat rather than ATP.[117-120] However, it is unknown whether dobutamine or dopamine have a thermogenic action in newborn animals, and to what extent any such action impacts on the relative alterations in systemic $O_2$ delivery and consumption.

Finally, there are only limited data, which address the role of nitric oxide in mediating responses to catecholamines in the young neonate. Again, one might speculate that these regulatory effects of NO may undergo considerable developmental change. For example, the role of NO in the regulation of pulmonary vascular function undergoes profound alterations in the transitional circulation[121-123] and NO plays a pivotal role in the regulation of the function of and blood flow through brown adipose tissue.[66,124-126]

### *1.5 The Purpose of This Thesis.*

Given the widespread use of inotropes in the young infant, against a background of limited information concerning their effects on integrated cardiovascular function, this thesis will develop a model for the assessment of integrated cardiovascular physiology in the neonatal lamb. It will examine the effects of the most commonly used inotropes, dobutamine and dopamine in this model.

# CHAPTER 2 METHODS

"When I first gave my mind to vivisections, as a means of discovering the motions and uses of the heart... I found the task so truly arduous, so full of difficulties, that I was almost tempted to think with Fracastorius, that the motion of the heart was only to be comprehended by God"

William Harvey.[127]

## *2.1 Study Objectives*

The aim of this work was to examine the effects of inotropic agents on integrated cardiovascular physiology in the neonate. A significant challenge therefore was to develop a model in which it would be possible simultaneously to study all of the following physiological variables in the young neonate:

- Left ventricular contractility
- Left ventricular myocardial blood flow, $O_2$ delivery and consumption
- Systemic and Pulmonary Vascular Resistance
- Systemic $O_2$ delivery and consumption

## *2.2 Choice of Experimental Animal*

Studies were performed in lambs, the species which is most commonly used for the study of perinatal cardiovascular physiology and has been employed in the classic studies of Barcroft,[128] Barclay,[129,130] Dawes,[131,132] and Rudolph.[100,107,133,134] The lamb shares many of the characteristics of the human perinatal circulation, with a similar birthweight, in utero dominance of the right ventricle, and an elevated pulmonary vascular resistance in the early neonatal period.[107] Lambs also have in common with humans a reduced myocardial tension development compared to adults,[135-137] as well as developmental changes in the expression of contractile proteins and in ventricular compliance characteristics.[138] Another important similarity with humans is the abundance of brown adipose tissue at birth and its marked reduction by the end of the first week of life.[114,139]

### *2.3 Ethical Considerations*

All experiments were performed in accordance with the guidelines of the National Health and Medical Research Council of Australia and were approved by the Monash University Animal Experimentation Ethics Committee.

### *2.4 Acute Surgical Preparation.*

For the purpose of this thesis, 76 lambs, of which 50 were between 1 and 2 days and 26 between 7 and 10 days old, were anesthetized with an intramuscular injection of ketamine ($5mg.kg^{-1}$) and xylazine ($0.1mg.kg^{-1}$), and an intravenous bolus of α-chloralose ($25\text{-}50mg.kg^{-1}$). Anaesthesia was maintained with a continuous intravenous infusion of α-chloralose ($12\text{-}25mg.kg^{-1}.hr^{-1}$; Appendix A). Animals were intubated with a cuffed endotracheal tube and ventilated with oxygen-enriched air using a large animal respirator (model 607, Harvard Apparatus Co., Dover, Mass.).

Ventilation was adjusted to maintain arterial oxygen ($O_2$) tension between 100 and 120 mm Hg, and arterial carbon dioxide ($CO_2$) tension between 35 and 40 mm Hg. Blood acid-base status and gas exchange was monitored with regular arterial blood gas determinations, and significant base deficits were corrected with sodium bicarbonate as required. Body temperature was maintained between 38.5 and 40°C with a combination of a heating pad and abundant towel covering.

The neck was incised in the midline and polyvinyl catheters were advanced through the right external jugular vein into the superior vena cava for fluid and drug infusion.

A left thoracotomy was performed in the fourth intercostal space, and the fourth and fifth ribs were then sectioned anteriorly and posteriorly to increase exposure of the heart and great vessels. The left hemiazygous vein was ligated near the anterior margin of the thoracic aorta, taking care not to damage any adjacent cardiac sympathetic nerve fibers. The proximal portion of the hemiazygous vein was cannulated with a silastic catheter (internal diameter 1.5 mm), which was advanced to the origin of the coronary sinus for blood sampling.[140,141] In the 1-2 day-old animals, the ductus arteriosus was ligated. A Teflon cannula was inserted through an adventitial purse-string suture into the proximal descending aorta and connected to a polyvinyl extension tubing for blood sampling and pressure measurement. After incision of the pericardium, an ultrasonic, perivascular flow probe (10-12 mm diameter; Transonics Systems Inc., Ithaca, New York) was placed around the ascending aorta to measure left ventricular output (Appendix C). The thermistor portion of a Swan-Ganz catheter (model 93A-754H-7.5F, Baxter Healthcare Corp., IL) was inserted through a purse-string suture in the distal part of the pulmonary trunk for measurement of cardiac output by thermodilution (Appendix D) and a Teflon cannula was inserted through an adventitial purse-string suture into the distal part of the pulmonary trunk and connected to a polyvinyl extension tubing for blood sampling and pressure measurement. A catheter was passed into the left atrial cavity via the appendage for pressure measurement, while a 1.5F micromanometer-tipped catheter (Model MPC-500) (Millar Instruments, Houston, TX) was inserted through the roof of the left atrium and passed across the mitral valve into the LV cavity to measure LV pressure. The circumflex coronary artery was dissected free of surrounding tissue near its origin and enclosed within a 2 mm diameter perivascular

ultrasonic flow probe (model 2SS, Transonics Systems, Ithaca, New York). The edges of the pericardial incision were then loosely re-approximated using a continuous suture ***(Figure 2:1)***.

The experimental preparation is depicted in the schematic diagram (*Figure 2:1*) and allowed measurement of:

- Arterial blood gas tensions and pressure
- Pulmonary arterial gas tensions and pressure
- Coronary sinus blood gas tensions
- Pulmonary blood flow by thermodilution
- Left atrial pressure
- Left ventricular pressure and
- The rate of rise of LV pressure (LV dP/dt)
- Left ventricular output (aortic flow)
- Circumflex coronary artery blood flow

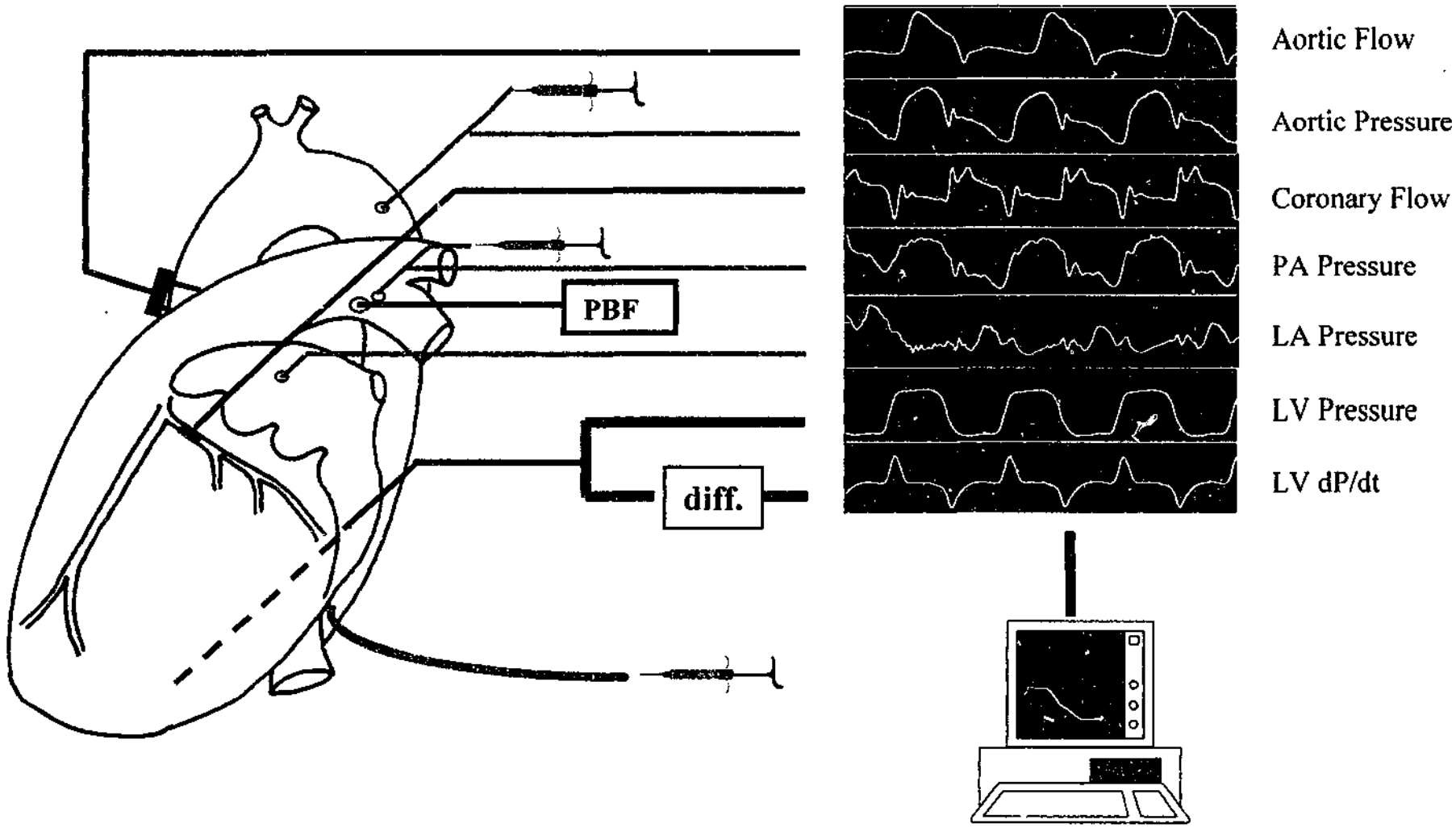


***Figure 2:1.*** *Instrumentation of the anaesthetised, open-chested lamb. All haemodynamic variables were acquired at 1000Hz and stored on computer for later off-line analysis. Pulmonary blood flow (PBF) was measured by thermodilution and the LV pressure signal was differentiated on-line (diff.). Blood samples were obtained from the aorta, pulmonary artery and coronary sinus for blood gas analysis.*

## *2.5 EXPERIMENTAL PROTOCOLS*

After completing surgery and instrumentation, haemodynamics were monitored for at least 15 min to ensure a cardiorespiratory steady state. Baseline haemodynamic variables and pulmonary blood flow (thermodilution) were measured and blood samples obtained anaerobically from the aortic, pulmonary arterial and coronary sinus catheters for blood gas analysis. Animals were then assigned to one of five protocols.

***Protocol 1. Incremental infusion of dobutamine.*** In seven, 1-2 day and eleven 7-10 day animals, dobutamine (David Bull, Victoria, Australia; Appendix B) was infused continuously into the superior vena cava in incremental steps of 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 30 and 40 $\mu g.kg^{-1}.min^{-1}$ using a roller pump (model MS 4-Reglo, Ismatec SA, Zürich, Switzerland). After steady-state conditions had been attained 5-10 min into each dobutamine dose, haemodynamics, pulmonary blood flow and blood gas measurements were repeated and the dobutamine infusion was then increased to the next dose.

***Protocol 2. Incremental infusion of dobutamine after selective adrenoreceptor blockade.*** In order to characterise the role of $\alpha_1$, $\beta_1$ and $\beta_2$ adrenoceptor activation on dobutamine responses in the immediate newborn period, nineteen 1-2 day lambs, incremental infusion of dobutamine up to $40\mu g.kg^{-1}.min^{-1}$ was performed after selective adrenoreceptor blockade (Appendix C) with the following agents:

*1)* the $\alpha_1$ adrenoreceptor antagonist prazosin (Sigma), 0.2 mg/kg intravenous bolus, followed by an intravenous infusion at a rate of 1 $mg.kg^{-1}.hr^{-1}$ (n=3),

*2)* the $\alpha_2$ adrenoceptor antagonist yohimbine (Sigma), 1mg/kg intravenous bolus, followed by an intravenous infusion at a rate of 1 $mg.kg^{-1}.hr^{-1}$ (n=3)

*3)* the $\beta_1$ adrenoreceptor antagonist CGP 20712A (Ciba Geigy, Basel), 50 μg/kg intravenous bolus followed by an infusion at a rate of $50\mu g.kg^{-1}.hr^{-1}$ (n=3),

*4)* the $\beta_2$ adrenoreceptor antagonist ICI 118551 (Imperial Chemicals), i.v. bolus of 0.2 mg/kg, followed by a continuous infusion of 0.2 $mg.kg^{-1}.hr^{-1}$ (n=3), or

*5)* simultaneous $\alpha_1$, $\beta_1$ and $\beta_2$ adrenoreceptor blockade with a combination of prazosin, CGP 20712A and ICI 118551 at the above doses (n=4).

In order to simplify the preparation, these animals were not instrumented with coronary flow probes or coronary sinus catheters. Furthermore, for the purpose of clarity, the data from only selected subgroups of these animals are included in each chapter.

***Protocol 3 Incremental infusion of dopamine.*** In nine 1-2 day and eight 7-10 day animals, dopamine (Appendix G) was infused into the superior vena cava at the following incrementally increasing rates: 0.5, 1, 2.5, 5, 7.5, 10, 15, 20, 30 and 40 $\mu g.kg^{-1}.min^{-1}$. Again, after steady-state conditions had been attained 5-10 min into each dose, haemodynamics, pulmonary blood flow and blood gas measurements were repeated and the dopamine infusion increased to the next dose.

***Protocol 4. Incremental infusion of dobutamine before and after inhibition of endogenous nitric oxide synthesis with L-NNA.*** In seven animals of each age group, dobutamine was infused continuously into the superior vena cava at the following incrementally increasing doses: 0.5, 1, 2.5, 5, 7.5 and 10 $\mu g.kg^{-1}.min^{-1}$. After steady-state conditions had been attained 5-10 min into each dobutamine dose,

measurements were repeated and the dobutamine infusion increased to the next dose. After completing measurements at the highest dose, the dobutamine infusion was progressively reduced over a period of 15 min and then stopped. A 30 min recovery period, which corresponded to more than 10 dobutamine circulating half times,[142,143] was then allowed to ensure adequate clearance of the dobutamine from the circulation.

Following the recovery period, NO synthesis was inhibited with the long-acting, stereospecific NO synthase inhibitor, $N^{\omega}$-nitro-L-arginine[144-147] (L-NNA; Sigma Chemical Co., St Louis MO). L-NNA was administered intravenously over 15 min at a dose of 25 mg/kg. This dose was associated with maximal haemodynamic response in our experimental preparation (Appendix E). Haemodynamics were allowed to stabilize for 10-15 min, after which measurements were repeated. A second incremental infusion of dobutamine was commenced and the measurement protocol repeated as before. Experiments were also performed to confirm the reproducibility of repeated dobutamine infusions (Appendix F).

***Protocol 5. Incremental infusion of dobutamine before and after partial aortic occlusion.*** In order to evaluate the contribution of an increase in aortic blood pressure per se on myocardial responses to dobutamine, in eight animals aged 1-2 days, dobutamine was infused continuously into the superior vena cava in incremental steps of 1, 2.5, 5 and 10 $\mu g.kg^{-1}.min^{-1}$ using a roller pump. After steady-state conditions had been attained 5-10 min into each dose, measurements were repeated and the dobutamine infusion increased to the next dose. After completing measurements at the highest dose, the dobutamine infusion was progressively reduced over a period of 15 min and then stopped. A 30 minute

recovery period was then allowed to ensure adequate clearance of the dobutamine from the circulation. Following the recovery period, mean arterial pressure was increased to a similar level as obtained with L-NNA by combining partial inflation of a 5F Fogarty atrial septostomy catheter (Baxter Healthcare Corp., IL, USA) which was passed into the brachiocephalic trunk via the left axillary artery, with subtotal occlusion of the descending thoracic aorta produced by tightening of a mechanical snare. Mechanical occlusion of distal segments of the central arteries was specifically chosen to increase aortic pressure because it generates a large arterial reflected wave in the latter part of systole that imposes a load on the left ventricle akin to that produced by an acute increase in peripheral resistance.[148,149] This manoeuvre therefore mimics the haemodynamic changes which would accompany pharmacological inhibition of NO synthesis.[150,151] Following partial aortic oclusion, haemodynamics were allowed to stabilize for 10-15 min, after which measurements were repeated. A second incremental infusion of dobutamine was commenced and the measurement protocol repeated as before.

At the end of each protocol, the coronary artery flow probe was removed and the animal was killed with an overdose of pentobarbitone sodium ($150mg.kg^{-1}$). The circumflex coronary artery was immediately cannulated at the site of flow probe placement, and infused with India ink solution to outline the anatomical limits of its perfusion territory. After checking for correct positioning of the tip of the coronary sinus catheter, the heart was excised and fixed in 10% buffered formol saline for 7-10 days. The stained portion of left ventricular myocardium was later carefully excised from the heart, and its weight used to normalize blood flows measured in the circumflex coronary artery. The normalized circumflex coronary flow was in turn

used to calculate total myocardial blood flow to the left ventricular free wall and left side of the interventricular septum.

### *2.6 PHYSIOLOGICAL MEASUREMENTS.*

***2.6.1 Haemodynamics.*** Aortic, pulmonary arterial and left atrial pressures were measured with silicon chip pressure transducers (model CDX-111, COBE Laboratories, Lakewood CO), which were calibrated against a water manometer before each experiment. Vascular pressures were referenced to atmospheric pressure at the level of the midthoracic vertebral spines. Ascending aortic and circumflex coronary artery blood flow were measured as described in Appendix G with an ultrasonic flowmeter (model T208, Transonic Systems Inc., Ithaca, NY). The maximal rate of rise of LV pressure ($dP/dt_{MAX}$) was obtained using an on-line differentiator (Baker Institute Model 100, Baker Institute, Victoria, Australia), in which output was directly proportional to frequency ($\pm$5%) up to 1000Hz (Appendix H). The outputs from the pressure and flow transducers were amplified using an 8-channel programmable signal conditioner (Cyberamp Model 380, Axon Instruments, Foster City, CA) and displayed continuously on a direct-writing recorder (Neotrace model 800Z, Neomedix Systems, New South Wales, Australia). Following passage through a 24 Hz low-pass filter to prevent aliasing, pressure and flow signals were digitized at a sampling rate of 500 Hz for 20 sec, and the data stored on computer for subsequent off-line analysis using customized interactive software. The pulmonary artery thermistor was connected to a pulmonary blood flow computer (model 9520, Edwards Laboratory, Santa Anna, CA) and pulmonary blood flow was measured in

duplicate or triplicate by injection of 3ml boluses of 20°C 5% dextrose into a superior vena caval catheter (Appendix I).

***2.6.2 Blood gas calculations.*** Blood pH, $PO_2$, $PO_2$ and base excess were measured at the temperature recorded with the pulmonary arterial thermistor using a blood gas analyser (model ABL 500, Radiometer, Copenhagen). Blood haemoglobin content and $O_2$ saturation were measured photometrically with a hemoximeter (model OSM2, Radiometer, Copenhagen, Denmark). The $O_2$ content ($ml \cdot dl^{-1}$) of aortic ($C_{Ao}O_2$), pulmonary arterial ($C_{PA}O_2$) and coronary sinus ($C_{CS}O_2$) blood was calculated as $(1.36 \cdot HbS \cdot Hb/100) + 0.003 \cdot PO_2$, where HbS = haemoglobin $O_2$ saturation (%), Hb = haemoglobin content of blood ($g.dl^{-1}$) and $PO_2$ = $O_2$ tension (mm Hg).

***2.6.3 Derived haemodynamic variables.*** Steady-state LV external work (mm Hg · $ml.min^{-1}.kg^{-1}$) was calculated as $(P_{Ao} - P_{LA}) \cdot CO$, where $P_{Ao}$ = mean aortic pressure (mm Hg), $P_{LA}$ = mean left atrial pressure and CO = cardiac output ($ml \cdot min^{-1} \cdot kg^{-1}$). Systemic vascular resistance ($mmHg/ml \cdot min^{-1} \cdot kg^{-1}$) was calculated as $P_{AO}$ / CO. Pulmonary vascular resistance ($mmHg/ml \cdot min^{-1} \cdot kg^{-1}$) was calculated as $(P_{PA}-P_{LA})$ / CO, where $P_{PA}$ = mean pulmonary arterial pressure and LV vascular resistance (mm Hg/ml/min/100g) was calculated as $(P_{Ao} - P_{LA})$ / $Q_{LV}$, where $Q_{LV}$ = blood flow per 100g of LV myocardium.

***2.6.4 Systemic and LV myocardial $O_2$ balance.*** Systemic $O_2$ delivery ($ml \cdot min^{-1} \cdot kg^{-1}$) was calculated as $CO \cdot C_{Ao}\ O_2$, systemic $O_2$ consumption, ($ml \cdot min^{-1} \cdot kg^{-1}$) as $CO \cdot (C_{Ao}\ O_2 - C_{PA}\ O_2)$ and the $O_2$ extraction coefficient was derived as the Systemic $O_2$ consumption/Systemic $O_2$ delivery ratio. LV myocardial $O_2$ delivery ($ml \cdot min^{-}$

$^{1}\cdot 100gLV^{-1}$) was computed as $Q_{LV} \cdot C_{Ao}O_2$, LV myocardial $O_2$ consumption ($ml \cdot min^{-1} \cdot 100gLV^{-1}$) as $Q_{LV} \cdot (C_{Ao}O_2 - C_{CS}O_2)$.

### *2.7 Statistical Analysis.*

***Effect of inotropic infusion on haemodynamic and blood gas variables.***

The effects of incremental inotropic infusions on haemodynamic and blood gas variables were assessed by one-way analysis of variance for repeated measures[152]. The total variability (sums of squares, SS) was partitioned into variability "between subjects", variability "between treatments" and "residual" variability. The null hypothesis of no variation during "treatment" (inotrope infusion) was rejected if the F statistic formed by the ratio of "between treatment" mean square (MS) to "residual" MS exceeded a critical F value, (F0.05, with degrees of freedom (treatments -1), [(treatments -1) · (n-1)]. The analysis was extended by post-hoc analysis, using multiple t-tests with Bonferroni correction, which allowed comparison between individual treatments and gave the extent that each treatment contributed independently to the total sums of squares of treatments.[152]

***Effect of adrenoreceptor blockade on the responses to dobutamine.***

Because of small subgroup sizes in the experiments in which adrenoreceptor blockers were used, these studies were not subjected to statistical analysis, as statistical power was inadequate.

***Differences between dobutamine and dopamine responses.***

The differences between the maximal responses to dobutamine and dopamine were compared with unpaired t-tests, preceded by appropriate tests for normality.

***Effect of L-NNA Infusion of Haemodynamic and Blood Gas Variables.*** Differences between measurements before and immediately after L-NNA were assessed with paired t-tests, preceded by appropriate testing for normality.

***Comparison of dobutamine effects before and after either L-NNA or arterial occlusion.***

In order to compare responses to dobutamine before and after L-NNA infusion or aortic occlusion, the differences between pre- and post- LNNA or occlusion measures at each dobutamine infusion rate were calculated and analysed with repeated measures analysis of variance ***(Figure 2:2)***.

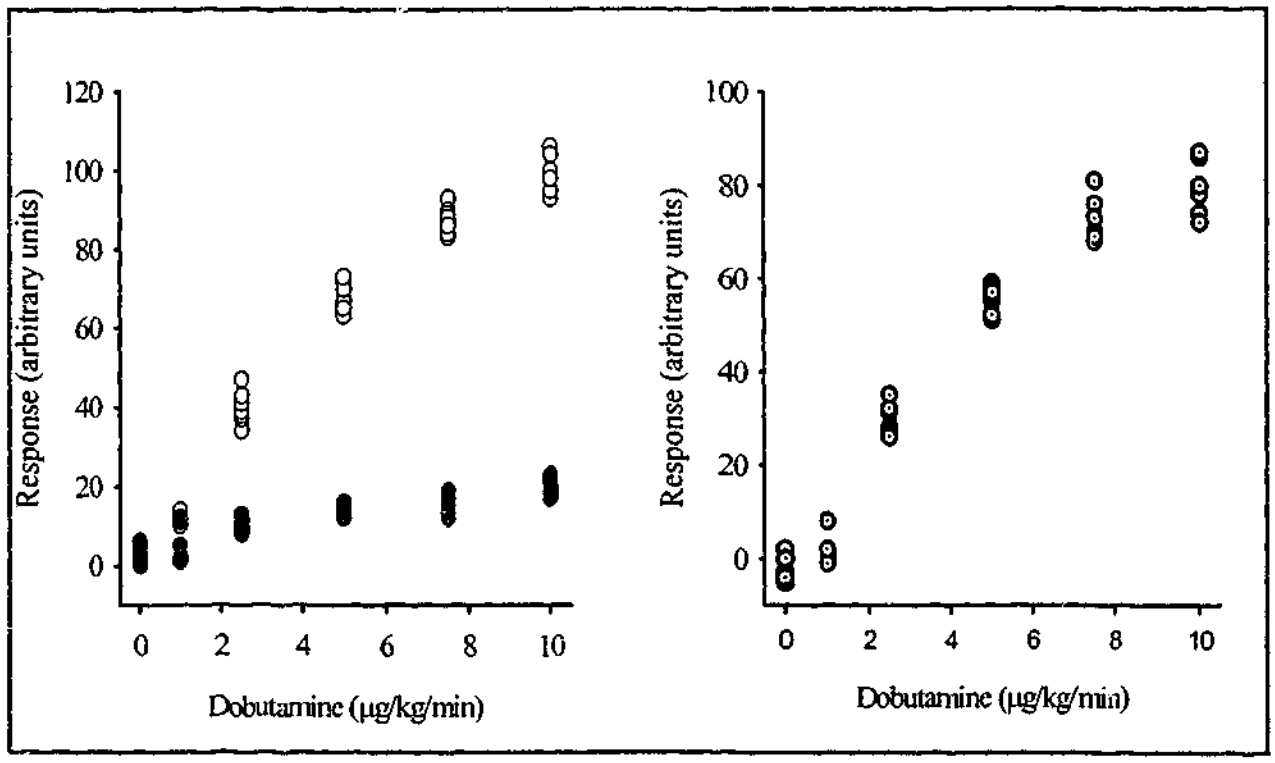


*Figure 2:2 Theoretical, individual dose-response curves to dobutamine before (●) and after (○) L-NNA infusion, (left-hand panel). In order to examine differences between pre-and post-L-NNA responses over the complete dobutamine infusion range, the differences between pre- and post- L-NNA responses for each subject at each infusion rate was plotted (right-hand panel) and analysed with ANOVA*

Throughout this thesis, results are expressed as mean ± SE and the null hypothesis was rejected at $p < 0.05$.

# CHAPTER 3 CENTRAL HAEMODYNAMICS AND LEFT VENTRICULAR PERFORMANCE

This chapter examines the changes in LV contractility, cardiac output and minute work during infusions of dobutamine and dopamine in the early postnatal period. It also evaluates the adrenoreceptor mechanisms and the regulatory role of NO in the effects of dobutamine.

There was no difference between 1-2 and 7-10 day groups in terms of the changes in cardiac performance during inotropic stimulation, but differences were apparent between dobutamine and dopamine,. While increases in heart rate, and LV performance during dobutamine stimulation in 1-2 day animals were blunted by $\beta_1$ adrenoreceptor blockade, considerable increases in cardiac output still occurred due to an increase in stroke volume. The changes in LV performance during dobutamine infusion were not altered by systemic inhibition of NO synthesis.

These results indicate that in the early days after birth, there are no major maturational changes in the central haemodynamic responses to dobutamine and dopamine. The responses to dobutamine, which appear to be predominantly related to $\beta_1$ adrenoreceptor effects, differ from those to dopamine and are not significantly modulated by systemic inhibition of NO synthesis.

## *3.1 INTRODUCTION*

By definition, one of the fundamental effects of inotropes on the myocardium is an increase in contractility. The cellular mechanisms underlying this increase in contractility during adrenergic stimulation are now well-established and the effects of inotropic stimulation on myocardial activation can be demonstrated in vivo, by examining the changes in any of a number of indices of left ventricular myocardial function.

Considerable changes occur in cardiac structure and function during fetal development, and these have already been outlined in Chapter 1. Perhaps more importantly, or at least of greater relevance in the clinical arena, are the less than clear-cut changes in the contractile response to inotropic stimulation which occur during early extra-uterine life. Indeed, there exists a degree of controversy in the literature regarding the nature of these changes and their pattern of clinical expression in the early neonatal period.

A blunted response of *in vitro* neonatal rabbit and canine myocardium to isoprenaline administration has been observed by some investigators,[97,99] whereas others demonstrated that the inotropic response to this agent was fully developed in isolated papillary muscle from neonatal rats.[98] In vivo studies of adrenergic stimulation on contractility and cardiac output in neonatal models are equally inconsistent, ranging from reports of a reduced cardiac output,[100] a similar cardiac output response with reduced contractility[96] to a similar contractile response.[101]

Accordingly, the aim of this chapter was to use our experimental preparation to explore the changes in left ventricular contractility, cardiac output and left

ventricular work during infusions of the inotropes dobutamine and dopamine in the early postnatal period. An additional goal was to examine the receptor mechanisms responsible for, and the regulatory role of nitric oxide, in modulating the effects of dobutamine.

## *3.2 Materials And Methods*

***Surgical preparation.*** Seventy lambs, of which 47 were aged 1-2 days and weighed 4.6±0.1kg and twenty-three were aged 7-10 days and weighed 6.2±0.3 kg, were surgically prepared under general anaesthesia, as described in Chapter 2. As part of the preparation, a teflon cannula was inserted through an adventitial purse-string suture in the descending thoracic aorta and a silastic catheter was advanced into the left atrium for pressure measurement. A 2F micomanometer-tipped catheter was placed in the left ventricle for high-fidelity pressure recording and an ultrasonic, perivascular flow probe was placed around the ascending aorta.

***Experimental protocol.*** The first protocol examined the effects of an incremental infusion of dobutamine at rates up to $40\mu g.kg^{-1}.min^{-1}$ in seven 1-2 day and eight 7-10 day old animals. In a second group of sixteen 1-2 day lambs, dobutamine was infused at similar rates after adrenoceptor blockade with either the $\alpha_1$-adrenocecptor antagonist prazosin, (n=3), the $\alpha_2$-adrenoceptor antagonist yohimbine (n=3), the $\beta_1$-adrenoceptor antagonist CGP 20712A (n=3), the $\beta_2$-adrenoceptor antagonist ICI 118551 (n=3) or combined $\alpha_1$–, $\beta_1$- and $\beta_2$-adrenoreceptor blockade (n=4).

In the third protocol, which included seventeen animals, of which nine were 1-2 day and eight were 7-10 days old, the effects of an incremental intravenous infusion of dopamine at rates up to 40 $\mu g.kg^{-1}.min^{-1}$ were studied.

In fourteen animals, of which seven were 1-2 days and seven, 7-10 days old, the responses to an incremental infusion of dobutamine at rates up to $10\mu g.kg^{-1}.min^{-1}$ were studied, before and after inhibition of NO synthesis with an intravenous infusion of $N^{\omega}$–nitro-L-arginine (L-NNA) 25 mg/kg. Finally, the responses to an incremental infusion of dobutamine at rates up to $10\mu g.kg^{-1}.min^{-1}$ were studied in 8 animals aged 1-2 days, before and after applying partial occlusion of the descending thoracic aorta (as described in Chapter 2) in order to increase mean systemic arterial pressure to a similar level as obtained with L-NNA.

***Physiologic measurements and calculations.*** Cardiac output was obtained by measuring ascending aortic blood flow with an ultrasonic flowmeter. Aortic and left atrial pressures were measured with silicon-chip pressure transducers. Left ventricular minute work was derived from the blood pressure and cardiac output measurements using the equation presented in Chapter 2. The left ventricular pressure recording was differentiated on-line with a differentiator, as described in Appendix H.

## *3.3 RESULTS*

### *3.3.1 Baseline variables.*

Within each age group, baseline variables did not significantly differ between animals assigned to the different protocols. For this reason, the baseline variables for the 1-2 day and the 7-10 day animals which were entered into matched protocols (either dobutamine or dopamine alone, or dobutamine and L-NNA) were compared. Compared to 1-2 day lambs, heart rate was higher ($p<0.001$) and cardiac output and stroke volume were lower ($p<0.05$ and $p<0.001$, respectively) in 7-10 day lambs. Mean aortic pressure tended to be higher in the older animals, although the observed differences did not achieve statistical significance ($0.1>p>0.05$; ***Table 3:1).***

| | **Group** | | |
|---|---|---|---|
| | **1-2 day (n=23)** | **7-10 day (n=23)** | **P Value** |
| **Heart Rate ($beats \cdot min^{-1}$)** | 178±4 | 212±6 | <0.001 |
| **LV $dP/dt_{MAX}$ ($mmHg \cdot s^{-1}$)** | 1856±70 | 2113±118 | n.s. |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 172±7 | 142±10 | <0.05 |
| **Stroke Volume ($ml \cdot kg^{-1}$)** | 0.98±0.04 | 0.67±0.04 | <0.001 |
| **Mean Aortic Pressure (mmHg)** | 62±2.0 | 69±3 | <0.1 |
| **Mean Left Atrial Pressure (mmHg)** | 5.3±0.3 | 5.1±0.5 | n.s. |
| **LV Minute Work ($mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$)** | 9.7±0.5 | 9.0±0.7 | n.s. |

***Table 3:1****. Baseline haemodynamic variables in 1-2 day and 7-10 day animals.*

### *3.3.2 Changes in Haemodynamics During Dobutamine Infusion.*

***Heart Rate and LV dP/dt$_{MAX}$.*** Incremental infusion of dobutamine to 40μg.kg$^{-1}$.min$^{-1}$ increased heart rate by 153±3 and 113±13 beats·min$^{-1}$ and LV dP/dt$_{MAX}$ by 1824±357 and 1757±270 mmHg·s$^{-1}$ in the 1-2 day and 7-10 day groups respectively (all $p<0.001$).

***Cardiac Output and Stroke Volume.*** Cardiac output increased by 168±25 ml·min$^{-1}$·kg$^{-1}$ in the 1-2 day, and by 102±15 ml·min$^{-1}$·kg$^{-1}$ in the 7-10 day group. However, this increase was entirely attributable to the elevation in heart rate during dobutamine infusion, as the stroke volume was unchanged in both groups ***(Figure 3:1)***.

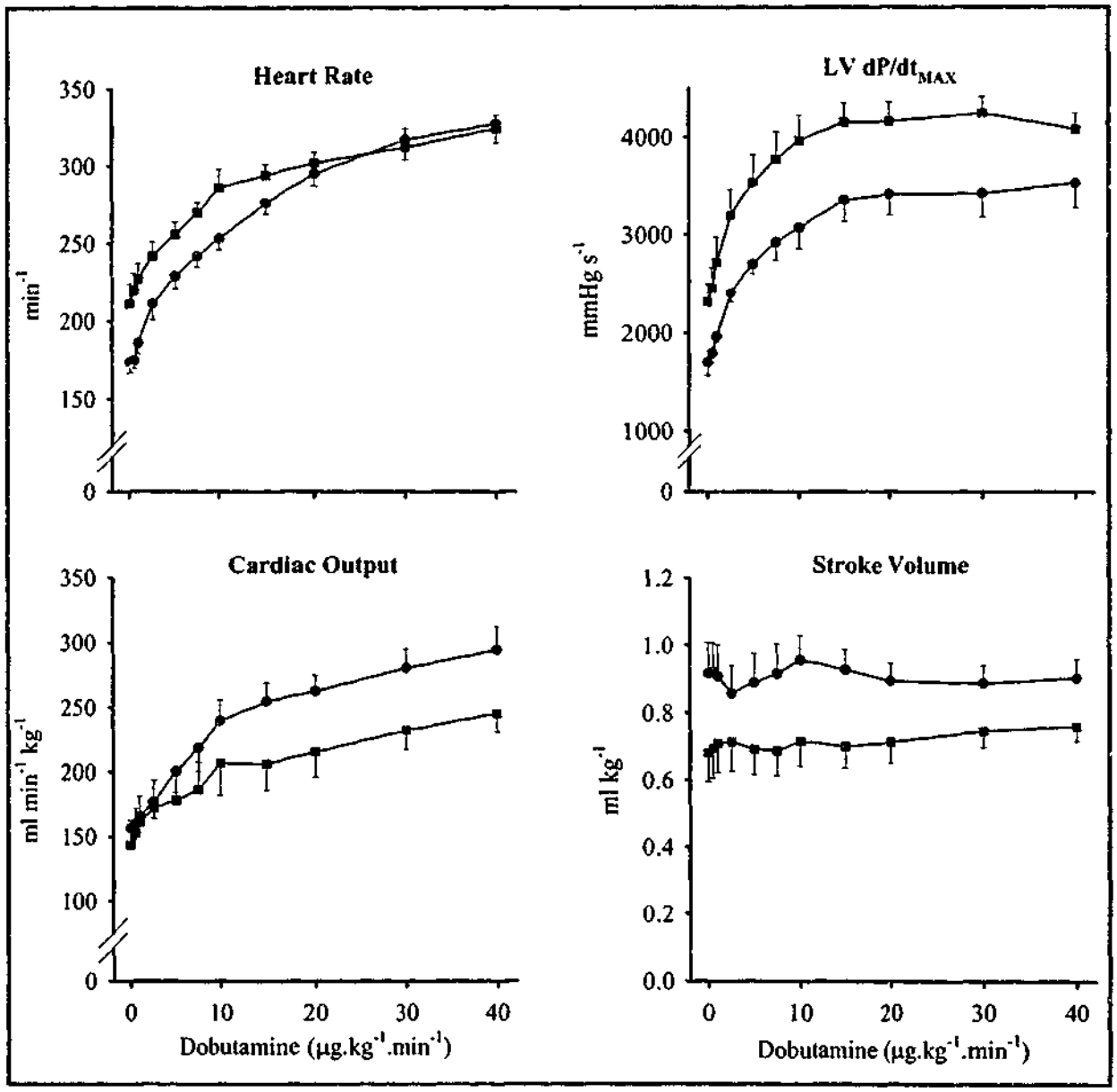


***Figure 3:1.*** *Heart rate, LVdP/dt$_{MAX}$, cardiac output and stroke volume responses during incremental infusion of dobutamine in 1-2 day (•) and 7-10 day (■) lambs.*

***Aortic, Left Atrial Pressures and LV Minute Work.*** Increasing dobutamine infusion progressively reduced mean aortic pressure in both groups to levels which were 7±5 mmHg (1-2 day) and 11±3 mmHg (7-10 day) below baseline at the peak infusion rate (both $p<0.05$). Mean left atrial pressure fell in response to dobutamine infusion at rates up to 7.5μg.kg$^{-1}$.min$^{-1}$ ($p<0.05$) but at higher infusion rates, progressively increased and reached levels similar to baseline at infusion rates exceeding >15μg.kg$^{-1}$.min$^{-1}$. As a result of the predominant increases in cardiac output, left ventricular minute work significantly increased by 5.9±2.4 and by 3.8±1.3 mmHg·ml·min$^{-1}$·kg$^{-1}$ in the 1-2 day and 7-10 day animals (both $p<0.001$; ***Figure 3:2).***

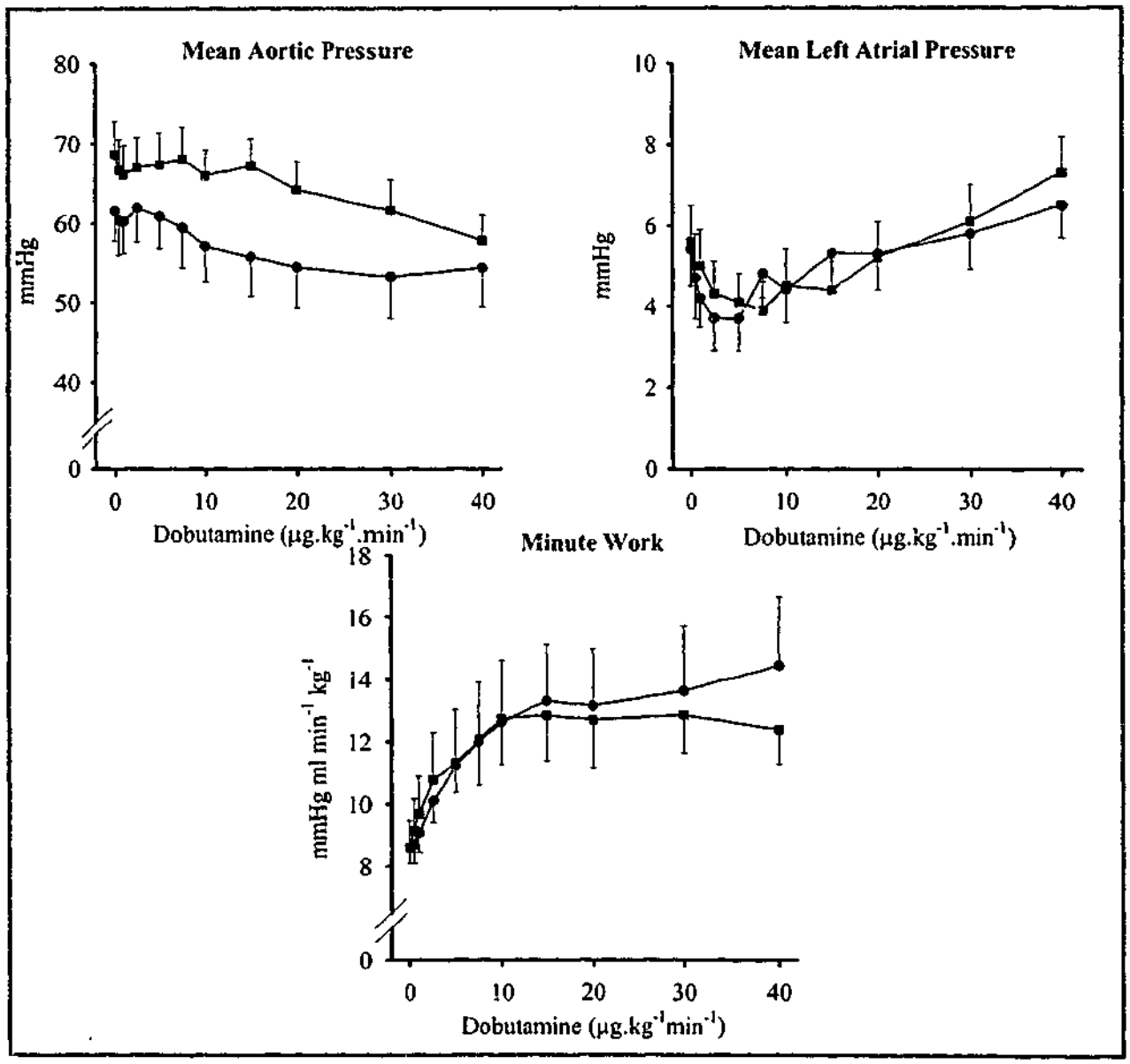


***Figure 3:2.*** *Mean aortic and left atrial pressures, and LV minute work responses during dobutamine infusion in 1-2 day* (●) *and 7-10 day* (■) *lambs.*

Overall, the changes in all haemodynamic indices measured were not significantly different in the two age groups investigated.

### *3.3.3 Effect of adrenoreceptor blockade on the responses to dobutamine*

***Effect of adrenoreceptor blockade on baseline variables*** Selective adrenoceptor blockade resulted in negligible changes in haemodynamics, apart from a 17% reduction in mean arterial pressure which followed selective $\alpha_1$ blockade, a 14% fall in LV external work which occurred after $\beta_1$ blockade and a 14% fall in $LVdP/dt_{MAX}$, combined with a 18% fall in LV external work which resulted from simultaneous blockade of the $\alpha_1$, $\beta_1$ and $\beta_2$ receptor.

| **Variable** | | **$\alpha_1$-blockade** (n=3) | **$\alpha_2$-blockade** (n=3) | **$\beta_1$-blockade** (n=3) | **$\beta_2$-blockade** (n=3) | **$\alpha_1/\beta_1/\beta_2$** (n=4) |
|---|---|---|---|---|---|---|
| **Heart Rate ($min^{-1}$)** | Before | 180±17 | 204±24 | 176±15 | 171±9 | 169±3 |
| | After | 171±20 | 213±41 | 170±13 | 166±9 | 157±4 |
| **LV $dP/dt_{MAX}$ (mm Hg $s^{-1}$)** | Before | 2242±425 | 1794±241 | 1969±254 | 2197±218 | 1662±119 |
| | After | 1902±300 | 2031±422 | 1862±260 | 2018±158 | 1441±163 |
| **Cardiac Output (ml $min^{-1}$ $kg^{-1}$)** | Before | 165±48 | 193±29 | 181±28 | 136±39 | 166±4 |
| | After | 164±46 | 178±45 | 170±27 | 156±37 | 151±6 |
| **Stroke Volume ($ml{\cdot}kg^{-1}$)** | Before | 0.9±0.2 | 0.9±0.1 | 1.0±0.1 | 0.8±0.2 | 1.0±0.0 |
| | After | 0.9±0.1 | 0.8±0.1 | 1.0±0.1 | 0.8±0.2 | 1.0±0.0 |
| **Mean Aortic Pressure (mm Hg)** | Before | 65±8 | 58±2 | 62±1 | 71±3 | 59±3 |
| | After | 53±3 | 60±4 | 56±4 | 68±1 | 54±7 |
| **Mean Left Atrial Pressure (mm Hg)** | Before | 3.2±1.3 | 3.9±0.8 | 3.9±0.3 | 3.7±0.2 | 5.3±0.1 |
| | After | 3.4±0.9 | 2.5±1.2 | 3.5±0.3 | 4.9±0.7 | 5.6±0.7 |
| **LV Minute Work (mm Hg $ml{\cdot}min^{-1}{\cdot}kg^{-1}$)** | Before | 10.1±2.9 | 10.6±2.0 | 10.6±1.8 | 9.0±2.3 | 8.8±0.6 |
| | After | 8.3±2.7 | 10.3±2.9 | 9.2±2.0 | 8.6±2.3 | 7.4±1.0 |

***Table 3:2*** *Comparison of haemodynamic variables before and after selective and combined adrenoreceptor blockade in 1-2 day lambs.*

***Effect of adrenoreceptor blockade on responses to dobutamine*** Subjective examination of the effects of dobutamine infusion in the presence of adrenoceptor blockade suggest that the $\beta_1$ receptor contributes to the increases in heart rate, and $LVdP/dt_{MAX}$ during dobutamine stimulation: these two responses were considerably blunted following $\beta_1$ blockade. Nonetheless, after $\beta_1$ blockade, considerable increases in cardiac output still occurred during dobutamine infusion, which reflected an increase in stroke volume ***(Figure 3:3)***.

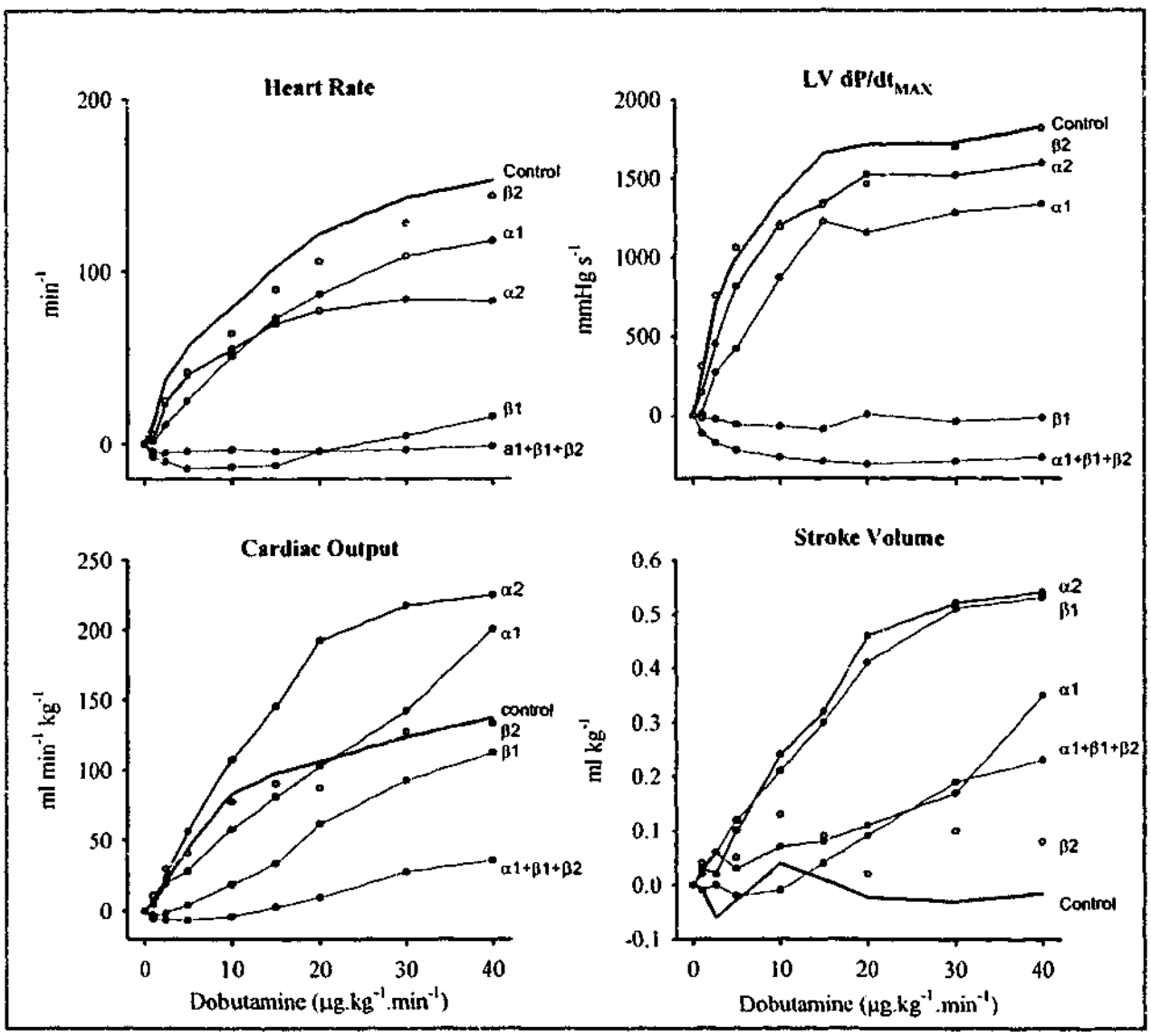


***Figure 3:3*** *Changes in heart rate, LV $dP/dt_{MAX}$, cardiac output and stroke volume in 1-2 day lambs during incremental dobutamine infusion in the absence of (control;–) or presence of $\alpha_1$ (–), $a_2$ (–), $\beta_1$ (–), $\beta_2$ ( ) or combined $\alpha_1,\beta_1,\beta_2$ (–) adrenoceptor blockade.*

The dobutamine-induced reduction in mean aortic pressure was blunted by blockade of $\beta_2$ and $\alpha_2$ receptors and the increase in LV minute work was blunted by both selective $\beta_1$ and combined blockade of $\alpha_1/\beta_1/\beta_2$ adrenoceptors ***(Figure 3:4)***.

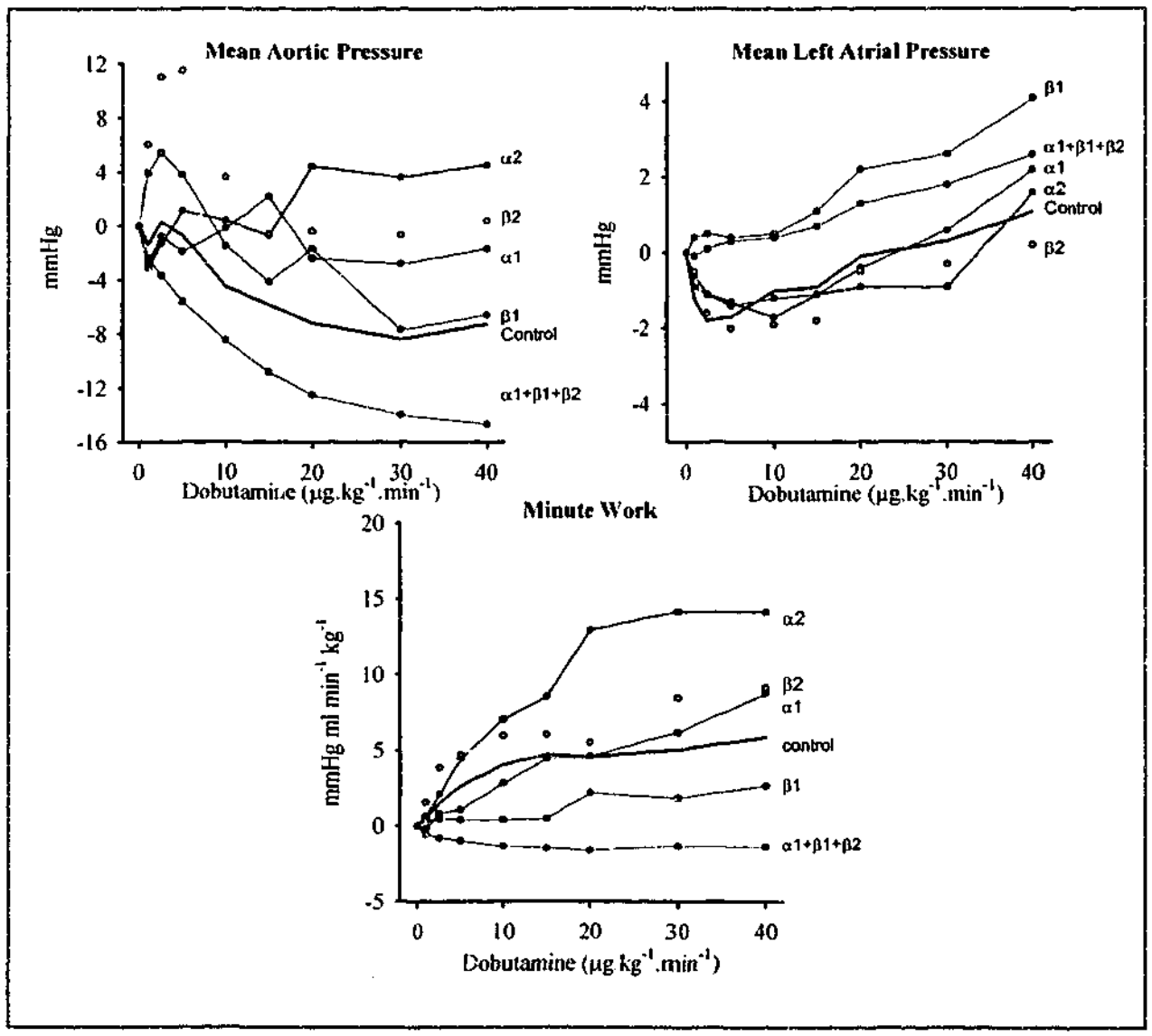


***Figure 3:4*** *Changes in mean aortic and left atrial pressures and LV minute work in 1-2 day lambs during incremental dobutamine infusion in the absence of (control:–) or presence of $\alpha_1$ (–), $a_2$ (–), $\beta_1$ (–), $\beta_2$ ( ) or combined $\alpha_1,\beta_1,\beta_2$ (–) adrenoceptor blockade.*

### *3.3.4 Changes in Haemodynamics During Dopamine infusion.*

***Heart Rate and LV dP/dt$_{MAX}$.*** Incremental infusion of dopamine increased heart rate by 109±8 and by 46±6 min$^{-1}$ in the 1-2 day and 7-10 day animals, while LV dP/dt$_{MAX}$ increased by 1888±193 and by 2533±323 mmHg·s$^{-1}$ (all $p<0.001$).

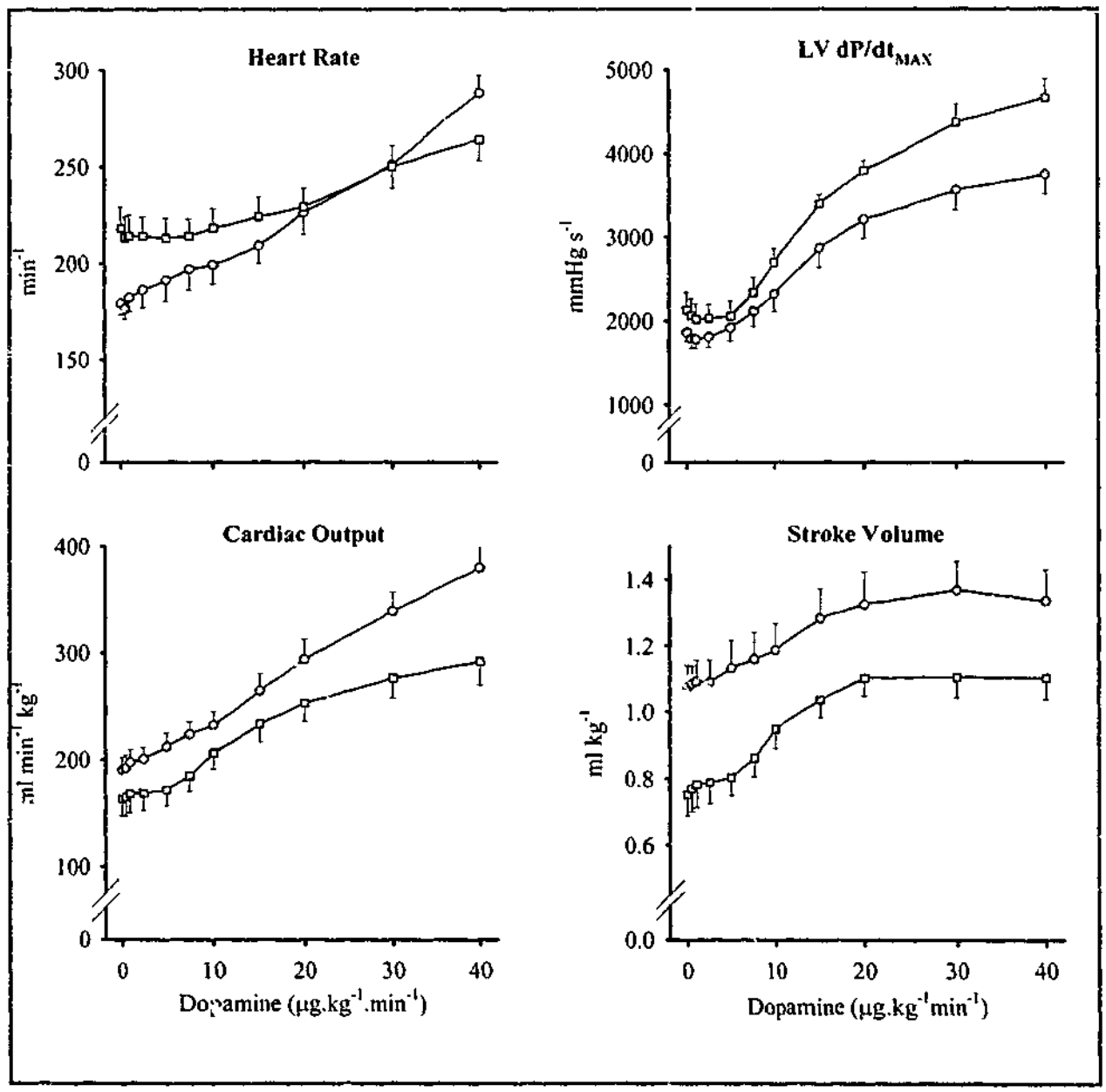


***Figure 3:5.*** *Heart rate, LVdP/dt$_{MAX}$, cardiac output and stroke volume responses during incremental infusion of dopamine in 1-2 day (•) and 7-10 day (■) lambs.*

***Cardiac Output and Stroke Volume.*** Dopamine infusion increased cardiac output by 189±17 and 159±13 ml·min$^{-1}$·kg$^{-1}$ in the younger and older animals, respectively ($p<0.001$). In contrast with dobutamine, this increase was attributed to increases in

both heart rate *and* in stroke volume (of approximately 25%) to peak levels which were 0.3±0.1 ml·kg$^{-1}$ (1-2 days) and 0.4±0.1 ml·kg$^{-1}$ (7-10 days) above baseline (both p<0.001) ***(Figure 3:5)***

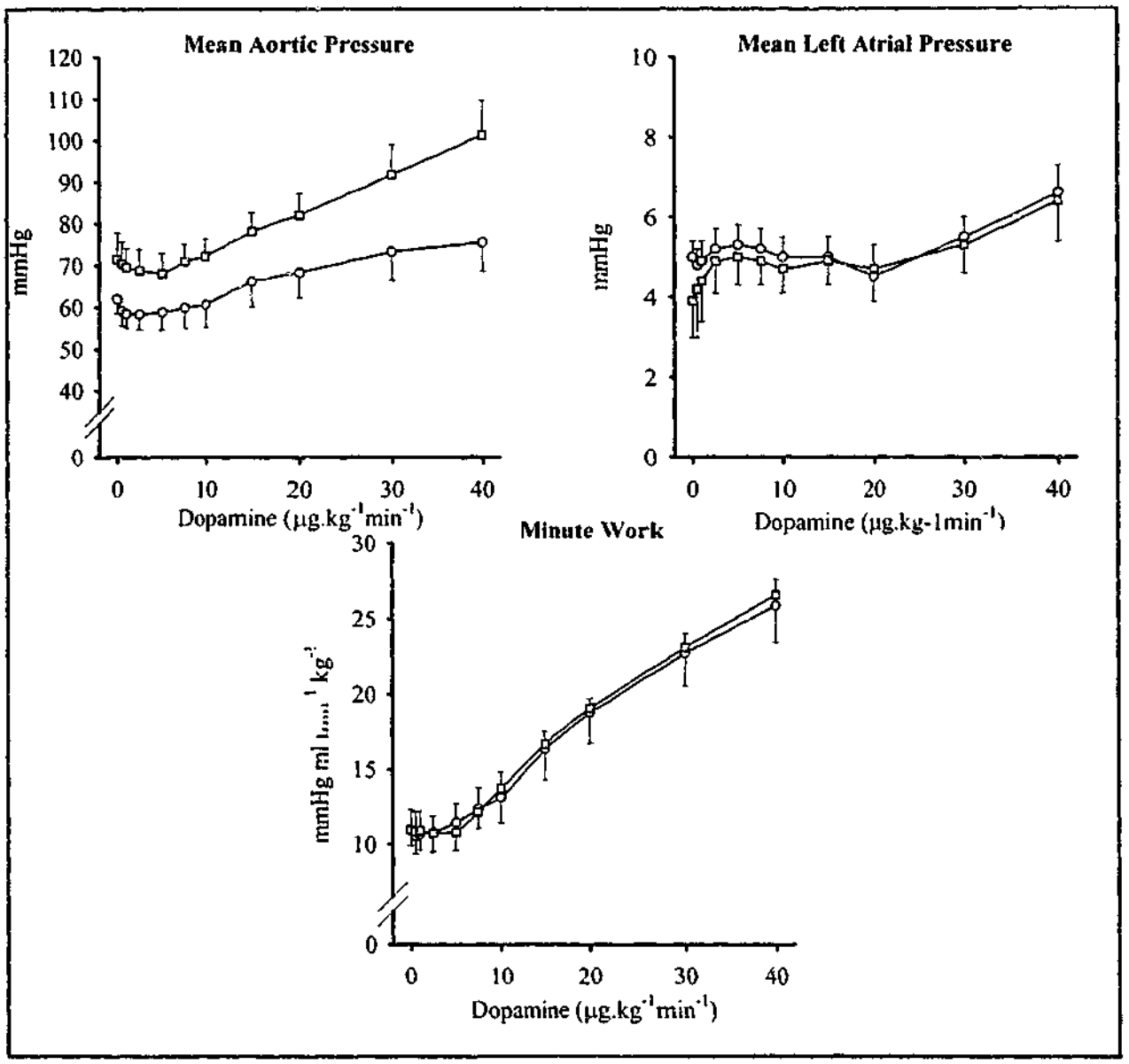


***Figure 3:6.*** *Mean aortic and left ventricular pressure and LV minute work responses during incremental infusion of dopamine in 1-2 day* (•) *and 7-10 day* (▪) *lambs.*

***Aortic, Left Atrial Pressures and LV Minute Work.*** Dopamine increased mean aortic pressure by 14±6 mmHg (1-2 day) and by 30±10 mmHg (7-10 day) (both p<0.001). Left atrial pressure was also increased (1.6±0.6 mmHg; 1-2 days and 2.5±1.0; 7-10 days; both p<0.01). The increases in cardiac output and mean arterial

pressure were reflected by elevations in left ventricular minute work output of 15.0±2.1 (1-2 days) and 15.7±1.2 $mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$ (7-10 days; both $p<0.001$; ***Figure 3:6)***

Thus, apart from minor differences in the heart rate response, which was slightly more exaggerated in the younger animals ($p<0.05$), the changes in haemodynamic indices during dopamine infusion were similar in the two age groups.

### *3.3.5 Comparison of dobutamine and dopamine responses.*

The haemodynamic responses in response to dobutamine and dopamine therefore differed in a number of ways. First, for some variables, the ***direction*** of change differed between treatments. Importantly, mean arterial pressure fell during dobutamine infusion in both groups, but increased in response to dopamine. Second, the ***magnitude*** of the peak response for some variables differed. Thus for animals receiving dopamine, the peak increase in heart rate was significantly lower ($p<0.001$ for both age groups), whereas the increase in LV minute work was significantly greater ($p<0.05$, 1-2 day and $p<0.001$, 7-10 day animals) than in response to dobutamine. Furthermore, while stroke volume was unchanged by dobutamine, it increased in response to dopamine in both groups.

Noticeably, and of relevance to the clinical setting, despite the similarity in the extent of the peak responses to both agents, it appeared that effects on most variables emerged at much lower infusion rates in animals given dobutamine, compared to those to which dopamine was administered. ***(Figure 3:7; Figure 3:8; Table 3:3).***

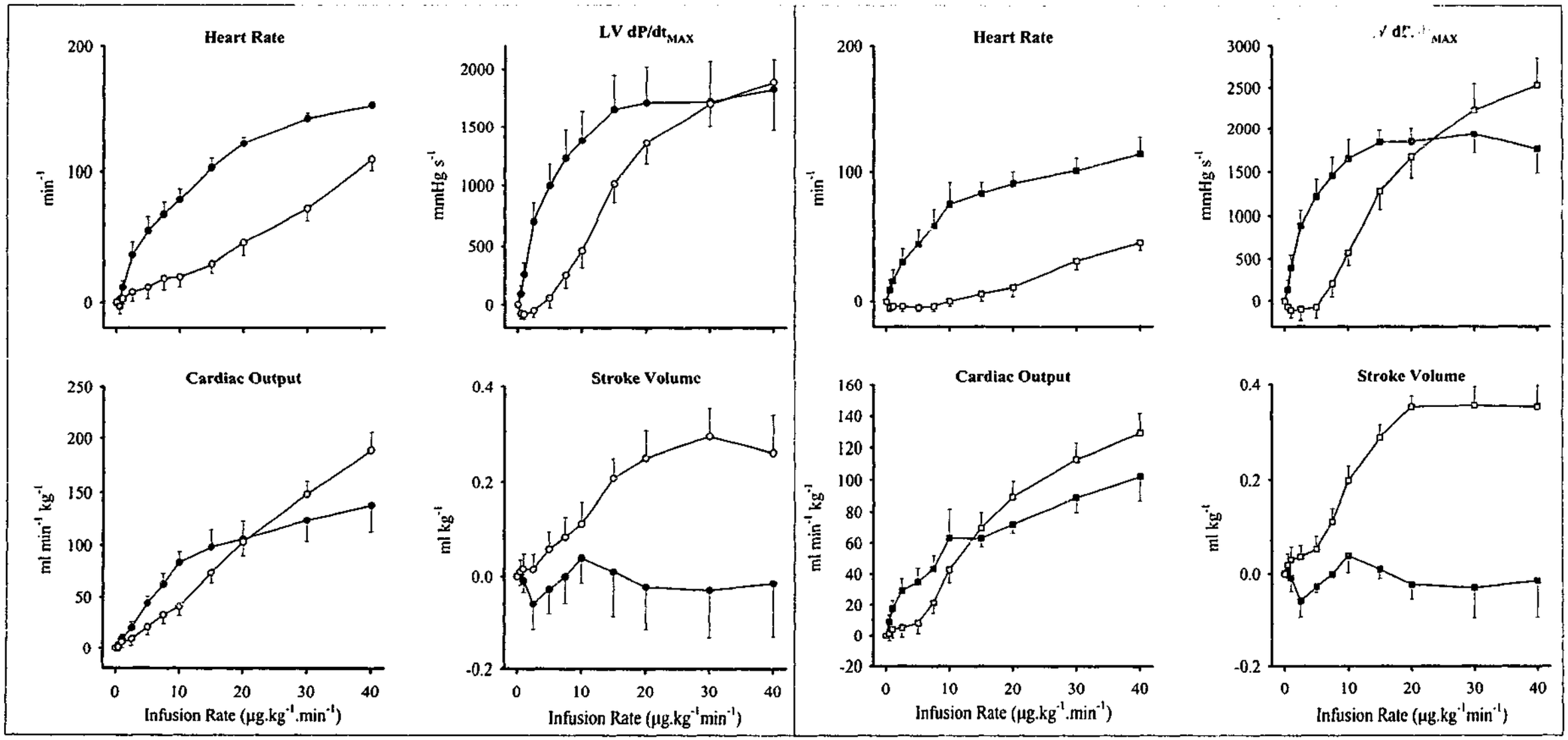


***Figure 3:7.*** *Changes in heart rate, LVdP/dt$_{MAX}$, cardiac output and stroke volume in 1-2 day(left-hand panels) and 7-10 day (right-hand panels) animals during incremental infusions of dobutamine (black symbols) and dopamine* (grey symbols).

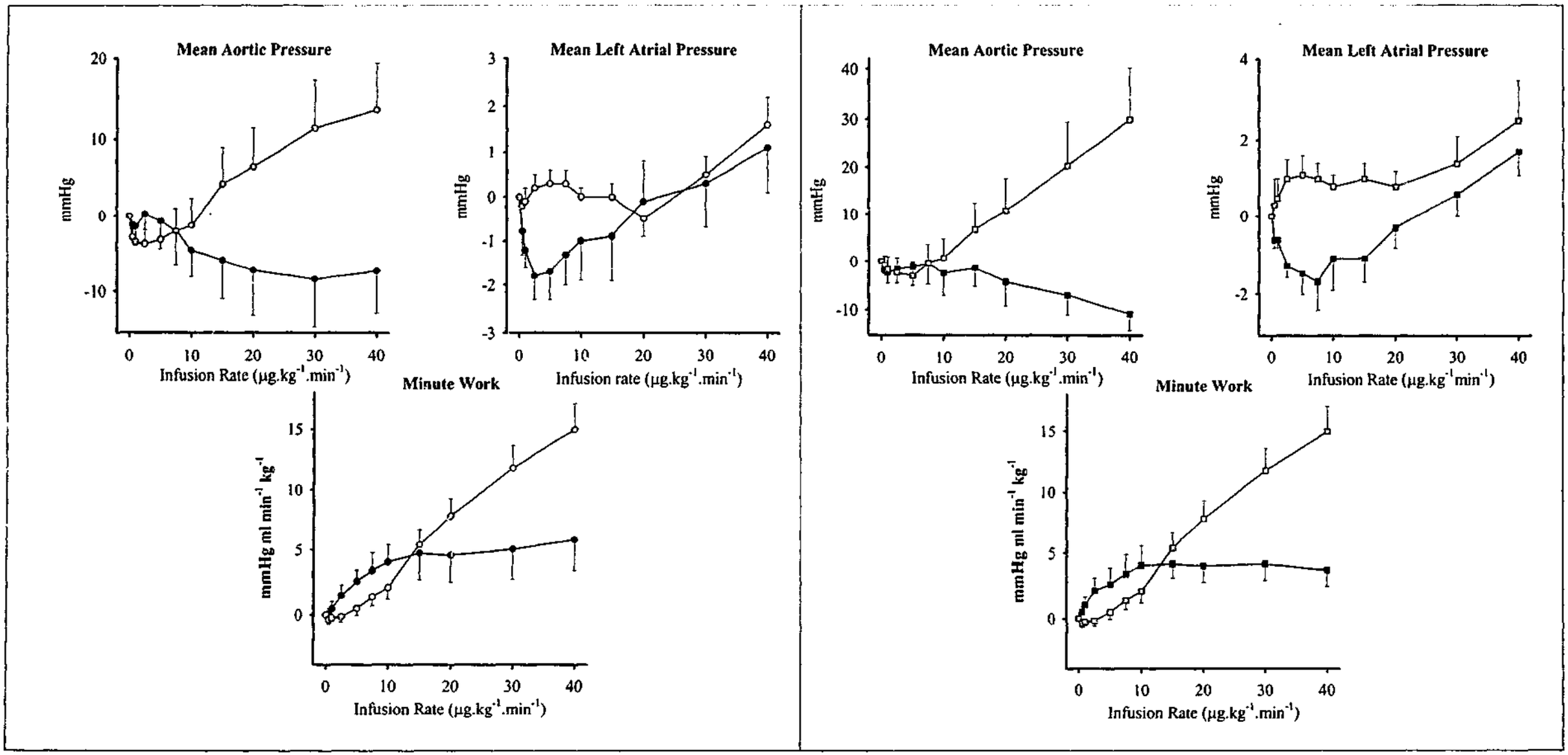


***Figure 3:8.*** *Changes in mean aortic and left atrial pressures and LV minute work in 1-2 day (left-hand panels) and 7-10 day (right-hand panels) animals during incremental infusions of dobutamine (black symbols) and dopamine (grey symbols)*

| | Change From Baseline | Dobutamine | Dopamine | P Value Dobut. Vs Dopa. |
|---|---|---|---|---|
| **Heart Rate ($min^{-1}$)** | 1-2 days | 153±3 | 109±8 | <0.001 |
| | 7-10 days | 113±13 | 46±6 | <0.001 |
| **LV $dP/dt_{MAX}$ ($mmHg \cdot s^{-1}$)** | 1-2 days | 1824±357 | 1888±193 | N.S. |
| | 7-10 days | 1757±270 | 2533±323 | N.S. |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 138±25 | 189±17 | N.S. |
| | 7-10 days | 102±15 | 129±13 | N.S. |
| **Stroke Volume ($ml \cdot kg^{-1}$)** | 1-2 days | -0.02±0.12 | 0.26±0.08 | <0.1 |
| | 7-10 days | 0.08±0.06 | 0.35±0.05 | <0.01 |
| **Mean Aortic Pressure (mmHg)** | 1-2 days | -7±5 | 14±6 | <0.05 |
| | 7-10 days | -11±3 | 30±10 | <0.01 |
| **Mean Left Atrial Pressure (mmHg)** | 1-2 days | 1.1±1.0 | 1.6±0.6 | N.S. |
| | 7-10 days | 1.7±0.6 | 2.5±1.0 | N.S. |
| **LV Minute Work ($mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 5.9±2.4 | 15.0±2.0 | <0.05 |
| | 7-10 days | 3.8±1.3 | 15.7±1.2 | <0.001 |

***Table 3:3.*** *Comparison of peak changes during incremental infusion of dobutamine and dopamine in neonatal lambs.*

### *3.3.6 Effect of NO Synthase Inhibition on Responses To Dobutamine.*

***Effect of NO synthase inhibition on baseline variables.*** The effects of intravenous L-NNA, 25mg/kg on haemodynamic variables were similar in magnitude and direction in both age groups. Although heart rate was not altered by L-NNA in either group, $LVdP/dt_{MAX}$ was increased in both. However, despite this, cardiac output and

stroke volume were diminished. Both aortic and left atrial pressures were elevated by L-NNA, with the former being associated with an increase in left ventricular minute work ***(Table 3.4)***.

| | | Before L-NNA | After L-NNA | P Value |
|---|---|---|---|---|
| **Heart Rate ($min^{-1}$)** | 1-2 days | 162±4 | 167±8 | n.s. |
| | 7-10 days | 206±5 | 198±5 | n.s. |
| **LV dP/dt $_{MAX}$ ($mmHg \cdot s^{-1}$)** | 1-2 days | 1457±112 | 1846±104 | <0.01 |
| | 7-10 days | 1818±190 | 2130±218 | <0.01 |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 136±4 | 123±3 | <0.01 |
| | 7-10 days | 126±9 | 108±7 | <0.05 |
| **Stroke Volume ($ml \cdot kg^{-1}$)** | 1-2 days | 0.84±0.04 | 0.74±0.04 | <0.05 |
| | 7-10 days | 0.61±0.04 | 0.55±0.04 | <0.1 |
| **Mean Aortic Pressure (mmHg)** | 1-2 days | 46±4 | 77±5 | <0.001 |
| | 7-10 days | 59±3 | 90±7 | <0.001 |
| **Mean Left Atrial Pressure (mmHg)** | 1-2 days | 4.6±0.4 | 6.8±0.4 | <0.001 |
| | 7-10 days | 5.9±0.8 | 7.9±0.7 | <0.05 |
| **LV Minute Work ($mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 5.5±0.5 | 8.5±0.5 | <0.001 |
| | 7-10 days | 6.7±0.7 | 8.9±0.9 | <0.01 |

*Table 3:4 Comparison of haemodynamic variables before and after L-NNA in 1-2 and 7-10 day lambs.*

***Responses to dobutamine, before and after NO synthase inhibition.***

Before L-NNA administration, dobutamine infusion increased heart rate by 72±6 $min^{-1}$ in the 1-2 day animals and by 79±7 $min^{-1}$ in the 7-10 day animals. $LVdP/dt_{MAX}$ was increased by 935±140 and by 2062±158 $mmHg \cdot s^{-1}$, and cardiac output by 70±16 and by 80±12 $ml \cdot min^{-1} \cdot kg^{-1}$ in the younger and older animals respectively. *After* L-NNA, in both groups, the rise in heart rate, $LVdP/dt_{MAX}$ and in cardiac output during dobutamine paralleled that during dobutamine alone ***(Figure 3:9)***

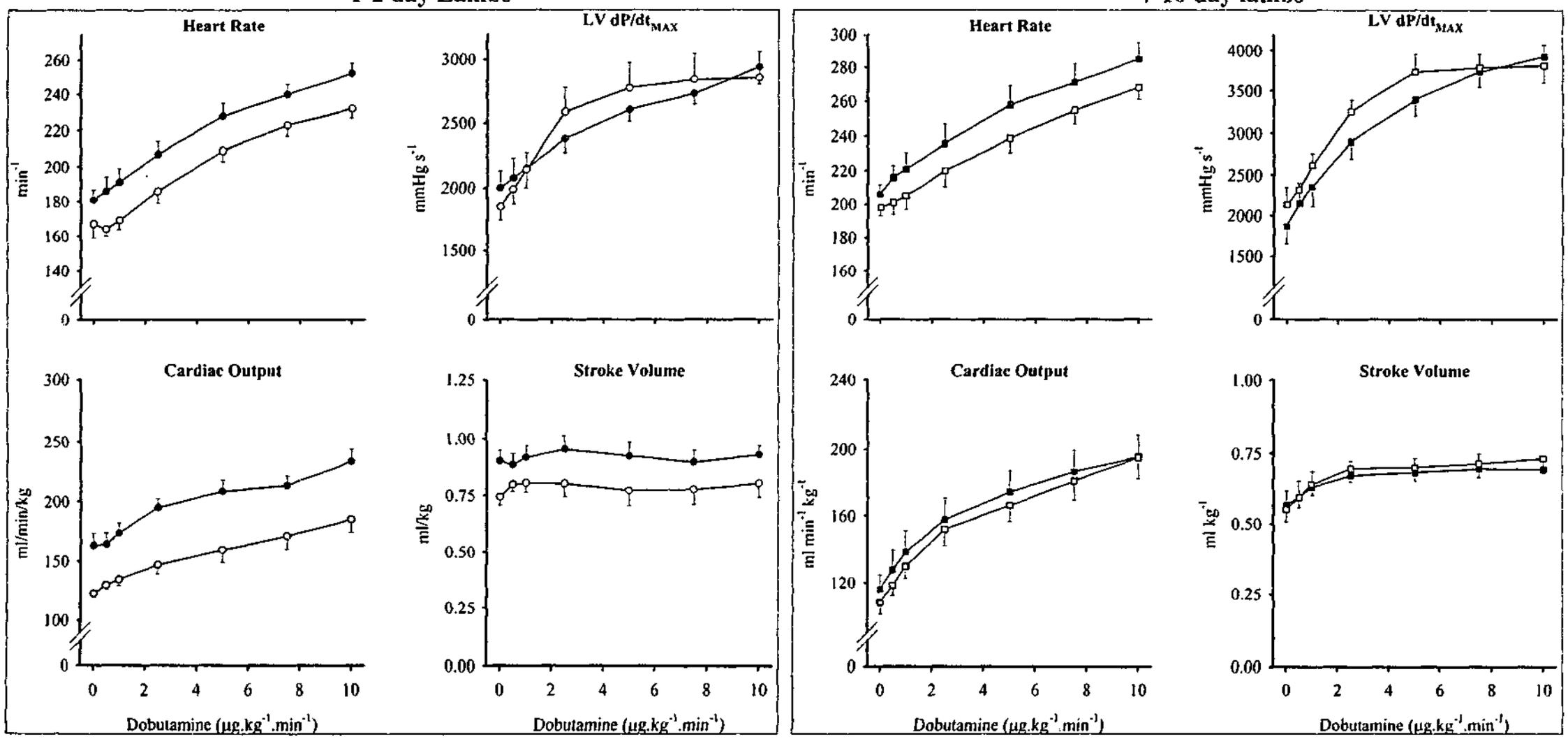


***Figure 3:9.*** *Heart rate, LV $dP/dt_{MAX}$, cardiac output and stroke volume responses during incremental infusions of dobutamine in 1-2 day (●) and 7-10 day (■) lambs, before (closed symbols) and after L-NNA (open symbols)*

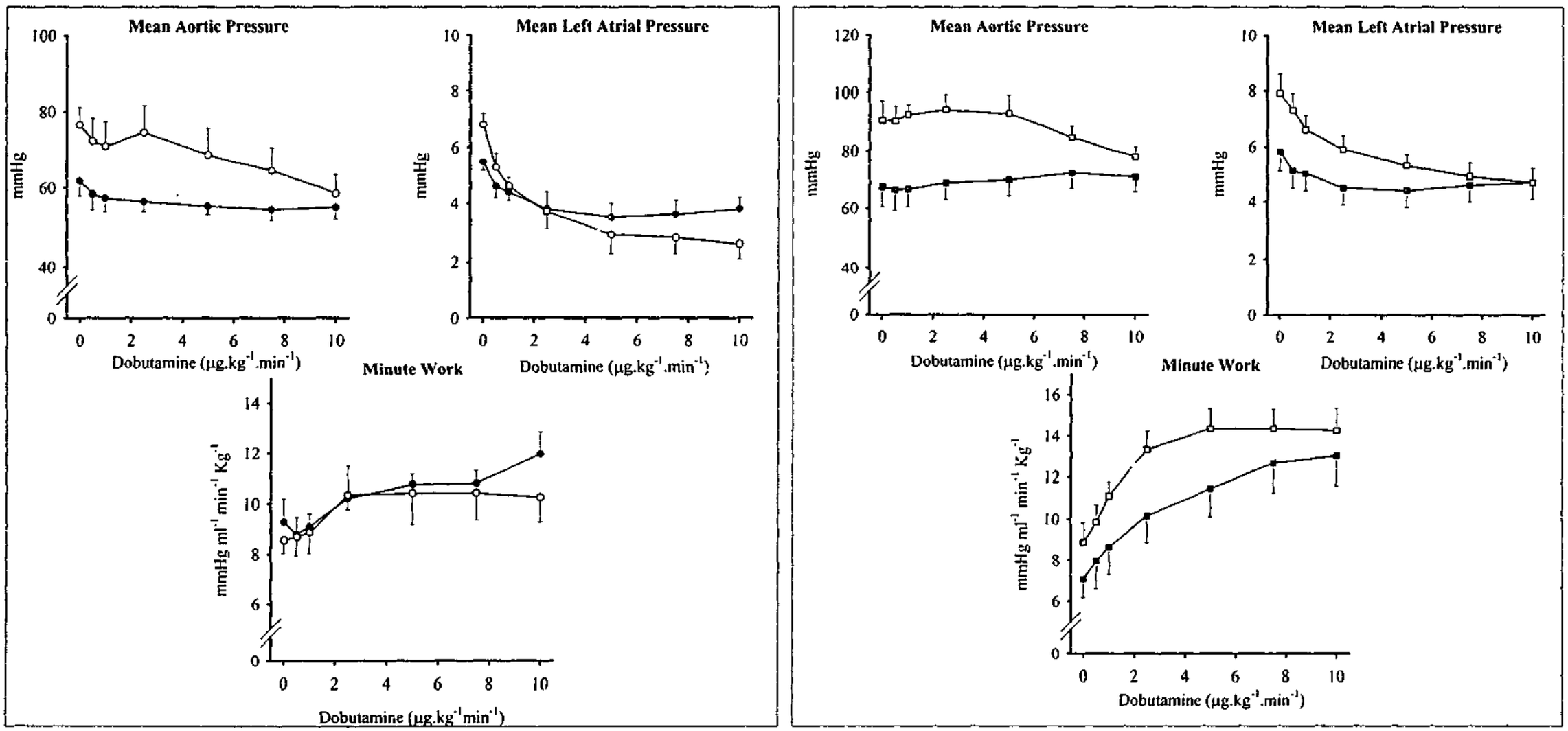


***Figure 3:10.*** *Mean aortic and left atrial pressures and LV minute work responses during incremental infusions of dobutamine in 1-2 day (●) and 7-10 day (■) lambs, before (closed symbols) and after L-NNA (open symbols)*

Beginning from higher baseline levels, reductions in aortic and left atrial pressures during dobutamine were greater after L-NNA, compared to without L-NNA, in both groups ($p<0.01$). However the dobutamine-related changes in LV external work were not modified significantly by L-NNA administration. ***(Figure 3:10).***

### *3.3.7 Effects of Partial Aortic Occlusion on Dobutamine-Related Changes in 1-2 Day Lambs*

***Effect of partial aortic occlusion on baseline variables*** Partial aortic occlusion did not change heart rate, but increased $LVdP/dt_{MAX}$ by 448±135 $mmHg \cdot s^{-1}$ ($p<0.05$). Despite the elevation in $LVdP/dt_{MAX}$, cardiac output and stroke volume were unaltered. Aortic and left atrial pressures were elevated by 39±4 mmHg and by 2±0.3 mmHg respectively (both $p<0.001$), with the former being associated with an increase in left ventricular minute work of 5.7±1.3 $mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$ ($p<0.01$).

***Responses to dobutamine, before and after aortic occlusion.***

Before aortic occlusion, dobutamine increased heart rate by 76±9 $min^{-1}$ ($p<0.001$). $LVdP/dt_{MAX}$ was increased by 938±145 $mmHgs^{-1}$ and cardiac output by 86±14 $ml \cdot min^{-1} \cdot kg^{-1}$ (both $p<0.001$). After aortic occlusion, dobutamine-related rises in heart rate (98±7 $min^{-1}$) and $LVdP/dt_{MAX}$ (1695±128 $mmHgs^{-1}$) were greater than during dobutamine alone ($p<0.05$ and $p<0.001$ respectively), while the dobutamine-related increase in cardiac output was unchanged. ***(Figure 3:11)***

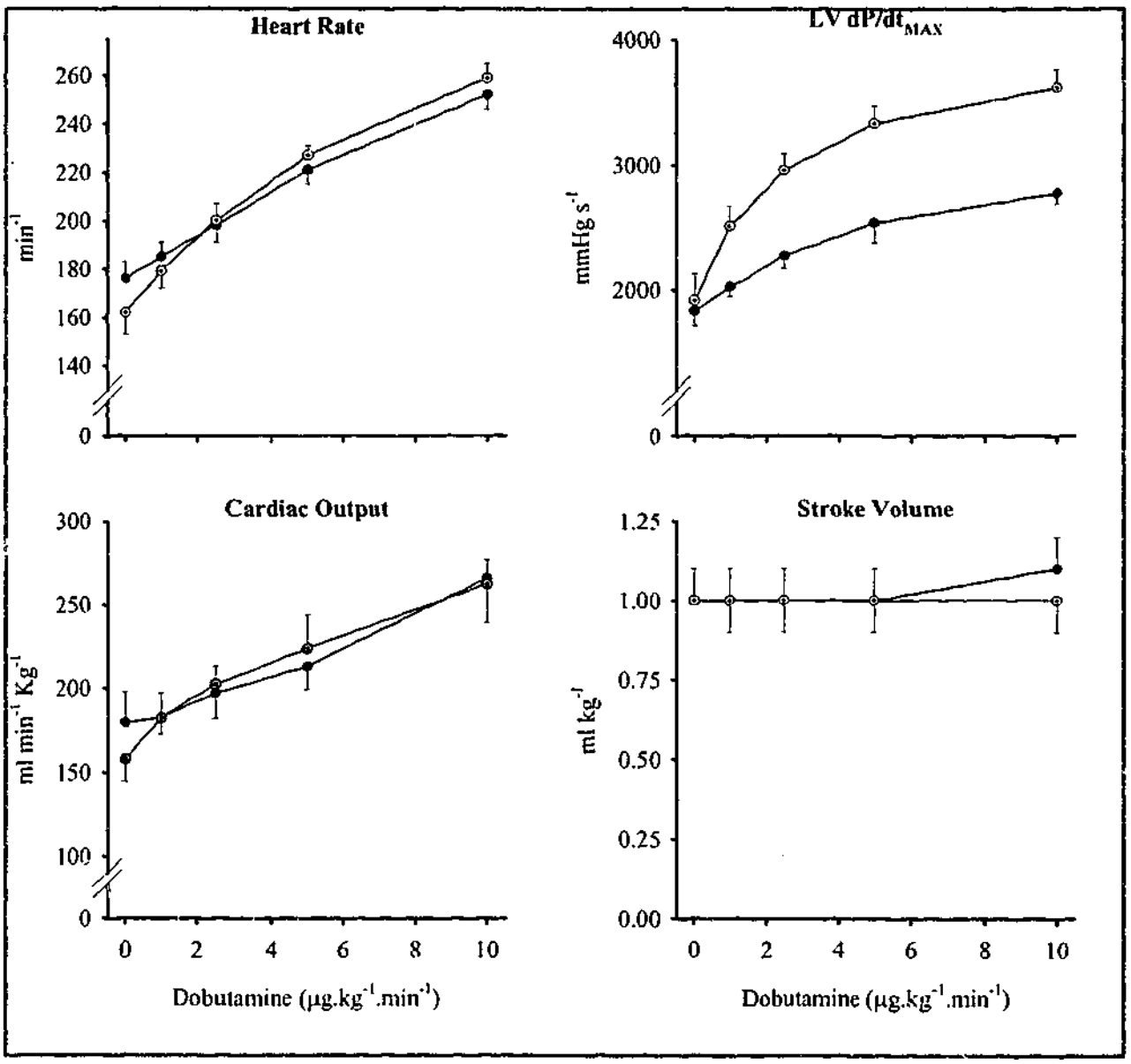


***Figure 3:11.*** *Heart rate, $LVdP/dt_{MAX}$, cardiac output and stroke volume responses in 1-2 day animals during incremental infusions of dobutamine before (•) and after partial aortic occlusion (⊙).*

Again, beginning from higher levels, dobutamine increased mean arterial pressure by 8±4 mmHg and left atrial pressure by 1.8±0.5 mmHg after aortic occlusion ($p<0.05$ for both). As a result, the dobutamine-related increase in LV minute work was significantly greater after aortic occlusion (11.1±1.8 $mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$) than before (2.9±1.3 $mmHg \cdot ml \cdot min^{-1} \cdot kg^{-1}$;$p<0.001$; ***Figure 3:12***).

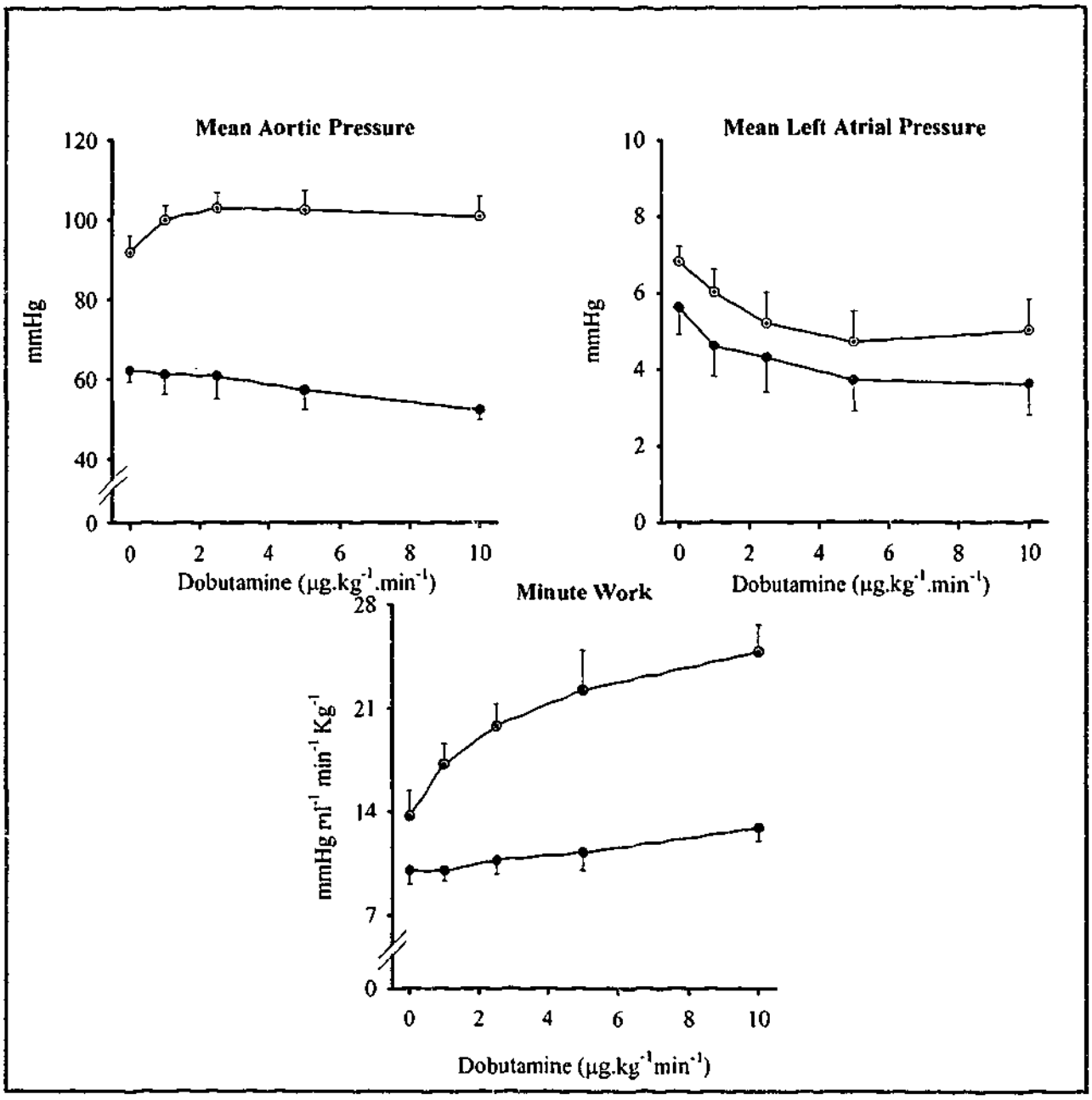


***Figure 3:12.*** *Mean aortic and left atrial pressures and LV minute work responses in 1-2 day animals during incremental infusions of dobutamine before (•) and after partial aortic occlusion* (⊙).

### *3.4 DISCUSSION.*

These studies, which examined the effects of the inotropes dobutamine and dopamine on central haemodynamics and left ventricular performance, have produced four main findings. First, in the neonatal lamb, the changes in left ventricular performance during infusions of dobutamine and dopamine were similar in 1-2 day animals, and those aged 7-10 days. Second, studies with selective

adrenoreceptor blockade studies suggested that in the very young neonate, some of the most important effects of dobutamine are mediated through its interactions with the $\beta_1$ adrenoreceptor. Third, distinctly different changes in central haemodynamics occurred in response to dopamine, compared to dobutamine, during early neonatal life. Finally, the effects of dobutamine on central haemodynamic responses did not appear to be modulated by inhibition of nitric oxide synthesis.

### *3.4.1 Comparison of Inotrope-Related Effects in 1-2 Day and 7-10 Day lambs.*

In the present studies, changes in LV contractility and mechanical performance during infusions of dobutamine and dopamine did not differ significantly between 1-2 day and 7-10 day animals. The reported effects of postnatal development on the contractile responses to inotropic stimulation are controversial. Some studies have demonstrated a reduced left ventricular response to inotropic stimulation in younger animals,[100,96] while others found no age-related difference.[101] It is important to examine these observations in light of these apparent inconsistencies. More detailed assessment of the available literature reveals there are important methodological differences, which may at least in part, explain these observations. General anesthesia has important effects on cardiovascular performance. One important study which demonstrated a reduced response to inotropic stimulation in younger animals was performed in *conscious* lambs,[96] in which baseline levels of contractility was shown to be higher in younger animals. A second study which demonstrated no age-related differences was carried out in animals receiving pentobarbitone anaesthesia[101], which may associated with profound myocardial depression.[153] The present studies were performed under α-chloralose anaesthesia. One of the main

reasons for this choice of agent was that it has been demonstrated to have a much lower myocardial depressant effect than other anaesthetic drugs.[154,153] However it is know that even it may alter endogenous catecholamine release[155] and have interactions with β-adrenergic receptors at high doses.[156] A second important consideration is the postnatal age at which the comparisons are being made. The literature suggests that the most important maturational changes in the contractile response to inotropic stimulation in lambs occurs between beyond the first week of life.[96] The present studies examined changes during the earlier postnatal period and it may well be that this pre-dated any significant postnatal developmental change.

***3.4.2 Modulation of dobutamine-related actions by selective adrenoceptor antagonists.*** Selective adrenoceptor antagonism profoundly altered the actions of dobutamine on cardiovascular performance. While the results from these studies must be considered with caution due to the small subject numbers, they do highlight a number of principles which may be important in the modulation of cardiovascular function by inotropic stimulation in the young neonate. First, they demonstrate the predominant role of the $\beta_1$ receptor in mediating dobutamine effects, as selective antagonism of the $\beta_1$ receptor almost completely abolished the dobutamine-related increases in LV $dP/dt_{MAX}$ and reduced the peak increase in heart rate by approximately 90% and the increase in LV external work by 60%. Second, they emphasise the dynamic interaction between contractility, arterial pressure and heart rate in mediating overall cardiovascular performance in the young neonate. Thus, while the increases in heart rate, LV contractility and external work during dobutamine infusion were significantly blunted by $\beta_1$ blockade, dobutamine-related increases cardiac output were minimally altered by this antagonist. It is interesting

that although stroke volume was unchanged during infusions of dobutamine alone, it increased during dobutamine infusion, after $\beta_1$ blockade. It is likely that this increase stroke volume was related to two factors. The first was the marked vasodilator response to dobutamine after $\beta_1$ blockade, which was manifest as a reduction in systemic arterial pressure (possibly mediated through the actions of the $\beta_2$ receptor) and which would be expected to mediate an increase in cardiac output by enhancing ventriculovascular coupling (See chapter 4). The second was a blunting of the heart rate response to dobutamine, which would have allowed for more efficient ventricular filling.

***3.4.3 Differences between dobutamine and dopamine-related actions.*** While both dobutamine and dopamine increased LV contractility, the overall responses to these agents differed markedly in our model. First, dobutamine lowered the systemic arterial pressure, whereas dopamine increased it. It is likely that this pressor response reflected the α-adrenoreceptor-mediated systemic vasoconstrictor action of dopamine,[157-161] rather than a direct effects mediated by dopaminergic receptor stimulation as it has been shown that the selective dopaminergic agonist fenoldopam reduces, rather than increases arterial pressure.[162] Cardiac output increased during infusions of both dobutamine and dopamine, although stroke volume increased *only* during dopamine infusions.

Marked differences in dose-related effects were observed in both age groups, when dobutamine and dopamine were compared. Relatively modest infusion rates of dobutamine produced substantial increases in LV contractility, while higher infusion rates of dopamine were required before to produce similar effects. These data

suggest that dobutamine may have a greater affinity for the β adrenoreceptor, compared to dopamine in the heart of the young neonate.

***3.4.4 Effect of systemic inhibition of NO synthesis on dobutamine-related actions.***

Recent in vitro and in vivo findings suggest that β-adrenergic actions of inotropes in the heart may be limited by nitric oxide (NO). Thus exogenous NO decreased the contractile responses to adrenergic agents in isolated preparations,[163,48] while inhibition of NO synthase enhanced the inotropic action of isoprenaline in ventricular myocytes[49] and intracoronary infusion of a NO synthase inhibitor augmented the contractile response to dobutamine.[164,50] It has been proposed that the augmented contractile response to β–adrenergic stimulation after exposure to a NO synthase inhibitor may reflect decreased levels of myoplasmic cGMP synthesis[49,50] with a resultant increase in myofilament sensitivity to calcium, and removal of the inhibitory effect of cyclic GMP on the slow inward calcium current.[165,166] In our previous study in an acute study of adult sheep, we demonstrated that systemic inhibition of NO synthesis enhanced the increases in left ventricular performance during dobutamine infusion.[65] However, we observed a similar enhancement after elevating aortic pressure by partial arterial occlusion to a level equivalent to that produced by inhibition of NO synthesis. This suggested that the rise in aortic blood pressure accompanying systemic inhibition of NO synthesis itself potentiates the LV responses to β–adrenergic stimulation.[65]

Given the important developmental changes in myofibrillar structure[91-94] and the marked sensitivity of the newborn heart to elevations in afterload[167,168] it was important to examine whether systemic inhibition of nitric oxide would have the

same impact on inotropic responses in the neonatal circulation. In contrast to the adult, systemic inhibition of NO synthesis did not enhance the inotropic effects of dobutamine. At first glance, this observation may lead to the conclusion that the neonatal myocardium responds very differently to the adult during inotropic stimulation with dobutamine after inhibition of nitric oxide synthesis. However, there is an alternative explanation for the finding in newborn lambs. Although inhibition of nitric oxide synthesis increased mean arterial pressure, this elevation was not maintained, as the dobutamine infusion was increased to levels greater than $2.5\mu g.kg^{-1}.min^{-1}$. Furthermore, dobutamine-related increases in LV $dP/dt_{MAX}$ and in external work were markedly enhanced by partial aortic occlusion. These data combined suggest that the contractile responses of the neonatal myocardium during elevated afterload may be similar to those in the adult and that the differences between neonatal and adult myocardial responsiveness after nitric oxide inhibition, do not primarily reflect developmental changes in the myocardium, but rather differences within the *vascular* effects of dobutamine after nitric oxide inhibition between the two groups. Clearly further studies, to delineate the cellular role of NO in the developing myocardium are warranted.

***3.4.5 Conclusions.*** These studies of the changes in central haemodynamics during infusion of dobutamine and dopamine have shown that while there are no major maturational changes in the response to these two agents during the first week of life, these agents are very different in their effects on myocardial performance. Furthermore, the effect of that systemic inhibition of nitric oxide synthesis on dobutamine-related increases in cardiac contractility or minute work output are not as

dramatic as in the adult, although this appears to be related to differences in vascular responses.

# CHAPTER 4 SYSTEMIC AND PULMONARY VASCULAR RESPONSES.

"The development of our knowledge of the circulation has been bedevilled by the fact that the measurement of blood flow is so complicated whereas that of pressure is so easy: Hence the blood pressure manometer has exerted an almost hypnotic influence, though most organs don't need pressure, but flow".

Adolf Jarish[169]

This chapter examines the pulmonary and systemic vascular effects of dobutamine and dopamine in the early neonatal period. It addresses the relative contributions of different adrenoreceptor subtypes to the vascular effects of dobutamine and investigates the extent to which NO modulates these changes.

Dobutamine and dopamine infusions reduced the pulmonary vascular resistance, but the magnitude of this pulmonary vasodilation was considerably blunted in the very early neonatal period. This differential age-dependent vasodilator response was specific to the pulmonary circulation and was not observed in the systemic vasculature. The changes in pulmonary vascular resistance during dobutamine infusion were not affected by pre-treatment with selective adrenoceptor antagonists. While *basal* systemic and pulmonary vascular resistances were increased by inhibition of NO synthesis, this did not significantly modulate the changes in vascular resistance during subsequent dobutamine infusion.

These findings indicate that the pulmonary vasodilator response to dobutamine and dopamine is blunted in the early neonatal period. This may be related to important structural features of the neonatal pulmonary vasculature. However, the absence of catecholamine-related NO release, which is an important contributor to the vasodilator effects of adrenergic stimulation in the adult circulation, may also play a role.

### *4.1 Introduction*

While until now, this thesis has concentrated on the changes in left ventricular function during infusions of dobutamine and dopamine, it is well recognised that these agents are also potent modulators of systemic and pulmonary vascular function. Importantly, it has been suggested that the changes within the vasculature during infusions of inotropic agents play a fundamental role in determining their overall effect on cardiovascular physiology and in turn, their therapeutic benefit.[14-16] In addition, vascular function is also regulated by nitric oxide and indeed it has been suggested by some that the vasoactive actions of adrenoceptor agonists may be mediated, at least in part, by their effects on nitric oxide release within the vascular endothelium.[62,64,63]

In contrast to the adult,[170-173] relatively few studies have examined the vascular effects of dobutamine or dopamine in the immature circulation and it remains unclear whether the systemic or pulmonary vascular actions of these agents undergo any developmental changes. Furthermore, it is unknown whether there is any change in the interaction between catecholamines such as dobutamine or dopamine and the nitric oxide pathway during early postnatal development.

However, with particular respect to the pulmonary circulation, there are strong grounds to expect that alterations in the vascular effects of adrenergic agonists might be present in the early postnatal period. First, the density of α-adrenoceptors is high in the fetal and early newborn period and subsequently declines during postnatal development, while conversely, the number of β-adrenoceptors rises progressively after birth.[104,105] Second, the pulmonary arterial vasculature is highly muscularized

at birth, presumably as a legacy of the high *in utero* pulmonary arterial pressures and this muscularization recedes in the initial postnatal weeks.[106] Third, the capillary bed in the lungs of newborn lambs is almost fully perfused at rest and has only a limited capacity for additional microvascular recruitment with rises in pulmonary blood flow.[108] It might also be expected that given the dramatic alterations in NO release by the pulmonary vascular endothelium[174,122,175] and the enormous endogenous catecholamine surge during the perinatal period,[176-178] important alterations in the interactions between catecholamines and NO might also occur at this time.

Accordingly, the aim of this chapter is to determine whether the responses of the systemic and pulmonary circulations to dobutamine and dopamine undergo developmental changes in newborn lambs. In order to investigate the relative contributions of individual receptor subtypes to the overall pulmonary vascular response to dobutamine, it will also examine its effects in the presence of selective adrenoceptor blockade and finally, it will evaluate the contribution of NO to the vascular effects of dobutamine.

## *4.2 METHODS*

***Surgical preparation*** Fifty-seven lambs, of which 35 were aged 1-2 days and weighed 4.6±0.2 kg and 22 were 7-10 days and weighed 6.2±0.3 kg were surgically prepared under general anaesthesia as described in Chapter 2. Briefly, as part of the preparation, teflon cannulae were inserted through adventitial purse-string sutures into the descending thoracic aorta and main pulmonary artery for measurement of systemic and pulmonary arterial pressures. A polyvinyl catheter was passed into the

left atrial cavity through a purse-string suture in the appendage. An ultrasonic, perivascular flow probe was placed around the ascending aorta. In forty-seven animals, the thermistor portion of a Swan-Ganz catheter was inserted directly into the main pulmonary artery through a purse string suture.

***Experimental protocol.*** The first protocol, which examined changes in systemic and pulmonary haemodynamics during an incremental infusion of dobutamine at rates up to 40$\mu g.kg^{-1}.min^{-1}$ was performed in seven 1-2 day and seven 7-10 day animals.

In another group of twelve 1-2 day lambs, dobutamine was infused at incremental rates of up to 40 $\mu g.kg^{-1}.min^{-1}$ after selective adrenoceptor blockade with one of the following four agents: *1)* the $\alpha_1$-adrenoceptor antagonist prazosin, (n=3), *2)* the $\alpha_2$-adrenoceptor antagonist, yohimbine (n=3) *3)* the $\beta_1$-adrenoceptor antagonist CGP 20712A, (n=3), or *4)* the $\beta_2$-adrenoceptor antagonist ICI 118551 (n=3), as described in Chapter 2.

In seventeen animals, of which nine were aged 1-2 days (weight 4.1±0.3kg) and eight, aged 7-10 days (weight 5.5±0.5kg), the effects of an incremental intravenous infusion of dopamine at rates up to 40 $\mu g.kg^{-1}.min^{-1}$ were studied.

The effects of inhibition of nitric oxide synthesis on dobutamine-related responses were investigated in fourteen animals, of which 7 were 1-2 days (weight 4.7±0.4kg) and 7 were 7-10 Days (weight 6.4±0.4kg). Responses to an incremental infusion of dobutamine at rates up to 10$\mu g.kg^{-1}.min^{-1}$ were studied before and after inhibition of endogenous nitric oxide synthesis, with an intravenous infusion of $N^{\omega}$-nitro-L-arginine (L-NNA) 25 mg/kg, as described in detail in Chapter 2.

***Physiologic measurements and calculations.*** Aortic, pulmonary arterial and left atrial pressures were measured with silicon-chip pressure transducers. Ascending aortic blood flow was measured continuously with an ultrasonic flowmeter. In all but seven animals, cardiac output was measured in triplicate at baseline and each infusion rate with thermodilution. In these seven animals, only ascending aortic flow was measured. However, a thermodilution-equivalent cardiac output was then calculated using an average relationship ($Y = 79 + 1.05X$, $r = 0.96 \pm 0.01$) obtained from simultaneous measurements of cardiac output by thermodilution (Y, ml) and ascending aortic flow measured with the flow probe (X, ml) in 32 lambs ranging in age from 1 day to 8 weeks, which underwent dobutamine infusion for this and other protocols in our laboratory.

Using pressure (mmHg) and cardiac output data ($L \cdot min^{-1}$), pulmonary vascular resistance per unit body weight was calculated as [(mean pulmonary arterial pressure - mean left atrial pressure) /cardiac output/body weight] and systemic vascular resistance as [mean aortic pressure /cardiac output/ body weight].

## *4.3 RESULTS*

### *4.3.1 Baseline Variables*

Under the conditions of our experiments, mean aortic, pulmonary arterial and left atrial blood pressures were similar in 1-2 and 7-10 Day old lambs, although cardiac output was higher in the 1-2 day group. As a result, both pulmonary and systemic vascular resistance were slightly higher in the 7-10 day animals ***(Table 4:1)***.

| | Group | | |
|---|---|---|---|
| | **1-2 day (n=23)** | **7-10 day (n=22)** | **P Value** |
| **Mean Aortic Pressure (mmHg)** | 62±2 | 68±3 | n.s. |
| **Mean Pulmonary Arterial Pressure (mm Hg)** | 27±1 | 26±1 | n.s. |
| **Mean Left Atrial Pressure (mm Hg)** | 5.3±0.3 | 5.2±0.5 | n.s. |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 183±7 | 143±8 | <0.001 |
| **Pulmonary Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | 0.12±0.01 | 0.16±0.01 | <0.01 |
| **Systemic Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | 0.32±0.02 | 0.66±0.04 | <0.001 |

*Table 4:1. Baseline vascular haemodynamic variables and cardiac output in 1-2 day and 7-10 day animals.*

### *4.3.2 Changes during dobutamine infusion*

***Haemodynamics*** Incremental infusion of dobutamine to a peak of $40\mu g.kg^{-1}.min^{-1}$ increased mean pulmonary arterial pressure in 1-2 day and 7-10 day old lambs ($p < 0.005$ for both groups). The increases reached a plateau between 15 and $40\mu g.kg^{-1}.min^{-1}$ and averaged 41 ± 10% (equivalent to 10.1±2.3 mm Hg) and 22±11% (equivalent to 4.7±2.9 mm Hg) for the younger and older lambs, respectively. Dobutamine infusion produced minor reductions in mean aortic pressure (of 4.6±5.4 mmHg for 1-2 day, and 2.6±4.6mmHg for 7-10 day lambs ($p<0.05$ for both) and produced dose-dependent increases in cardiac output of 150±20 $ml \cdot min^{-1} \cdot kg^{-1}$ and of 170±15 $ml \cdot min^{-1} \cdot kg^{-1}$ in the 1-2 and 7-10 day groups respectively ($p<0.005$ for both).

Mean left atrial pressure fell in both groups in response to dobutamine infusion at rates up to 5μg.kg$^{-1}$.min$^{-1}$ ($p<0.05$) but at higher infusion rates, this pressure progressively increased, exceeding baseline levels by 1.1±1.0 mmHg (1-2 day) and 2.2±0.6 mmHg (7-10 days; both $p<0.05$) at the peak infusion rate ***(Figure 4.1)***.

***Systemic and pulmonary vascular resistance*** Dobutamine significantly reduced pulmonary vascular resistance in both groups ($p < 0.005$), reaching a plateau over 15-40μg.kg$^{-1}$.min$^{-1}$. However, over this plateau, the fall in pulmonary vascular resistance was significantly lower for 1-2 day than for 7-10 day old lambs (0.06±0.02 vs 0.09±0.03 mmHg/ml·min$^{-1}$·kg$^{-1}$; $p<0.02$). Dobutamine also lowered systemic vascular resistance in both age groups ($p<0.005$). However, the decrement in systemic vascular resistance between infusion rates of 15 and 40 μg.kg$^{-1}$.min$^{-1}$ was similar in 1-2 day (0.23±0.05 mmHg/ml·min$^{-1}$·kg$^{-1}$) and 7-10 day animals (0.27±0.06 mmHg/ml·min$^{-1}$·kg$^{-1}$; ***Figure 4.1)***.

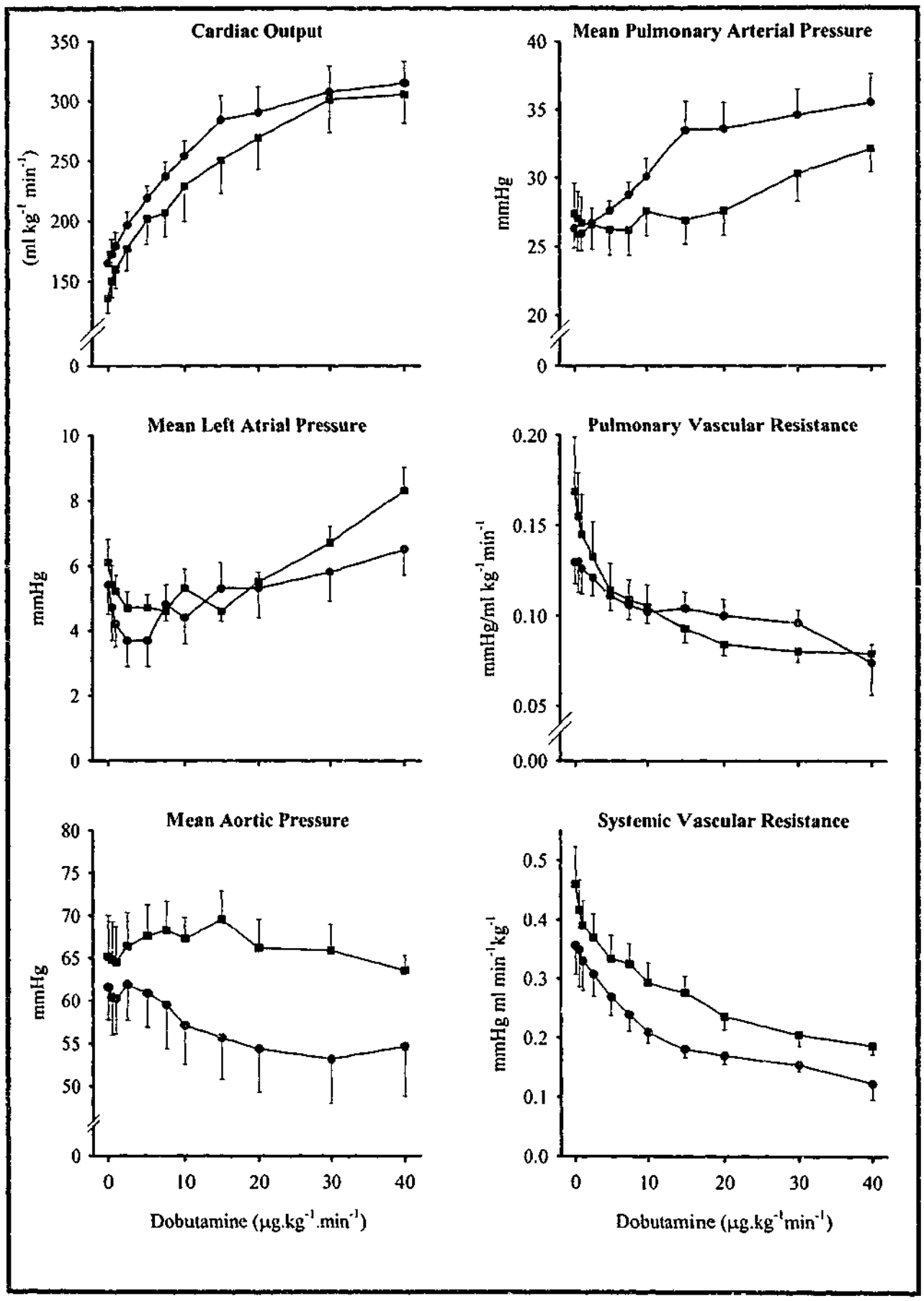


***Figure 4:1.*** *Vascular haemodynamic and cardiac output responses during dobutamine infusion in 1-2 day (●) and 7-10 day (■) lambs.*

### *4.3.3 Effect of adrenoreceptor blockade on the responses to dobutamine*

***Effect of adrenoreceptor blockade on baseline variables*** Adrenoceptor blockade in 1-2 day old lambs was not associated with any major haemodynamic changes, apart from a possible reduction in mean aortic pressure after α1-blockade ***(Table 4:2)***.

| Variable | | $\alpha_1$-blockade (n=3) | $\alpha_2$-blockade (n=3) | $\beta_1$-blockade (n=3) | $\beta_2$-blockade (n=3) |
|---|---|---|---|---|---|
| **Mean Aortic Pressure (mmHg)** | Before | 65±8 | 58±2 | 62±1 | 71±3 |
| | After | 53±3 | 60±4 | 57±4 | 68±1 |
| **Mean Pulmonary Arterial Pressure (mm Hg)** | Before | 26±3 | 26±1 | 26±1 | 29±3 |
| | After | 25±2 | 25±1 | 24±1 | 29±3 |
| **Mean Left Atrial Pressure (mm Hg)** | Before | 3.2±1.3 | 3.8±0.8 | 3.9±0.3 | 3.7±0.2 |
| | After | 3.4±0.9 | 2.5±1.2 | 3.5±0.3 | 4.9±0.7 |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | Before | 267±65 | 206±36 | 308±118 | 261±95 |
| | After | 273±82 | 185±50 | 282±107 | 255±83 |
| **Systemic Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | Before | 0.29±0.13 | 0.28±0.03 | 0.24±0.07 | 0.33±0.09 |
| | After | 0.24±0.09 | 0.35±0.07 | 0.24±0.07 | 0.30±0.08 |
| **Pulmonary Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | Before | 0.13±0.05 | 0.11±0.01 | 0.09±0.03 | 0.16±0.03 |
| | After | 0.11±0.05 | 0.13±0.02 | 0.10±0.03 | 0.11±0.02 |

***Table 4:2*** *Comparison of vascular haemodynamic variables and cardiac output before and after selective adrenoreceptor blockade in 1-2 day lambs.*

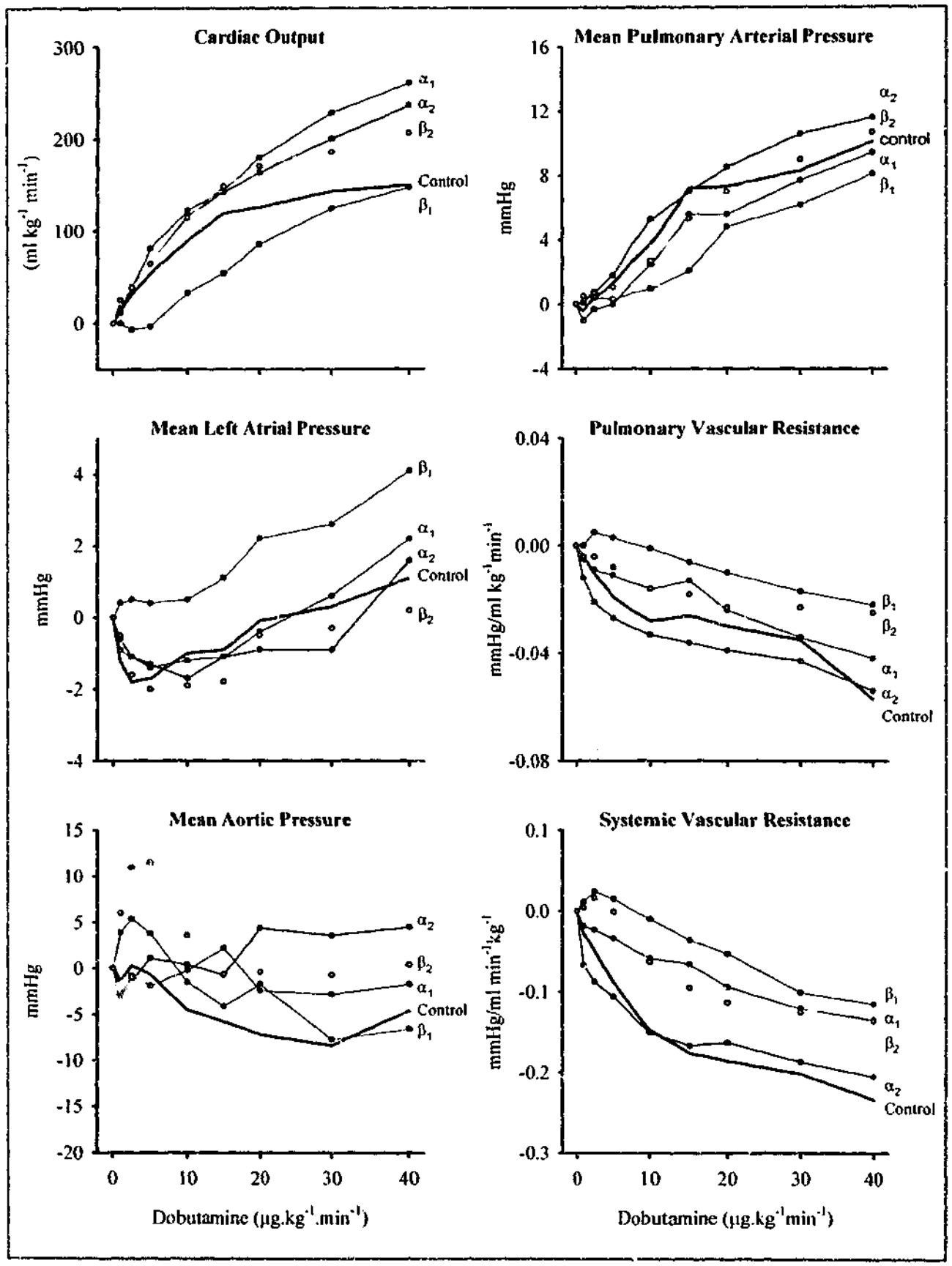


***Figure 4:2*** *Changes in vascular haemodynamics and cardiac output during incremental dobutamine infusion in the absence of (control; –) or presence of selective* $\alpha_1$ (–), $\alpha_2$ (–), $\beta_1$ (–), $\beta_2$ ( ) *adrenoceptor blockade. Error bars have not been included in order to maintain clarity.*

***Effect of adrenoreceptor blockade on responses to dobutamine*** Following selective blockade of $\alpha_1$, $\alpha_2$, $\beta_1$ or $\beta_2$-adrenoceptors in 1-2 day old lambs, responses in mean pulmonary arterial and aortic blood pressures, and cardiac output, occurring with dobutamine infusion were similar to those observed in unblocked animals. Similarly, the changes in pulmonary and systemic vascular resistances with dobutamine were not different to those occurring before adrenoceptor blockade ***(Figure 4:2)***.

### *4.3.4 Changes during dopamine infusion*

***Haemodynamics.*** Dopamine infusions at rates exceeding 20μg.kg$^{-1}$.min$^{-1}$ increased aortic pressure in both the 1-2 day and 7-10 day animals (both $p<0.001$), achieving peak increments of 14±6 and 30±10 mm Hg respectively. Dopamine also increased pulmonary arterial pressure by 11±1 (1-2 day) and 7±2 mm Hg (7-10 day) (both $p<0.001$), while minor increases in left atrial pressure of 1.6±0.6 mmHg and 2.5±1.0 mmHg respectively ($p<0.05$ for both) were observed. Although the initial infusion rates of dopamine had little effect on cardiac output, rates exceeding 10μg.kg$^{-1}$.min$^{-1}$ progressively increased this variable in both groups to peak levels which were 169±14 (1-2 days) and 125±10 ml·min$^{-1}$·kg$^{-1}$ (7-10 days) above baseline (both $p<0.001$).

***Systemic and pulmonary vascular resistances.*** At infusion rates exceeding 10-15 μg.kg$^{-1}$.min$^{-1}$, dopamine reduced the systemic and pulmonary vascular resistances in both age groups ($p<0.001$ for both). However, while the falls in systemic vascular resistance were similar in the two groups (0.09±0.02 and 0.08±0.06 mmHg/ml·min$^{-1}$·kg$^{-1}$ for 1-2 day and 7-10 day animals respectively), the fall in pulmonary vascular resistance in the 1-2 day old animals (0.03±0.01 mmHg/ml·min$^{-1}$·kg$^{-1}$) was

significantly lower than the reduction in the 7-10 day group (0.05±0.01 mmHg/ml·min$^{-1}$·kg$^{-1}$; p<0.01) ***(Figure 4:3)***.

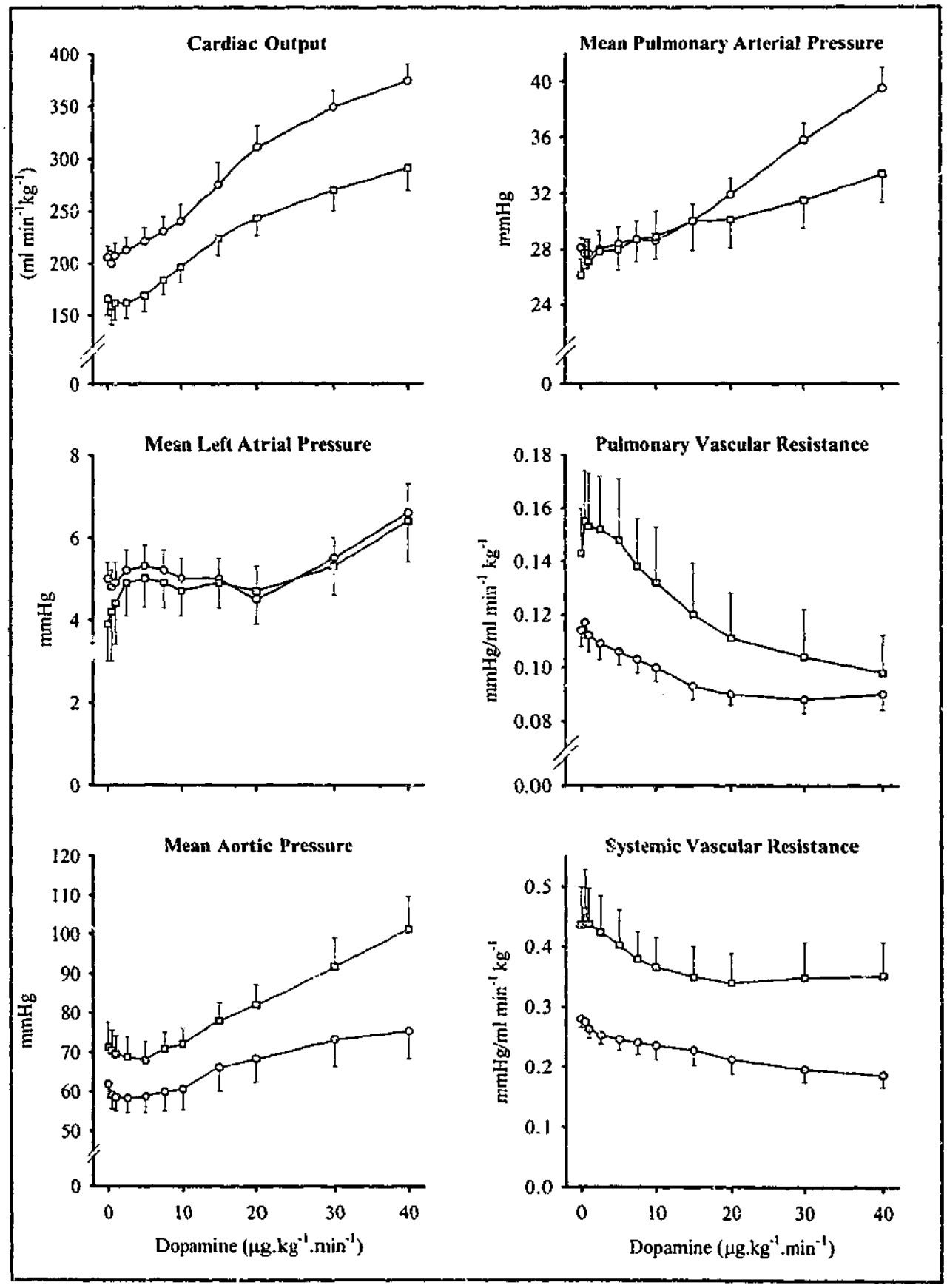


***Figure 4:3.*** *Vascular haemodynamic and cardiac output responses during incremental infusion of dopamine in 1-2 day* (•) *and 7-10 day* (■) *lambs.*

### *4.3.5. Comparison of dobutamine and dopamine responses.*

The responses to dobutamine and dopamine differed in a number of respects. First, for some variables, the direction of change differed between treatments. Thus, mean arterial pressure fell during dobutamine infusion, but increased in response to dopamine. Second, the magnitude of the reduction in systemic vascular resistance was significantly greater in the animals given dobutamine, compared to those given dopamine ($p<0.05$). Third, dose-reponse curves differed for the two drugs, indicating that increases in cardiac output and in mean pulmonary arterial pressure appeared to become evident at lower infusion rates for animals given dobutamine compared to those given dopamine. ***(Table 4:3, Figure 4:4 & 4.5).***

| | | **Dobutamine** | **Dopamine** | **P Value Dobut. Vs Dopa.** |
|---|---|---|---|---|
| **Mean Pulmonary Arterial Pressure (mmHg)** | 1-2 days | 10.0±2.3 | 11.4±1.4 | N.S. |
| | 7-10 days | 4.7±2.9 | 7.3±1.9 | N.S. |
| **Cardiac Output** | 1-2 days | 150±20 | 169±14 | N.S. |
| **(ml·min$^{-1}$·kg)** | 7-10 days | 170±15 | 125±10.0 | <0.05 |
| **Systemic Vascular Resistance** | 1-2 days | -0.23±0.06 | -0.09±0.02 | <0.05 |
| **(mm Hg/ml·min$^{-1}$·kg)** | 7-10 days | -0.27±0.06 | -0.08±0.06 | <0.05 |
| **Pulmonary Vascular Resistance** | 1-2 days | -0.04±0.01 | -0.03±0.01 | N.S. |
| **(mm Hg/ml·min$^{-1}$·kg)** | 7-10 days | -0.09±0.03 | -0.05±0.01 | N.S. |

***Table 4:3.*** *Comparison of peak changes in vascular haemodynamics and cardiac output during incremental infusion of dobutamine and dopamine in neonatal lambs.*

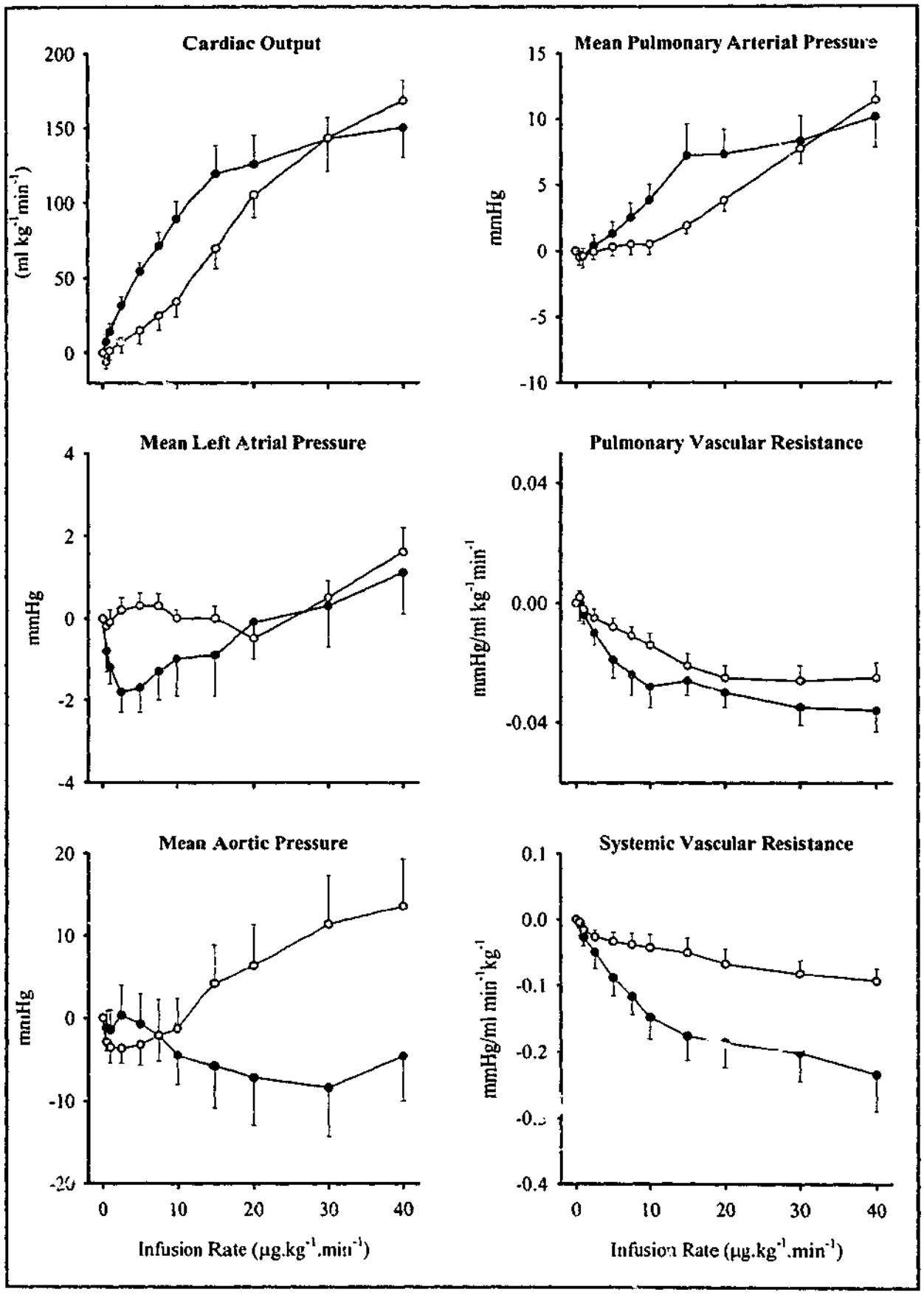


***Figure 4:4.*** *Vascular haemodynamic and cardiac output responses in 1-2 day animals during incremental infusions of dobutamine (•) and dopamine (•).*

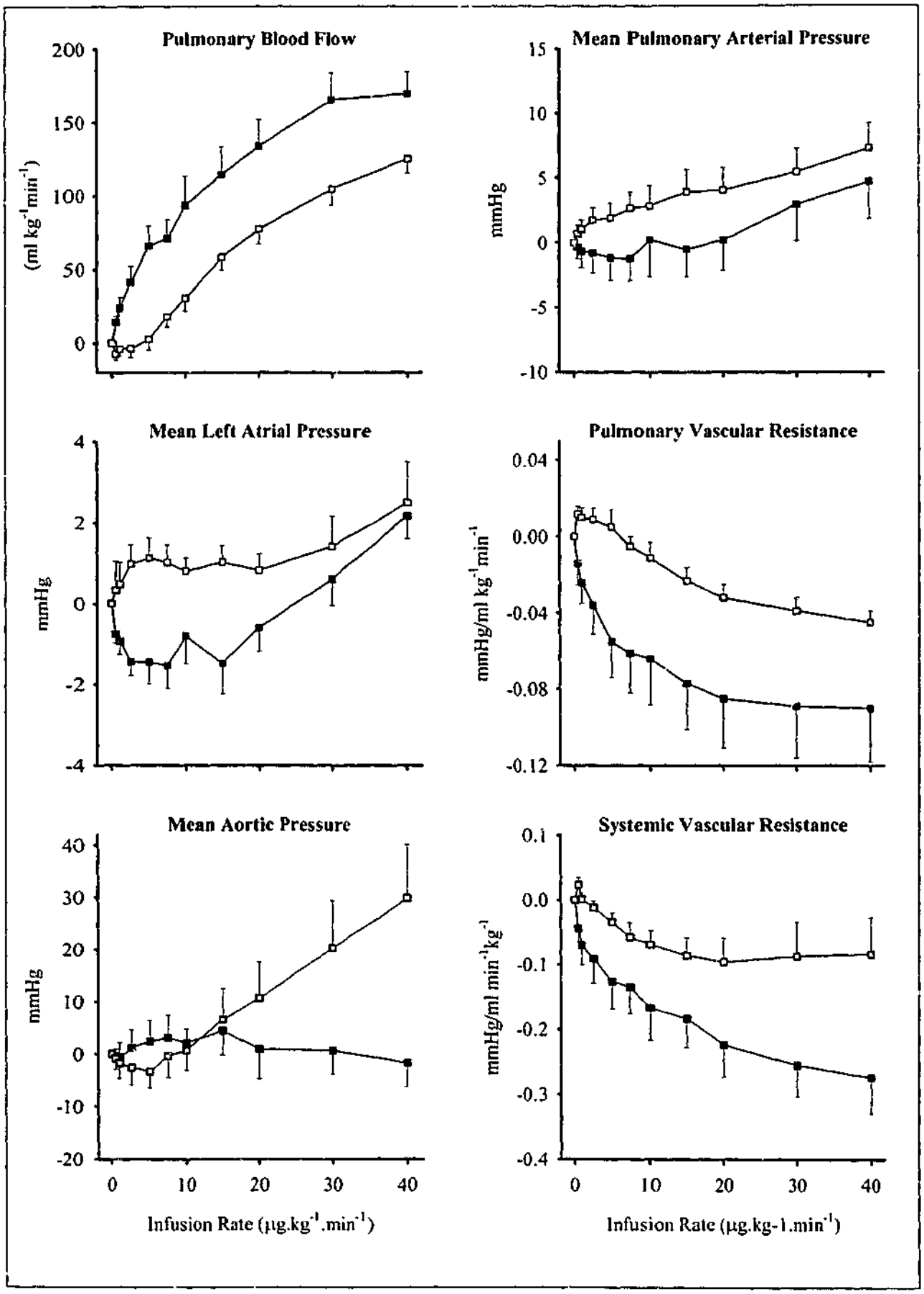


***Figure 4:5.*** *Vascular haemodynamic and cardiac output responses in 7-10 day animals during incremental infusions of dobutamine* (■) *and dopamine* (■).

### *4.3.6 Effects of NO Synthase Inhibition on The Responses to Dobutamine*

***Effect of NO synthase inhibition*** Intravenous administration of L-NNA increased mean aortic, pulmonary arterial and left atrial pressures in all animals. Although the increases in mean aortic pressure were similar between in the two groups, the increases in pulmonary arterial pressure were greater in the older (14.0±1.1 mmHg), compared to the younger animals (6.0±0.7 mmHg; $p<0.001$). Cardiac output was unaltered by L-NNA in the 1-2 day animals, but was significantly reduced in the 7-10 day group (12±3% reduction; $p<0.01$;***Table 4:4).***

| | | Before L-NNA (n=7) | After L-NNA (n=7) | P Value |
|---|---|---|---|---|
| **Mean Aortic Pressure (mmHg)** | 1-2 D | 46±4 | 77±5 | <0.001 |
| | 7-10 D | 59±3 | 90±7 | <0.001 |
| **Mean Pulmonary Arterial Pressure (mm Hg)** | 1-2 D | 23±1 | 29±1 | <0.001 |
| | 7-10 D | 23±2 | 37±2 | <0.001 |
| **Mean Left Atrial Pressure (mm Hg)** | 1-2 D | 4.6±0.4 | 6.8±0.4 | <0.001 |
| | 7-10 D | 5.9±0.8 | 7.9±0.7 | <0.05 |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 D | 156±5 | 148±8 | n.s. |
| | 7-10 D | 131±6 | 115±7 | <0.01 |
| **Systemic Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 D | 0.27±0.03 | 0.48±0.04 | <0.001 |
| | 7-10 D | 0.41±0.03 | 0.72±0.06 | <0.001 |
| **Pulmonary Vascular Resistance ($mm\ Hg/ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 D | 0.12±0.01 | 0.15±0.01 | <0.001 |
| | 7-10 D | 0.14±0.02 | 0.27±0.03 | <0.001 |

***Table 4:4*** *Comparison of vascular haemodynamic variables and cardiac output before and after L-NNA in 1-2 and 7-10 day lambs.*

Coincident with these changes in both groups, systemic and pulmonary vascular resistances were increased by L-NNA ($p<0.001$;***Table 4:4).*** The increase in pulmonary vascular resistance of 0.13±0.02 $mmHg/ml \cdot min^{-1} \cdot kg^{-1}$ which occurred in the 7-10 day group was greater than the increase of 0.03±0.01 $mmHg/ml \cdot min^{-1} \cdot kg^{-1}$

which was observed in the younger animals ($p<0.001$). Furthermore, there was a tendency towards a greater increase in systemic vascular resistance in the older animals, although this difference between groups (0.22±0.03 vs 0.31±0.04 $mmHg/ml \cdot min^{-1} \cdot kg^{-1}$) was not statistically significant ($p<0.07$)

***Responses to dobutamine before and after NO synthase inhibition.*** Before L-NNA infusion, dobutamine reduced mean aortic pressure, but increased pulmonary arterial pressure and cardiac output in the 1-2 day animals. In this group, L-NNA enhanced dobutamine-related reductions in mean aortic pressure (7±3 mmHg before vs 18±3 after L-NNA $p<0.05$), but prevented the increase in mean pulmonary arterial pressure. L-NNA also and blunted the dobutamine-induced increase in cardiac output (78±12 before vs 34±8 $ml \cdot min^{-1} \cdot kg^{-1}$ after L-NNA: $p<0.05$). Consequently, after L-NNA, the reductions in systemic and pulmonary vascular resistance in the 1-2 day group during dobutamine paralleled those during dobutamine alone ***(Figure 4:5)***.

In the 7-10 day animals, infusion of dobutamine alone increased cardiac output, but did not significantly alter mean aortic or pulmonary arterial pressures. However, after administration of L-NNA, dobutamine reduced mean aortic pressure by 12±7 mmHg ($p<0.01$) and pulmonary arterial pressure by 4.5±2.3 mmHg ($p<0.01$). L-NNA did not influence cardiac output responses to dobutamine in the older animals ***(Figure 4:6)***. In this group, beginning from higher levels, the reductions in systemic and pulmonary vascular resistance during dobutamine infusion were enhanced after L-NNA, compared to dobutamine alone ($p<0.001$; ***Figure 4:6)***.

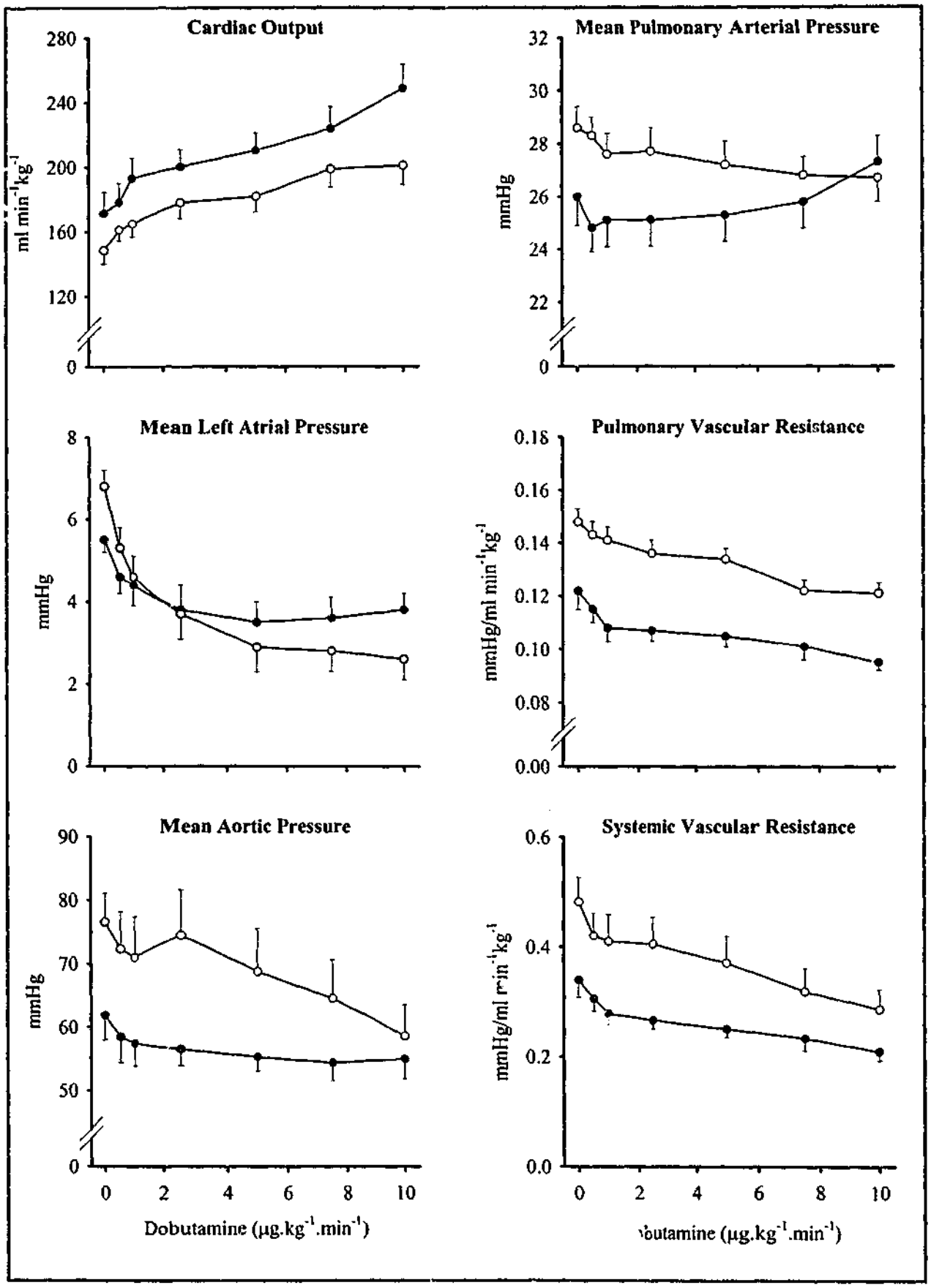


***Figure 4:6.*** *Vascular haemodynamic and cardiac output responses in 1-2 day animals during incremental infusions of dobutamine before* (•) *and after L-NNA administration* (○).

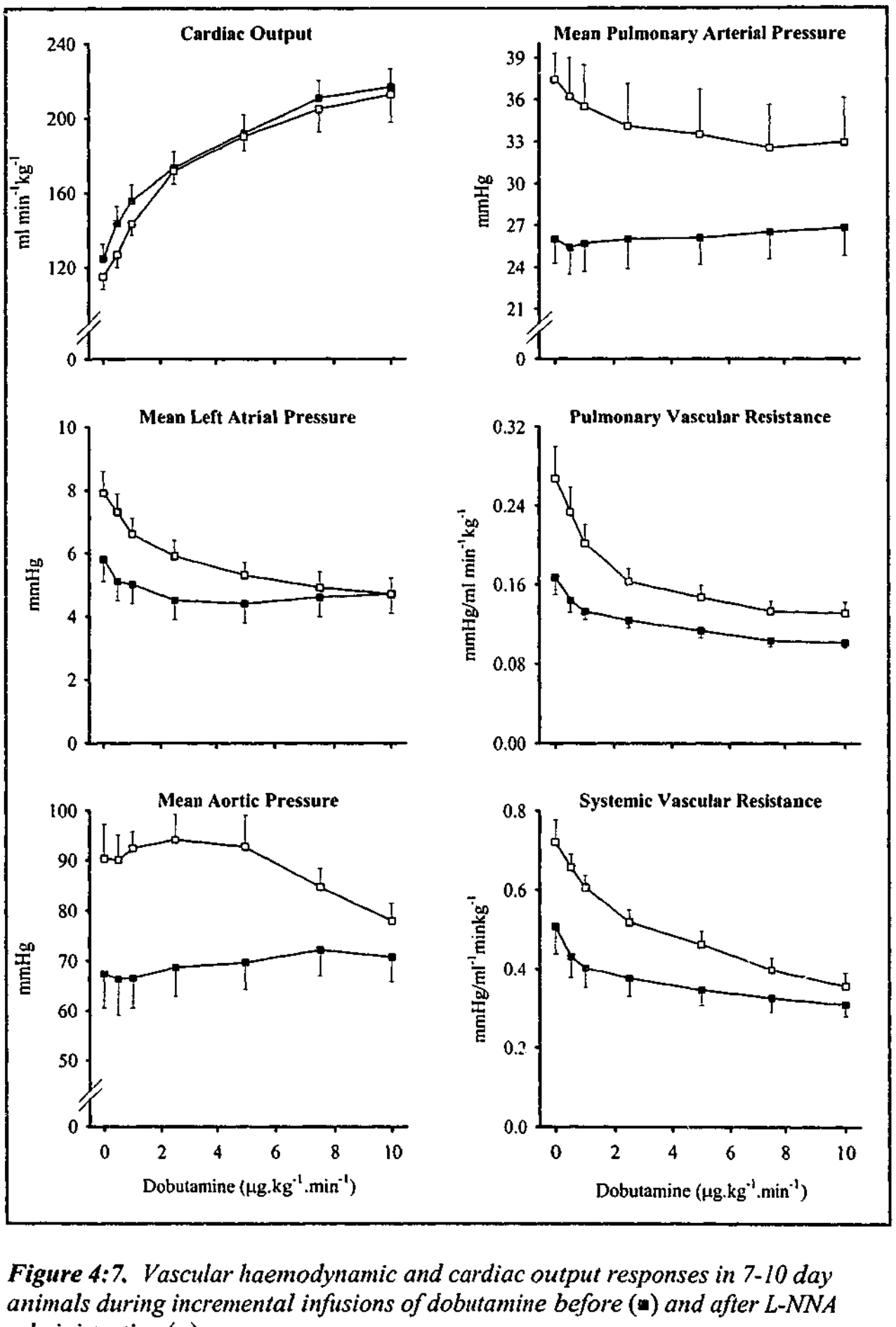


***Figure 4:7.*** *Vascular haemodynamic and cardiac output responses in 7-10 day animals during incremental infusions of dobutamine before* (■) *and after L-NNA administration* (□).

## *4.4 DISCUSSION*

Experiments in this chapter have examined the pulmonary and systemic vascular effects of dobutamine and dopamine in the early neonatal period, the relative contributions of different adrenoreceptor subtypes to the vascular effects of dobutamine, and the extent to which NO modulates these changes .

They have produced four main findings. First dobutamine and dopamine infusions reduced pulmonary vascular resistance, but the magnitude of this pulmonary vasodilation was considerably blunted in the very early neonatal period. Second, this differential age-dependent vasodilator response to dobutamine and dopamine appeared to be specific to the pulmonary and not the systemic vasculature. Third, the changes in pulmonary vascular resistance during dobutamine infusion in newborn lambs were not affected by pre-treatment with selective $\alpha_1$, $\alpha_2$, $\beta_1$ or $\beta_2$-adrenoceptor antagonists. Finally, while inhibition of NO synthesis increased *basal* systemic and pulmonary vascular resistances in even very young lambs, in these it did not significantly modulate changes in vascular resistance during dobutamine infusions.

***4.4.1 Effects of catecholamines on neonatal systemic and pulmonary vascular resistance.*** Reports of the effects of catecholamines on systemic and pulmonary vascular resistance in the postnatal period are limited and quite divergent. Thus, in an early study performed in anaesthetised newborn puppies, dobutamine increased systemic arterial pressure and cardiac output without changing systemic vascular resistance.[179] In a subsequent study using a similar preparation, dobutamine did not influence cardiac output or systemic blood pressure, suggesting that systemic vascular resistance was also unchanged.[180] Conversely, in conscious 1-2 month old

pigs, dobutamine reduced mean systemic arterial pressure and resistance without changing pulmonary arterial pressure or pulmonary vascular resistance.[181] In a clinical study in newborn infants, dobutamine increased cardiac output without changing systemic blood pressure, suggesting that systemic vascular resistance was reduced.[182] In another study, dobutamine increased mean arterial pressure and cardiac output without changing in systemic vascular resistance in hypotensive preterm infants.[183]

Data on the effects of dopamine are equally diverse. In neonatal lambs, dopamine increased both pulmonary and systemic vascular resistances, although in this study extremely high doses - up to $400\mu g.kg^{-1}.min^{-1}$ - were used.[184] In another study in lambs, dopamine infusion did not alter pulmonary vascular resistance either before or after α-adrenoreceptor blockade,[185] while in piglets an infusion of dopamine had no effect on systemic or pulmonary vascular resistance.[186] Clinical studies of dopamine's systemic effects have shown either no change in systemic vascular resistance[187] or an increase in arterial pressure coupled with a reduction in cardiac output (suggesting an increase in systemic vascular resistance).[183]

Our own previous clinical studies in the sick preterm neonate confirmed that the precise haemodynamic response to dopamine is somewhat diverse in the sick, preterm neonate. We observed that dopamine increased the systemic blood pressure in all infants, however, in some, this reflected an increase in systemic vascular resistance, and in others, an increase in cardiac output.[83]

In the current study, infusions of dobutamine and dopamine both reduced pulmonary vascular resistance. However, the most striking finding was that the pulmonary

vasodilator response to both agents in 1-2 day old lambs was blunted compared to older animals, and was associated with a substantial rise in pulmonary artery pressure. This may have reflected an age-related difference in the balance between $\alpha_1$-mediated vasoconstrictor and $\beta_2$-mediated vasodilator effects of these agents. At first glance, this hypothesis appears attractive because we know that there are relatively more $\alpha_1$- than β-adrenoceptors in the lung in the immediate newborn period and, whereas the density of α-adrenoceptors remains static, β-receptor numbers continue to increase during the early postnatal period.[104,105] Indeed, this explanation would be consistent with the observation that isolated pulmonary artery ring segments of immature pigs had a heightened contractile response to α-stimulation compared to adults,[188] and the finding that aortic and pulmonary artery strips from newborn rabbits showed less smooth muscle relaxation than adult rabbits in response to the β-agonist isoproteronol.[189]

However, if an age-related change in the balance between adrenoceptor-mediated vasoconstrictor and vasodilator actions of dobutamine was the main reason for the attenuated pulmonary vasodilator response in the 1-2 day lambs, then one would expect that any pulmonary vasodilator action would be enhanced by $\alpha_1$- blockade and reduced by $\beta_2$-blockade. However, neither the magnitude of the fall in pulmonary vascular resistance nor the rise in pulmonary arterial blood pressure occurring with dobutamine were affected by pre-treatment with specific $\alpha_1$- and $\beta_2$-adrenoceptor antagonists, or by $\beta_1$-adrenoceptor blockade. Taken together, these results suggest that the attenuated pulmonary vasodilator responses to dobutamine in 1-2 day old lambs was not simply related to a developmental shift in the

adrenoceptor-mediated balance between its pulmonary vasodilator and vasoconstrictor actions.

How, therefore, can the limitation of pulmonary vasodilation observed in younger animals be explained? First, two structural features of the newborn lamb lungs point to a likely contributing factor. Thus, morphometric analysis of the neonatal lung has shown that the pulmonary arteries of >200 μm diameter and intra-acinar arteries <100 μm diameter are highly muscularized at birth, but that this muscularization recedes over approximately a two week period.[106] This muscularization may not only contribute to a higher resting pulmonary vascular resistance in the newborn, but might also limit the degree of pulmonary vasodilation during increases in pulmonary blood flow. Additional evidence exists which suggests that the pulmonary capillary bed of the newborn lamb is almost fully perfused at rest and has little additional capacity to recruit vascular reserve during increases in pulmonary blood flow.[108] It is likely that these structural properties of newborn lamb lungs and an inotrope-induced increase in cardiac output together give rise to the increase in pulmonary arterial pressure and the attenuated fall in pulmonary vascular resistance during dobutamine infusion in 1-2 day old lambs. Indeed, consistent with this hypothesis is the demonstration that the increase in pulmonary arterial pressure in response to a rise in pulmonary flow in an isolated perfused preparation of lamb lungs was accentuated in the initial days after birth.[190]

In addition to the contribution of pulmonary vascular *structure* in limiting the vasodilator response to catecholamines during the early neonatal period, the observations of the effects of NO inhibition may provide further insights into a

*functional* mechanism for this limited vasodilator response. This will be addressed in section 4.4.4.

***4.4.2 Comparison of dobutamine and dopamine effects.*** The most striking difference between the effects of dobutamine and dopamine in the younger group relates to the magnitude of the vasodilator response. While systemic and pulmonary vascular resistance fell during infusion of both agents, the reduction in systemic vascular resistance in response to dopamine was considerably less than the fall during dobutamine infusion. As a result, mean arterial pressure fell during dobutamine, but increased during dopamine infusion. It is likely that this pressor response reflects the α-adrenoreceptor-mediated systemic vasoconstrictor action of dopamine,[157-161] rather than a direct effects mediated by dopaminergic receptor stimulation as it has been shown that the selective dopaminergic agonist fenoldopam reduces, rather than increases arterial pressure.[162]

***4.4.3 Changes in basal pulmonary and systemic vascular resistance after inhibition of NO synthesis.*** Inhibition of nitric oxide synthesis with intravenous L-NNA increased systemic and pulmonary vascular resistance in both age groups studied. These observations suggest that endogenous nitric oxide plays a role in the regulation of basal systemic and pulmonary vascular tone. This is consistent with data from isolated, perfused neonatal guinea pig lungs in which inhibition of NO synthesis increased pulmonary artery pressure[121] and also agrees with observations in healthy adult humans in whom systemic infusion of a NO synthase inhibitor caused dose-dependent increases in systemic and pulmonary vascular resistances.[151] Furthermore, in piglets aged 4 to 7 days under the condition of constant lung perfusion, inhibition

of NO synthesis doubled the pulmonary vascular resistance.[191] The observation in the present studies that the effects of L-NNA were greater in the older animals is consistent with the literature which demonstrates an increasing role of endogenous NO in the regulation of pulmonary vascular resistance during early postnatal development.[174,122,175]

***4.4.4 Effect of systemic inhibition of NO synthesis on vascular responses to dobutamine.*** While endogenous nitric oxide clearly has a role in the regulation of *basal* vascular tone, recent evidence suggests an additional role for endothelial nitric oxide in modulating and even mediating catecholamine-induced vasorelaxation in the systemic and pulmonary circulations. For example, the relaxation of rat aorta in response to the β-adrenoceptor agonist isoproterenol was blunted by removal of endothelial cells or treatment with the NOS inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME). Furthermore, the relaxant response of endothelium-intact arterial segments to isoproterenol was associated with increases in tissue cGMP content, which was markedly reduced by removal of endothelium or pre-treatment with L-NAME.[192] In another model, dobutamine produced vasodilation of *in vivo* pial artery, and an increased level of cGMP in cerebrospinal fluid, both of which were blunted by L-NNA.[63] In isolated rat pulmonary arteries, the vasodilator response to the $\beta_2$-adrenoceptor agonist salbutamol was reduced substantially by L-NMMA.[193] In anaesthetised, mechanically ventilated rabbits, intravenous infusions of adrenaline elicited dose-dependent increases in exhaled nitric oxide, which were inhibited by the β-adrenoceptor antagonist propranolol. Prenalterol, a $\beta_1$-agonist, and terbutaline, a $\beta_2$-agonist, also caused dose-dependent increases in exhaled NO.[194] Finally, our own data from adult sheep, using an identical preparation to the current demonstrated

that while under basal conditions, dobutamine infusion resulted in a fall in pulmonary and systemic vascular resistances, after pre-treatment with L-NNA, dobutamine infusion resulted in an *increase* in pulmonary vascular resistance.

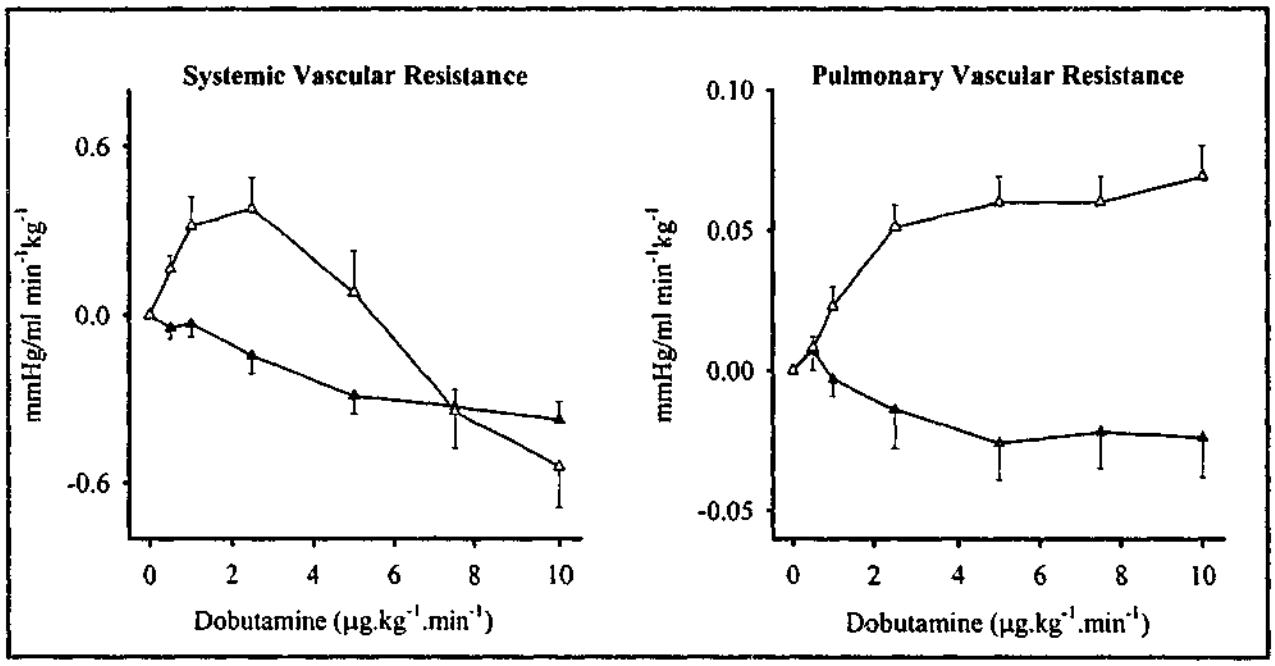


***Figure 4:8.*** *Changes in systemic and pulmonary vascular resistance during incremental infusion in anaesthetised, open-chested adult sheep, before* (▲) *and after* (△) *L-NNA administration (own data).*

Given the role of NO in mediating the vasodilator response to catecholamines, it was significant that in the 1-2 day animals, the reductions in systemic and pulmonary vascular resistance were not altered by L-NNA infusion. In the older animals, the reduction in vascular resistances at low infusion rates of dobutamine was enhanced by NO inhibition. However, it is likely that this enhanced vasodilation after L-NNA reflects a non-specific effect of elevated tone, which is known to augment the vasodilator response to catecholamines,[195-197] rather than an effect of L-NNA per se. This hypothesis is supported by the observation that in this group, both systemic and pulmonary vascular resistances were similar before and after L-NNA during

dobutamine infusion at rates exceeding 2.5μg.kg$^{-1}$.min$^{-1}$. Taken together these observations suggest that the important contribution of NO to enhancing vascular dilation during catecholamine infusion does not occur in the neonatal vasculature. While the absence of this important catecholamine-related increase in NO synthesis may potentially contribute to the blunting of the vasodilator response which was observed in response to adrenergic stimulation in the perinatal period, further studies to specifically examine the function-structure relationships of the systemic and pulmonary vasculature during postnatal development will be required to address this hypothesis.

*4.4.5 Conclusions.* These studies have demonstrated that the vasodilator response to catecholamine infusions undergoes considerable change during the early postnatal period. The vasodilator response to dobutamine and dopamine were blunted in the early neonatal period and this appeared to be specific to the pulmonary vascular bed. It is likely that this blunted pulmonary vasodilator response was related not only to important structural features of the neonatal pulmonary vasculature, but also to an apparent absence of catecholamine-related NO release, which appears to make an important contribution to the vasodilator response to adrenergic stimulation in the adult circulation.

# CHAPTER 5. SYSTEMIC OXYGEN DELIVERY AND CONSUMPTION

"All the vital mechanisms, however varied they may be, have only one object, that of preserving constant, the conditions of life in the internal environment"

Claude Bernard.[198]

An important goal of inotropic therapy is to promote an environment conducive to adequate tissue oxygenation by increasing systemic $O_2$ delivery relative to $O_2$ consumption. This chapter tests the hypothesis that in the newborn, the balance between systemic $O_2$ delivery and consumption is altered during infusions of dobutamine or dopamine by a prominent thermogenic response. It examines the relative contributions of specific adrenoreceptor subtypes to this thermogenic response and explores the role of NO in modulating it.

Infusion of both dobutamine and dopamine in 1-2 day old lambs was accompanied by a profound thermogenic response coupled with a substantial rise in systemic $O_2$ consumption, which consumed much of the increase in systemic $O_2$ delivery. The profound thermogenic response and the exaggerated increase in systemic $O_2$ consumption disappeared by the end of the first postnatal week. These effects were not significantly affected by individual $\alpha_1$, $\beta_1$ or $\beta_2$ adrenoceptor blockade, but were markedly blunted by combined blockade of these receptors and by systemic inhibition of NO synthesis with intravenous L-NNA.

These observations suggest that a prominent thermogenic response to dobutamine and dopamine in the early neonatal period may have adverse effects on the balance between systemic $O_2$ delivery and consumption, thereby diminishing the effectiveness of inotropic therapy in the newborn.

## 5.1 INTRODUCTION

A important goal of inotropic therapy is to promote an environment conducive to adequate tissue oxygenation by increasing systemic $O_2$ delivery relative to systemic $O_2$ consumption.[199-203] Dobutamine and dopamine both increase cardiac output and augment systemic $O_2$ delivery in adults,[35,32-34] but through their effects on metabolic rate they also increase $O_2$ consumption.[37-39] However, this increase in $O_2$ consumption is relatively minor, compared to the increase in $O_2$ delivery.[35,32,37,34] While it has been suggested that another important regulator of basal tissue metabolism and $O_2$ consumption is NO,[71,74,73,204] we have shown that the changes in systemic $O_2$ delivery or consumption during dobutamine infusion are *not* altered by NO inhibition in adult sheep.[27]

In contrast to the adult, little or no information is available about the effects of catecholamine-related inotropes such as dobutamine or dopamine, on systemic $O_2$ delivery and consumption in the young neonate. This is of particular importance because neonatal survival is in part dependent on the maintenance of body temperature by the catecholamine-dependent mechanism of non-shivering thermogenesis in brown adipose tissue (BAT).[205] Thermogenesis in BAT is normally activated by the release of noradrenaline from sympathetic nerve terminals and its subsequent interaction with adrenoreceptors within it.[206] In vitro studies have demonstrated the ability of dobutamine[207-209] and dopamine[210,211] to activate lipolysis in isolated brown adipocytes. However, it is unknown whether these agents have any thermogenic actions in the newborn, and if so, to what extent these may impact on the relative alterations in $O_2$ delivery and consumption in this setting. Finally, while it has been suggested that endogenous NO may regulate

thermogenesis[66,212-214] in BAT and the influence of catecholamines on such thermogenesis,[125] the potential modulation by NO of the changes in systemic $O_2$ balance during catecholamine infusion in the young neonate remain unexplored.

cordingly, this chapter will test the hypothesis that infusions of the catecholamines obutamine and dopamine elicit a thermogenic response in the newborn, and that this response may alter the balance between systemic $O_2$ delivery and consumption. In order to determine whether the response of the newborn lamb to dobutamine was related to $\alpha_1$-, $\beta_1$- or $\beta_2$-adrenoceptor effects or to combinations of these adrenoceptor subtypes, additional experiments were performed in subgroups of animals following pretreatment with selective adrenoceptor blockade. Finally, the role of NO in modulating the effects of dobutamine was examined in a further subgroup of animals, in which the response to dobutamine was examined, before and after inhibition of NO synthesis.

## *5.2 Methods*

***Surgical preparation.*** Fifty-eight lambs, of which 36 were aged 1-2 days and weighed 4.6±0.8 kg and 22 were aged 7-10 days and weighed 6.2±0.3kg, were surgically prepared under general anaesthesia, as described in Chapter 2. As part of the preparation, teflon cannulae were inserted through adventitial purse-string sutures in the descending thoracic aorta and pulmonary trunk for blood sampling and an ultrasonic, perivascular flow probe was placed around the ascending aorta. Central temperature was measured in 51 animals with a thermistor in the pulmonary trunk, while rectal temperature was measured in the remaining 7 animals.

***Experimental protocol.*** The first protocol examined the changes in systemic oxygenation variables and body temperature during an incremental infusion of dobutamine at rates up to $40\mu g.kg^{-1}.min^{-1}$ in seven 1-2 day and seven 7-10 day old animals. In a further group of thirteen 1-2 day lambs, dobutamine was infused at similar rates after adrenoceptor blockade with either the $\alpha_1$-adrenoceceptor antagonist prazosin (n=3), the $\beta_1$-adrenoceptor antagonist CGP 20712A (n=3), the $\beta_2$-adrenoceptor antagonist ICI 118551 (n=3) or combined $\alpha_1$-, $\beta_1$- and $\beta_2$-adrenoreceptor blockade with simultaneous infusion of prazosin, CGP 20712A and ICI 118551 (n=4). In sixteen animals, of which nine were 1-2 day and seven, 7-10 days old, the effects of an incremental intravenous infusion of dopamine at rates up to $40\mu g.kg^{-1}.min^{-1}$ were studied. Finally, the effects of inhibition of NO synthesis on dobutamine-related responses were investigated in fourteen animals, of which seven were 1-2 days and seven 7-10 days old. In this last group of studies, the responses to an incremental infusion of dobutamine at rates up to $10\mu g.kg^{-1}.min^{-1}$ were studied, before and after inhibition of NO synthesis with an intravenous infusion of $N^{\omega}$-nitro-L-arginine (L-NNA; 25 mg/kg), as described in detail in Chapter 2.

***Physiologic measurements and calculations.*** Cardiac output was obtained by measuring ascending aortic blood flow with an ultrasonic flowmeter. Blood gases were analyzed at the measured central temperature. Haemoglobin (Hb) and haemoglobin $O_2$ saturation (HbS) were measured photometrically with a haemoximeter. Oxygen content ($ml.dl^{-1}$) in the aorta ($C_{Ao}O_2$) and pulmonary artery ($C_{PA}O_2$) were calculated as $(1.36 \cdot Hb \cdot HbS/100) + (0.003 \cdot PO_2)$. The systemic arteriovenous (a-v $O_2$) content difference was calculated as $C_{Ao}O_2 - C_{PA}O_2$ and the

systemic $O_2$ extraction coefficient as $(C_{A_o}O_2 - C_{PA}O_2) / C_{A_o}O_2$, while systemic $O_2$ delivery was derived from the product of cardiac output and $C_{A_o}O_2$, and systemic $O_2$ consumption from the product of cardiac output and the systemic a-v $O_2$ content difference.

## *5.3 RESULTS*

### *5.3.1 Baseline Variables.*

Within each age group, baseline variables did not significantly differ between animals assigned to the different protocols. For this reason, the baseline variables for the twenty-three 1-2 day and the twenty-two 7-10 day animals which were entered into matched protocols (either dobutamine or dopamine alone, or dobutamine and L-NNA), were compared. Cardiac output and systemic $O_2$ delivery were lower ($p<0.05$ and $p<0.001$ respectively) in the 7-10 Day lambs. The a-v $O_2$ content difference ($p<0.05$), $O_2$ extraction ratio ($p<0.05$) and temperature were higher ($p<0.01$) in the 7-10 day group, while systemic $O_2$ consumption was similar in the two groups ***(Table 5:1)***

### *5.3.2 Changes in Systemic $O_2$ Delivery and Consumption During Dobutamine.*

***Cardiac output.*** Dobutamine produced a rise in cardiac output in both age groups ($p< 0.001$ for both). However, the increment at the peak infusion rate in 1-2 day lambs ($138 \pm 25$ ml·min$^{-1}$·kg$^{-1}$) was more pronounced than in the 7-10 day group ($97 \pm 17$ml·min$^{-1}$·kg$^{-1}$; $p < 0.02$).

| | Group | | |
|---|---|---|---|
| | **1-2 day (n=23)** | **7-10 day (n=22)** | **P Value** |
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 182±7 | 152±9 | <0.05 |
| **A-V $O_2$ Content Difference ($ml \cdot dl^{-1}$)** | 3.9±0.2 | 4.6±0.3 | <0.05 |
| **Systemic $O_2$ Extraction Ratio** | 0.27±0.01 | 0.38±0.02 | <0.001 |
| **Systemic $O_2$ Delivery ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 26.2±1.2 | 18.0±0.8 | <0.001 |
| **Systemic $O_2$ Consumption ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 6.8±0.3 | 6.6±0.2 | N.S. |
| **Core Temperature ($^{o}C$)** | 38.9±0.2 | 39.9±0.2 | <0.01 |

*Table 5:1. Baseline cardiac output, systemic oxygenation and core temperature in 1-2 day and 7-10 day animals.*

***A-V$O_2$ Content Difference and Systemic $O_2$ extraction.*** The a-v $O_2$ content difference in 1-2 day lambs rose progressively to a level, which was 1.8 ± 0.6 $ml.dl^{-1}$ above baseline at the highest dobutamine infusion rate ($p<0.001$). In contrast, it fell in 7-10 day animals with dobutamine to levels which, on average, were 1.1 ± 0.3 $ml.dl^{-1}$ below baseline ($p<0.001$). Systemic $O_2$ extraction ratio in 1-2 day animals increased to 0.19 ± 0.03 above baseline at the peak dobutamine infusion rate ($p< 0.001$), but fell by 0.07 ± 0.01 in 7-10 day group ($p = 0.001$).

***Systemic $O_2$ delivery, $O_2$ consumption and body temperature.*** Systemic $O_2$ delivery increased progressively during dobutamine infusion in both groups ($p < 0.001$), with no significant difference between increments in 1-2 day (13.8 ± 3.2 $ml \cdot min^{-1} \cdot kg^{-1}$) and 7-10 day (8.8 ± 3.0 $ml \cdot min^{-1} \cdot kg^{-1}$) animals. Systemic $O_2$ consumption also increased in both groups. Importantly, at infusion rates ≥10 $\mu g.kg^{-1}.min^{-1}$ in 1-2 day lambs, dobutamine progressively increased $O_2$ consumption ($p < 0.001$) to a peak level which was 11.2 ± 1.6 $ml \cdot min^{-1} \cdot kg^{-1}$ above baseline. This response, which

'consumed' around 80% of the corresponding rise in $O_2$ delivery, was 9-fold greater ($p < 0.001$) than the increment of $1.8 \pm 1.2$ ml·min$^{-1}$·kg$^{-1}$ in the 7-10 day group and importantly, was not associated with shivering.

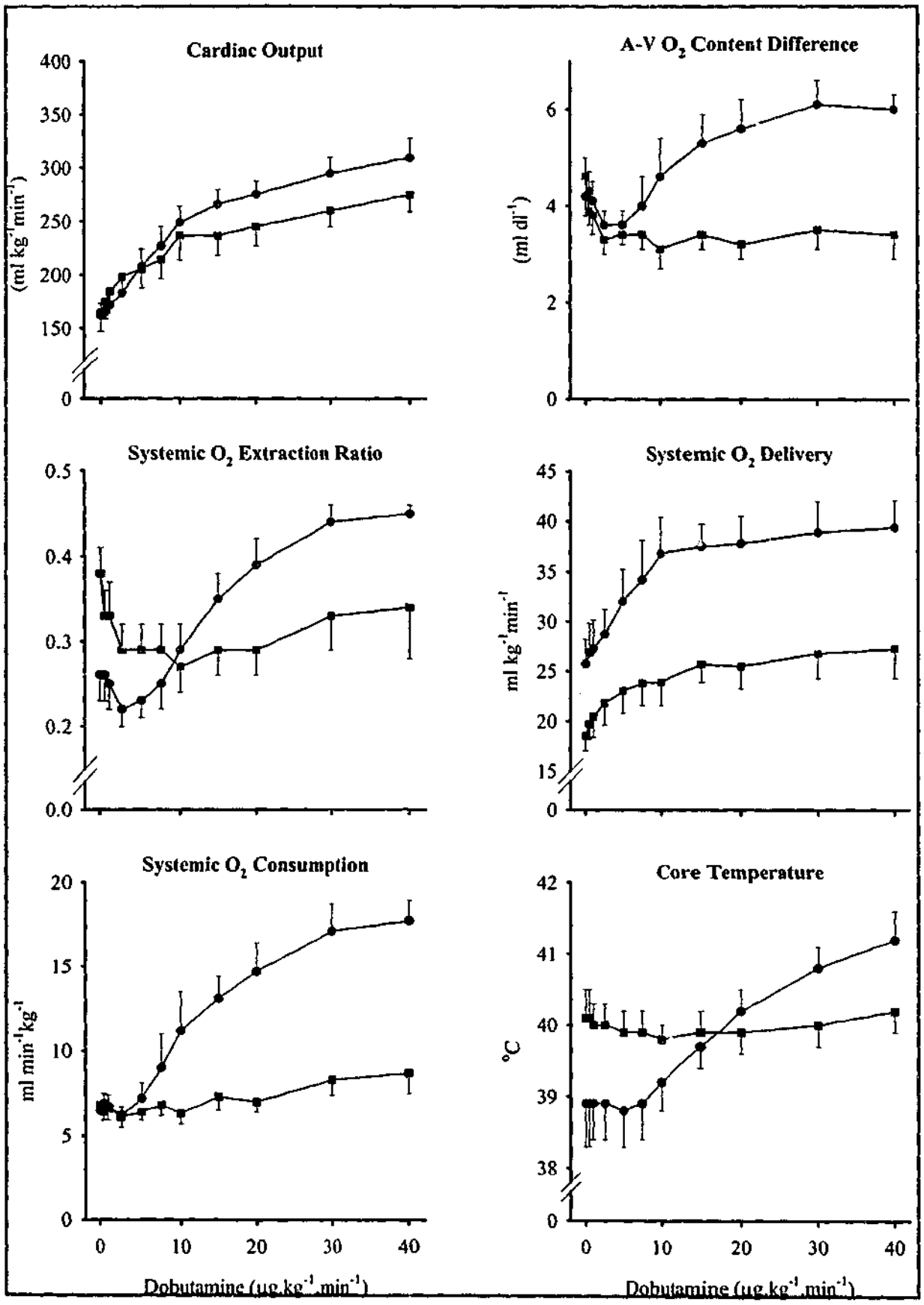


***Figure 5:1.*** *Cardiac output, systemic oxygenation and core temperature responses during incremental infusion of dobutamine in 1-2 day (●) and 7-10 day (■) lambs.*

This rise in systemic $O_2$ consumption, was accompanied by a significant increase in body temperature in 1-2 day animals to peak at 2.2 ± 0.6 °C above baseline ($p<0.001$), while temperature did not change significantly in the 7-10 day lambs.

### *5.3.3 Effect of adrenoreceptor blockade on the responses to Dobutamine in 1-2 day Lambs.*

Individual or combined $\alpha_1$, $\beta_1$ and $\beta_2$ adrenoceptor blockade was not associated with any significant changes in the baseline cardiac output, a-v $O_2$ content difference, systemic $O_2$ extraction ratio, delivery, consumption or core temperature ***(Table 5:2)***.

| Variable | State | $\alpha_1$-blockade (n=3) | $\beta_1$-blockade (n=3) | $\beta_2$-blockade (n=3) | $\alpha_1/\beta_1/\beta_2$-blockade (n=4) |
|---|---|---|---|---|---|
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | Pre-block | 165 ± 48 | 181 ± 28 | 135 ± 39 | 165 ± 4 |
| | Post-block | 163 ± 46 | 170 ± 27 | 136 ± 37 | 151 ± 6 |
| **A-V $O_2$ Content Difference ($ml \cdot dl^{-1}$)** | Pre-block | 4.1 ± 0.3 | 3.4 ± 0.8 | 4.2 ± 0.2 | 4.4 ± 0.5 |
| | Post-block | 3.8 ± 0.3 | 3.5 ± 0.6 | 4.5 ± 0.4 | 4.9 ± 0.6 |
| **Systemic $O_2$ Extraction Ratio** | Pre-block | 0.28 ± 0.03 | 0.22 ± 0.03 | 0.27 ± 0.02 | 0.26 ± 0.03 |
| | Post-block | 0.27 ± 0.02 | 0.24 ± 0.01 | 0.29 ± 0.03 | 0.28 ± 0.04 |
| **Systemic $O_2$ Delivery ($ml \cdot min^{-1} \cdot kg^{-1}$)** | Pre-block | 24.3 ± 7.1 | 26.1 ± 4.0 | 20.8 ± 4.7 | 22.8 ± 1.0 |
| | Post-block | 23.5 ± 6.6 | 24.2 ± 3.9 | 20.9 ± 4.6 | 20.9 ± 1.1 |
| **Systemic $O_2$ Consumption ($ml \cdot min^{-1} \cdot kg^{-1}$)** | Pre-block | 6.4 ± 1.3 | 5.8 ± 0.9 | 5.5 ± 1.4 | 5.9 ± 0.6 |
| | Post-block | 6.0 ± 1.2 | 5.6 ± 0.6 | 5.8 ± 1.1 | 5.8 ± 0.7 |
| **Core Temperature (°C)** | Pre-block | 39.6 ± 0.9 | 38.4 ± 0.3 | 39.6 ± 0.7 | 39.1 ± 0.4 |
| | Post-block | 39.4 ± 0.8 | 38.4 ± 0.4 | 39.6 ± 0.7 | 39.3 ± 0.3 |

***Table 5:2*** *Comparison of cardiac output, systemic oxygenation and core temperature before and after selective and combined adrenoreceptor blockade in 1-2 day lambs.*

However, pre-treatment with combined $\alpha_1$, $\beta_1$ and $\beta_2$ adrenoceptor blockade markedly reduced the cardiac output response to dobutamine, and also prevented

significant rises in the a-v $O_2$ content difference, extraction ratio, $O_2$ delivery and consumption, as well as body temperature during dobutamine therapy ***(Figure 5:2)***.

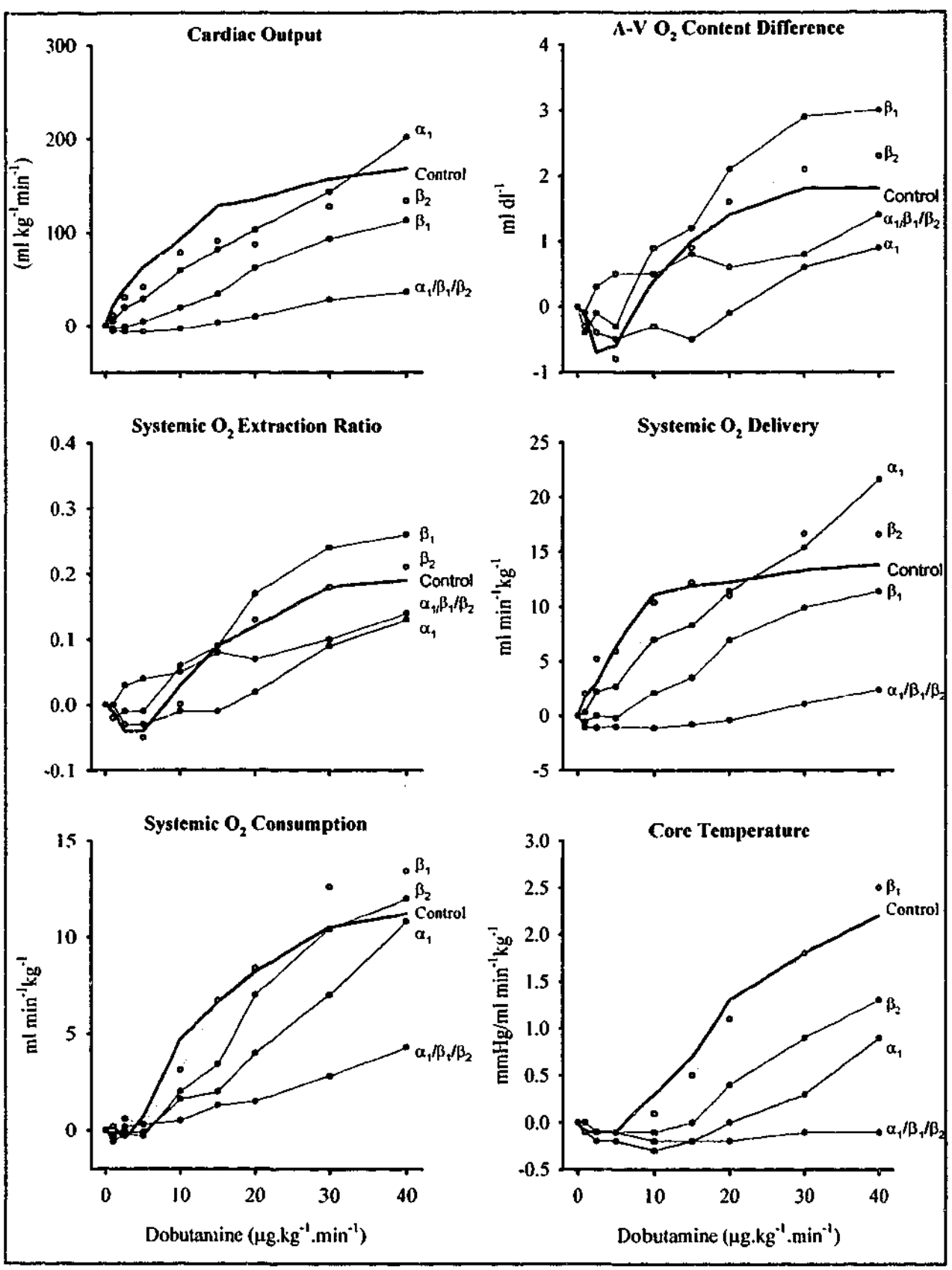


***Figure 5:2*** *Changes in cardiac output, systemic oxygenation and core temperature in 1-2 day lambs during dobutamine infusion in the absence of (control; –) or presence of* $\alpha_1$ (–), $\beta_1$ (–), $\beta_2$ ( ) *or combined* $\alpha_1,\beta_1,\beta_2$ (–) *adrenoceptor blockade.*

### *5.3.4 Changes in systemic $O_2$ delivery and consumption during Dopamine Infusion.*

***Cardiac output.*** Cardiac output was unchanged by lower infusion rates (up to 10μg.kg$^{-1}$.min$^{-1}$) of dopamine in both the 1-2 day and 7-10 day animals. Higher infusion rates (greater than 10μ.kg$^{-1}$.min$^{-1}$) progressively increased cardiac output in both groups (both $p<0.001$). The peak increment, which occurred at the highest infusion rate was more pronounced in the 1-2 day (169±14 ml·min$^{-1}$·kg$^{-1}$) than in the 7-10 day animals (115±13 ml·min$^{-1}$·kg$^{-1}$; $p<0.05$; ***Figure 5:3)***.

***Systemic A-V $O_2$ Content Difference and $O_2$ extraction.*** In the 1-2 day animals a biphasic change in the a-v $O_2$ content difference occurred with an increasing rate of dopamine infusion. The a-v $O_2$ content difference initially fell at infusion rates up to 10 μg.kg$^{-1}$.min$^{-1}$ to a level which was 1.1±0.1 ml·dl$^{-1}$ below the baseline value ($p<0.05$). However, it then progressively rose at higher infusion rates ($p<0.001$) reaching a peak of 5.0±0.4 ml·dl$^{-1}$ at the highest infusion rate, which was significantly greater than baseline ($p<0.01$). In contrast, in the 7-10 day group, the a-v $O_2$ content difference was initially unchanged by dopamine, and then progressively fell at doses greater than 7.5μg.kg$^{-1}$.min$^{-1}$ ($p<0.001$; ***Figure 5:3***).

Changes in $O_2$ extraction followed a similar pattern, with the systemic $O_2$ extraction ratio falling in the 1-2 day animals at infusion rates up to 10 μg.kg$^{-1}$.min$^{-1}$ and then progressively rising at higher infusion rates ($p<0.001$) to a peak level of 0.45±0.03, which was significantly higher than baseline ($p<0.001$). However, in the 7-10 day group, the systemic $O_2$ extraction ratio progressively fell with increasing infusion

rates above 7.5μg.kg$^{-1}$.min$^{-1}$ ($p<0.001$) to reach a level, which was 0.22±0.02 below baseline ***(Figure 5:3)***.

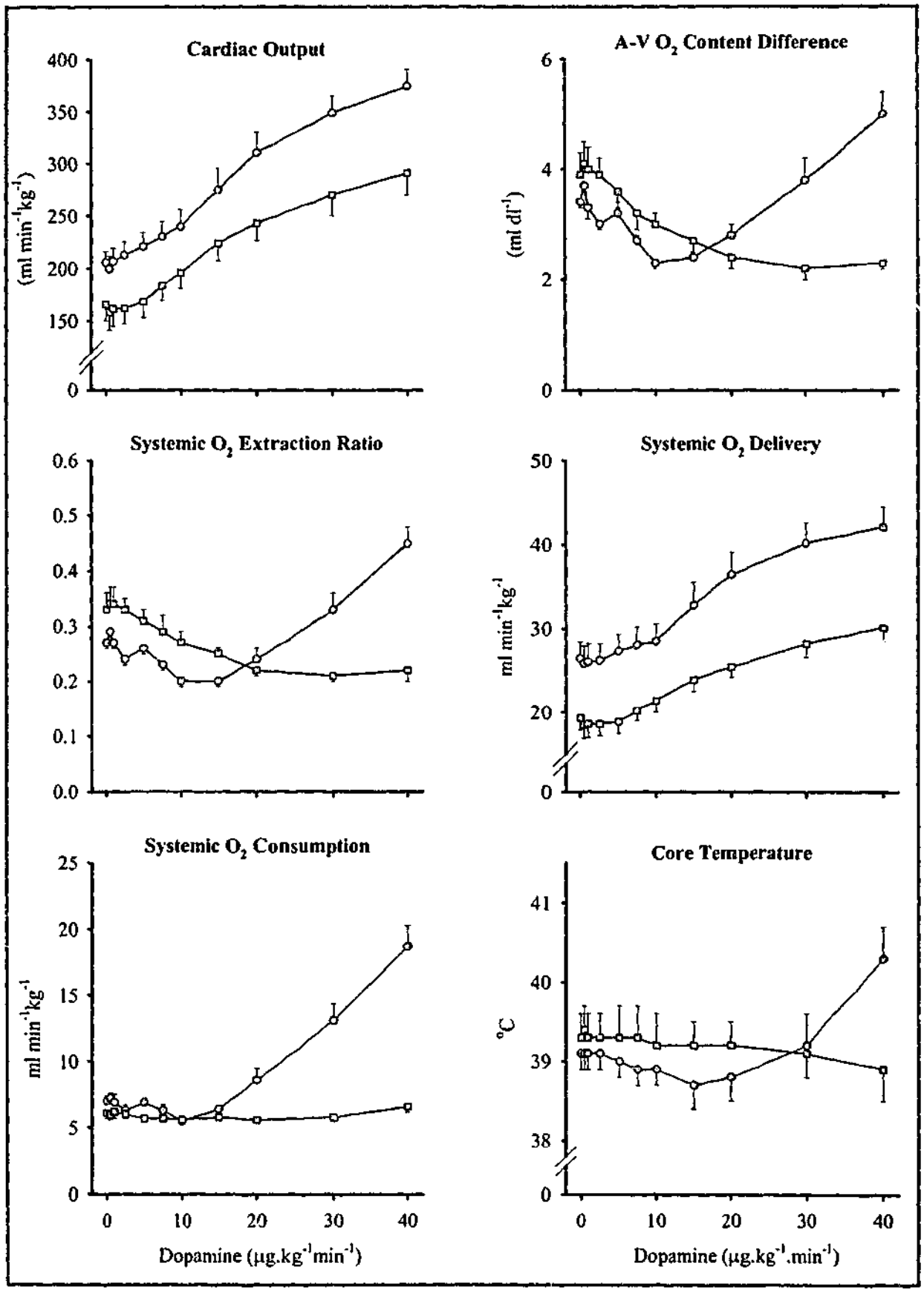


***Figure 5:3.*** *Cardiac output, systemic oxygenation and core temperature responses during incremental infusion of dopamine in 1-2 day (•) and 7-10 day (▪) lambs.*

***Systemic $O_2$ delivery, $O_2$ consumption and body temperature during dopamine infusion.*** Systemic $O_2$ delivery was initially unchanged at lower infusion rates of dopamine, but increased progressively in both groups during infusion rates above 10μg.kg$^{-1}$.min$^{-1}$ ($p<0.001$). The peak increase of 15.6±1.8 ml·min$^{-1}$·kg$^{-1}$ in the 1-2 day group was greater than the 10.8±0.6 ml.min$^{-1}$.kg$^{-1}$ , which occurred in the 7-10 day animals ($p<0.05$). Systemic $O_2$ consumption also increased significantly in both groups with infusion rates above 10μg.kg$^{-1}$.min$^{-1}$. However, the peak increase in $O_2$ consumption of 11.7±1.6 ml·min$^{-1}$·kg$^{-1}$ in the 1-2 day animals was more than 20 fold greater ($p<0.001$) than the increase of 0.5±0.5 ml.min$^{-1}$.kg$^{-1}$ in the 7-10 day group. Finally, body temperature was increased by 1±0.4 °C in the 1-2 day group ($p<0.001$), but was unchanged by dopamine infusion in the 7-10 day animals ***(Figure 5:3)***.

### *5.3.5 Comparison of Dobutamine and Dopamine Responses.*

Peak changes in cardiac output, systemic $O_2$ delivery, a-v $O_2$ content difference, as well as systemic $O_2$ extration ratio and O2 consumption were similar during dobutamine and dopamine infusions in both groups studied,but in the 1-2 day animals, the peak increase in core temperature tended to be higher after dobutamine infusion (2.2±0.6 °C) than after dopamine (1.0±0.4 °C; $p<0.1$). However, despite the similarity in the magnitude of the peak responses to dobutamine and dopamine, effects on most variables appeared to emerge at much lower infusion rates in animals given dobutamine. ***(Figure 5:4 & 5:5; Table 5:3)***.

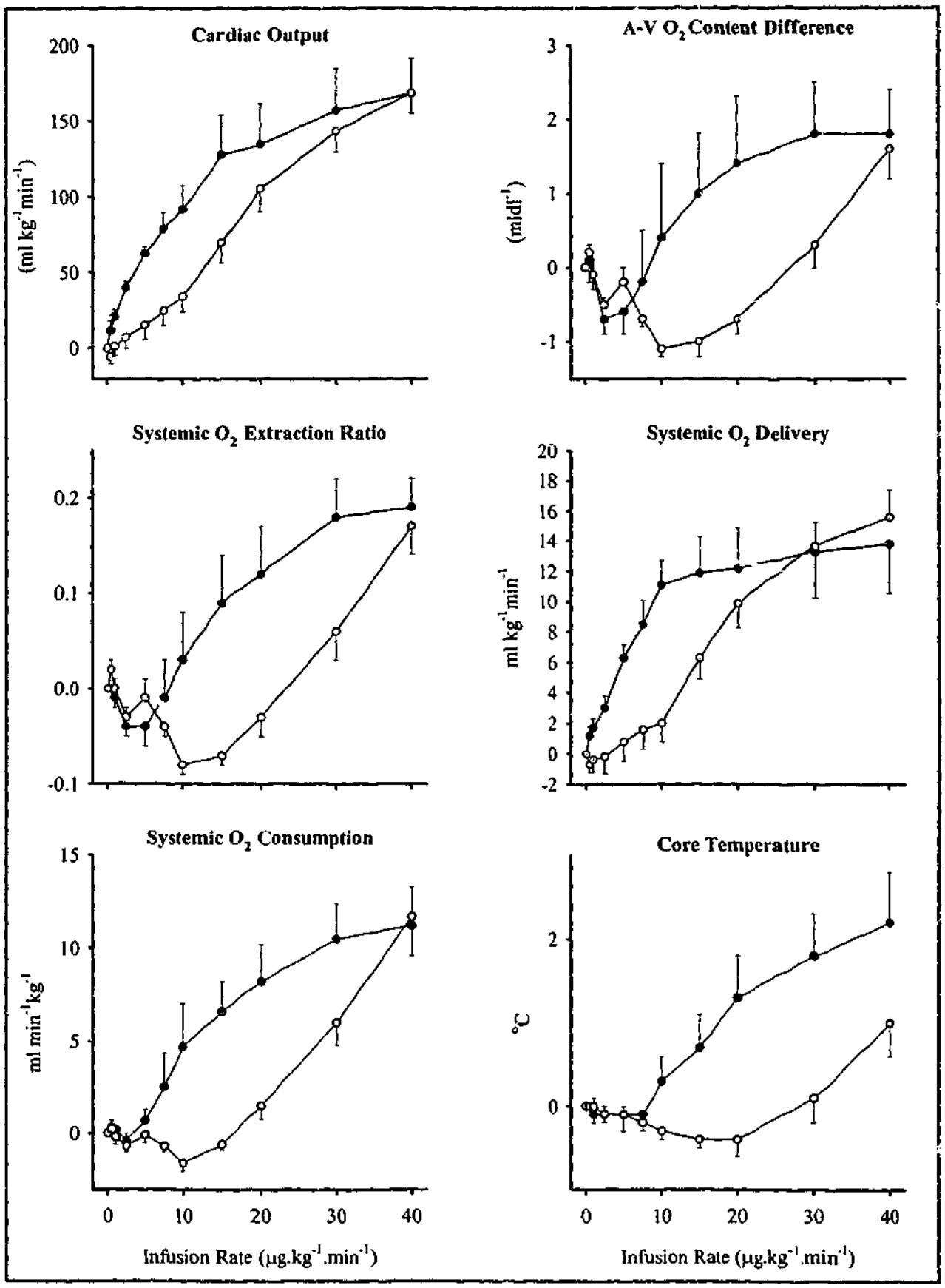


***Figure 5:4.*** *Cardiac output, systemic oxygenation and core temperature responses in 1-2 day animals during incremental infusions of dobutamine* (•) *and dopamine* (•).

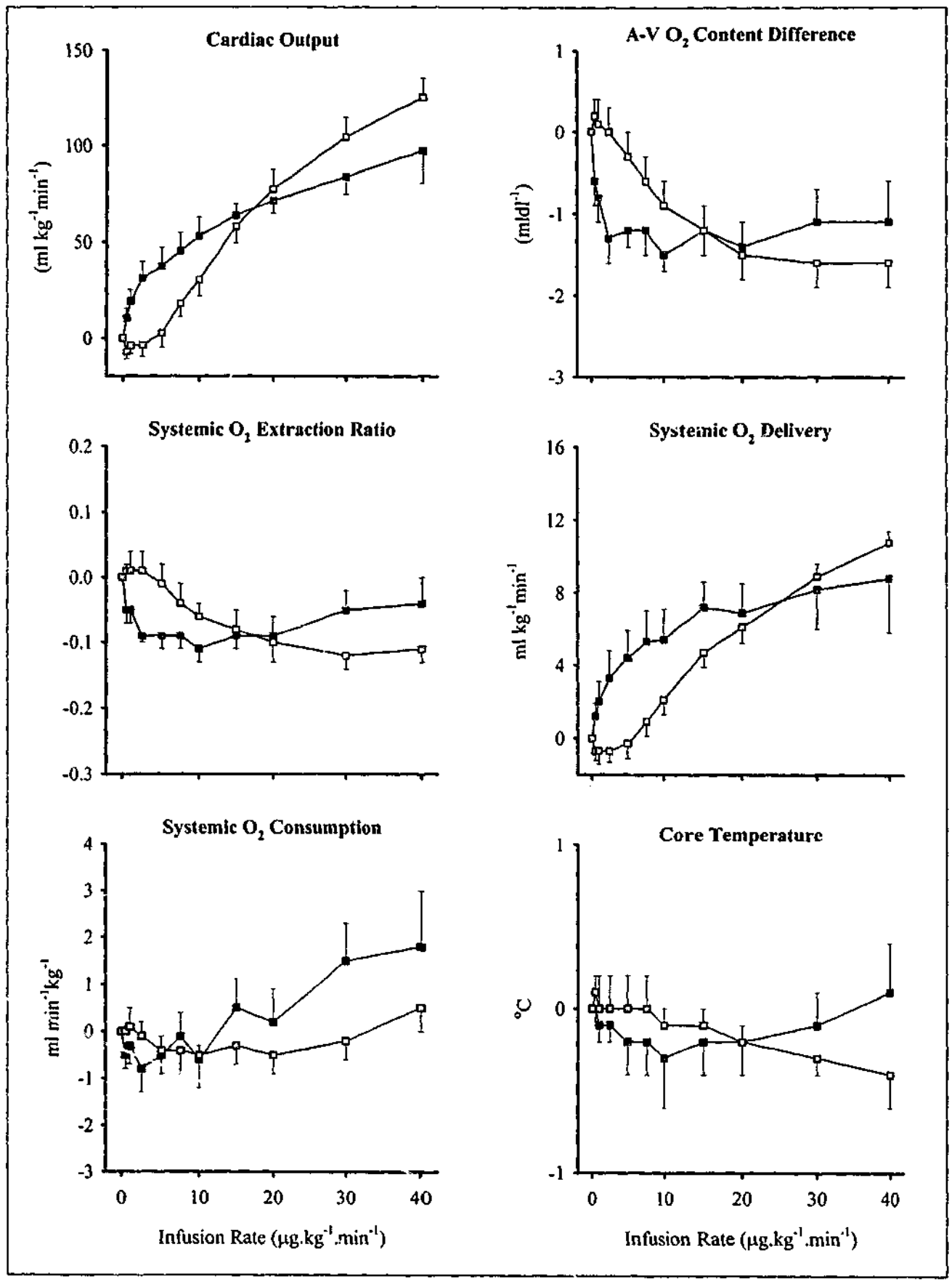


***Figure 5:5.*** *Cardiac output, systemic oxygenation and core temperature responses in 7-10 day animals during incremental infusions of dobutamine* (■) *and dopamine* (■).

| | | Dobutamine | Dopamine | P Value |
|---|---|---|---|---|
| **Cardiac Output ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 138±25 | 169±14 | N.S. |
| | 7-10 days | 98±17 | 125±10 | N.S. |
| **A-V $O_2$ Content Difference ($ml \cdot dl^{-1}$)** | 1-2 days | 1.8±0.6 | 1.6±0.4 | N.S. |
| | 7-10 days | -1.1±0.5 | -1.6±0.3 | N.S. |
| **Systemic $O_2$ Extraction Ratio** | 1-2 days | 0.19±0.03 | 0.17±0.03 | N.S. |
| | 7-10 days | -0.04±0.04 | -0.1±0.0 | N.S. |
| **Systemic $O_2$ Delivery ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 13.8±3.2 | 15.6±1.8 | N.S. |
| | 7-10 days | 8.8±3.0 | 10.8±0.6 | N.S. |
| **Systemic $O_2$ Consumption ($ml \cdot min^{-1} \cdot kg^{-1}$)** | 1-2 days | 11.2±1.6 | 11.7±1.6 | N.S. |
| | 7-10 days | 1.8±1.2 | 0.5±0.5 | N.S. |
| **Core Temperature (°C)** | 1-2 days | 2.2±0.6 | 1.0±0.4 | <0.1 |
| | 7-10 days | 0.1±0.3 | -0.4±0.2 | N.S. |

***Table 5:3.*** *Comparison of peak changes in cardiac output, systemic oxygenation and core temperature during incremental infusion of dobutamine and dopamine in neonatal lambs*

### *5.3.6 Effects of NO inhibition on the responses to Dobutamine.*

***Effect of NO synthase inhibition on baseline variables.*** Intravenous administration of L-NNA at a dose of 25 mg/kg did not alter any of the measured variables in the 1-2 day animals, but reduced cardiac output ($p<0.01$) and systemic $O_2$ delivery ($p<0.05$) in the 7-10 Day group, while increasing increasing the a-v $O_2$ content difference and $O_2$ extraction ratio (both $p<0.001$), with no significant effects on systemic $O_2$ consumption or core temperature ***(Table 5:4).***

| | | Before L-NNA | After L-NNA | P Value |
|---|---|---|---|---|
| **Cardiac Output** | 1-2 days | 156±5 | 148±8 | N.S. |
| **($ml \cdot min^{-1} \cdot kg^{-1}$)** | 7-10 days | 131±6 | 115±7 | <0.01 |
| **A-V $O_2$ Content Difference** | 1-2 days | 4.1±0.2 | 4.3±0.3 | N.S. |
| **($ml \cdot dl^{-1}$)** | 7-10 days | 4.8±0.3 | 5.7±0.3 | <0.001 |
| **Systemic $O_2$ Extraction Ratio** | 1-2 days | 0.30±0.02 | 0.30±0.02 | N.S. |
| | 7-10 days | 0.43±0.03 | 0.51±0.04 | <0.001 |
| **Systemic $O_2$ Delivery** | 1-2 days | 21.7±1.1 | 21.4±1.7 | N.S. |
| **($ml \cdot min^{-1} \cdot kg^{-1}$)** | 7-10 days | 15.0±1.1 | 13.4±1.3 | <0.05 |
| **Systemic $O_2$ Consumption** | 1-2 days | 6.4±0.4 | 6.3±0.4 | N.S. |
| **($ml \cdot min^{-1} \cdot kg^{-1}$)** | 7-10 days | 6.2±0.2 | 6.5±0.2 | N.S. |
| **Core Temperature** | 1-2 days | 39.0±0.5 | 38.9±0.4 | N.S. |
| **($^oC$)** | 7-10 days | 40.0±0.3 | 40.0±0.3 | N.S. |

***Table 5:4*** *Comparison of cardiac output, systemic oxygenation and core temperature, before and after L-NNA in 1-2 and 7-10 day lambs.*

***Responses to dobutamine before and after NO synthase inhibition.*** In the 1-2 day animals, the increase in cardiac output of 78±12 $ml \cdot min^{-1} \cdot kg^{-1}$ which occurred during infusion of dobutamine alone, was attenuated by pre-administration of L-NNA (53±10 $ml \cdot min^{-1} \cdot kg^{-1}$; $p<0.05$). While in this group, the a-v $O_2$ content difference was unchanged by the first dobutamine infusion, after L-NNA, dobutamine resulted in a progressive fall in the a-v $O_2$ content difference to a level which was 0.9±0.3 $ml \cdot dl^{-1}$ below baseline ($p<0.001$). Furthermore, in this group, systemic $O_2$ extraction

ratio was unchanged by dobutamine alone, but was significantly reduced by 0.05±0.02 $ml \cdot dl^{-1}$ during dobutamine infusion after L-NNA ($p<0.001$). Systemic $O_2$ delivery increased by 8.4±1.3 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p<0.001$) during the first dobutamine infusion in the 1-2 day animals and the dobutamine-related increase in $O_2$ delivery was unaltered by L-NNA administration. By contrast, the increases in systemic $O_2$ consumption of 2.7±1.2 $ml \cdot min^{-1} \cdot kg^{-1}$ ($p<0.01$) and in central temperature of 0.5±0.2 °C ($p<0.05$) which occurred during the first dobutamine infusion in the 1-2 day animals, were abolished by prior L-NNA administration ***(Figure 5:6)***.

In the 7-10 day animals, dobutamine infusion resulted in an increase in cardiac output and reductions in a-v $O_2$ content difference and systemic $O_2$ extraction ratio, which were unaltered by L-NNA administration. Furthermore, in this group, systemic $O_2$ consumption and core termperature were unaltered by dobutamine infusion both before, or after L-NNA administration ***(Figure 5:7)***.

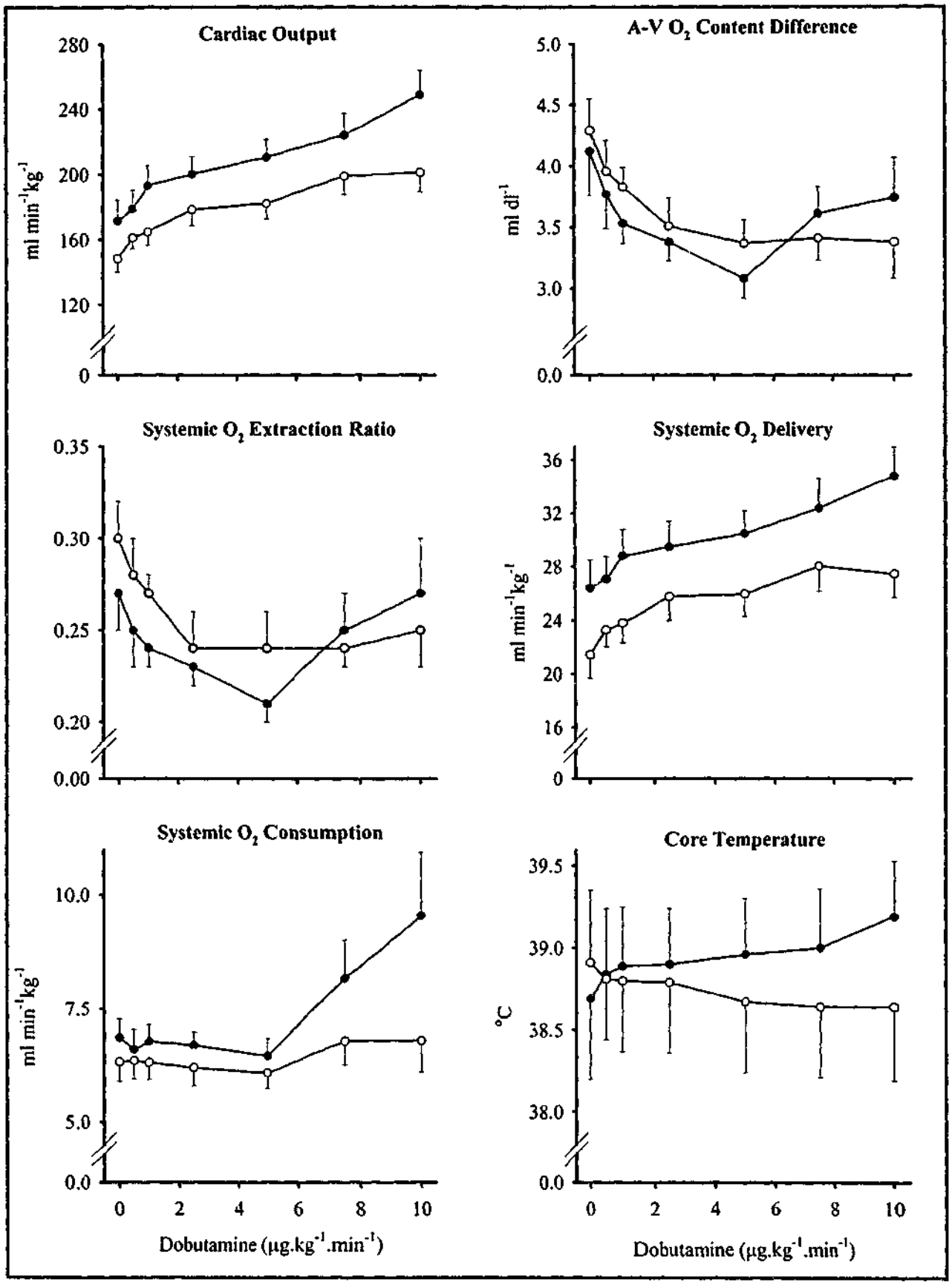


***Figure 5:6.*** *Cardiac output, systemic oxygenation and core temperature responses in 1-2 day animals during incremental infusions of dobutamine before* (•) *and after L-NNA administration* (○).

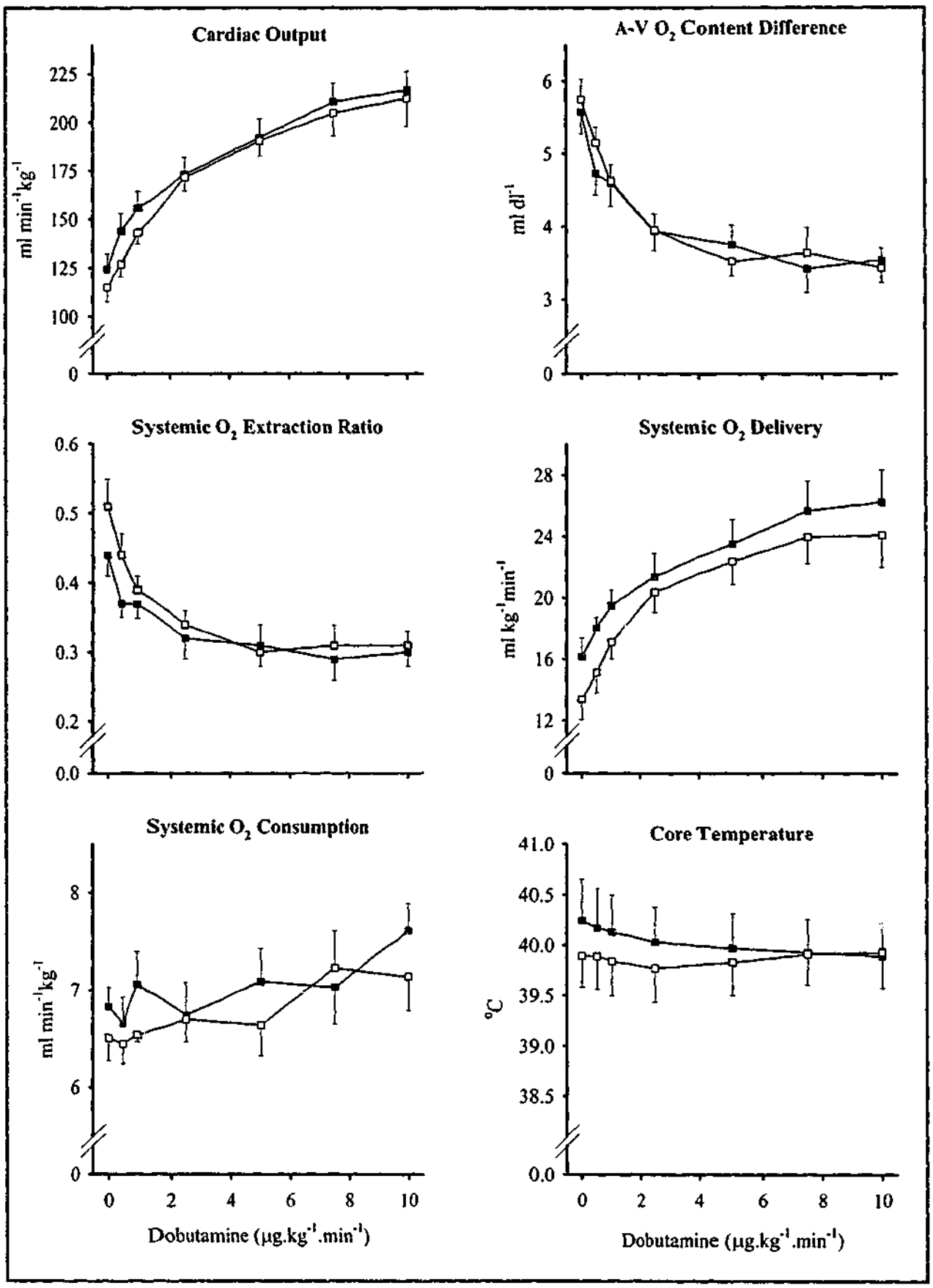


*Figure 5:7. Cardiac output, systemic oxygenation and core temperature responses in 7-10 day animals during incremental infusions of dobutamine before* (■) *and after L-NNA administration* (□).

## 5.4 Discussion

These studies have produced three main findings. First, infusion of both dobutamine and dopamine in 1-2 day old lambs produced a profound thermogenic response associated with a substantial rise in systemic $O_2$ consumption that 'utilized' or consumed a major portion of the increase in systemic $O_2$ delivery. This thermogenic response and the associated, exaggerated increase in systemic $O_2$ consumption disappeared by the end of the first postnatal week. Second, the increase in body temperature and systemic $O_2$ consumption in 1-2 day lambs was not significantly affected by individual $\alpha_1$, $\beta_1$ or $\beta_2$ adrenoceptor blockade, but was blunted by combined blockade of these receptors. Finally, inhibition of NO synthesis with intravenous L-NNA prevented the thermogenic response and increase in systemic $O_2$ consumption during dobutamine infusion.

***5.4.1 Effects of catecholamines on neonatal thermogenesis and systemic $O_2$ consumption.*** In newborn mammals, thermogenic responses occur through two mechanisms, namely via shivering in skeletal muscle, and non-shivering thermogenesis in BAT.[205] The developmental features of BAT in lambs have previously been well-characterized, and we know that its thermogenic activity increases within several hours of birth.[215,205] The thermogenic properties of BAT depend on a unique 'uncoupling protein', located on the inner membrane of the mitochondrion, which uncouples oxidative phosphorylation to produce heat rather than ATP[216] ***(Figure 5:8)***.

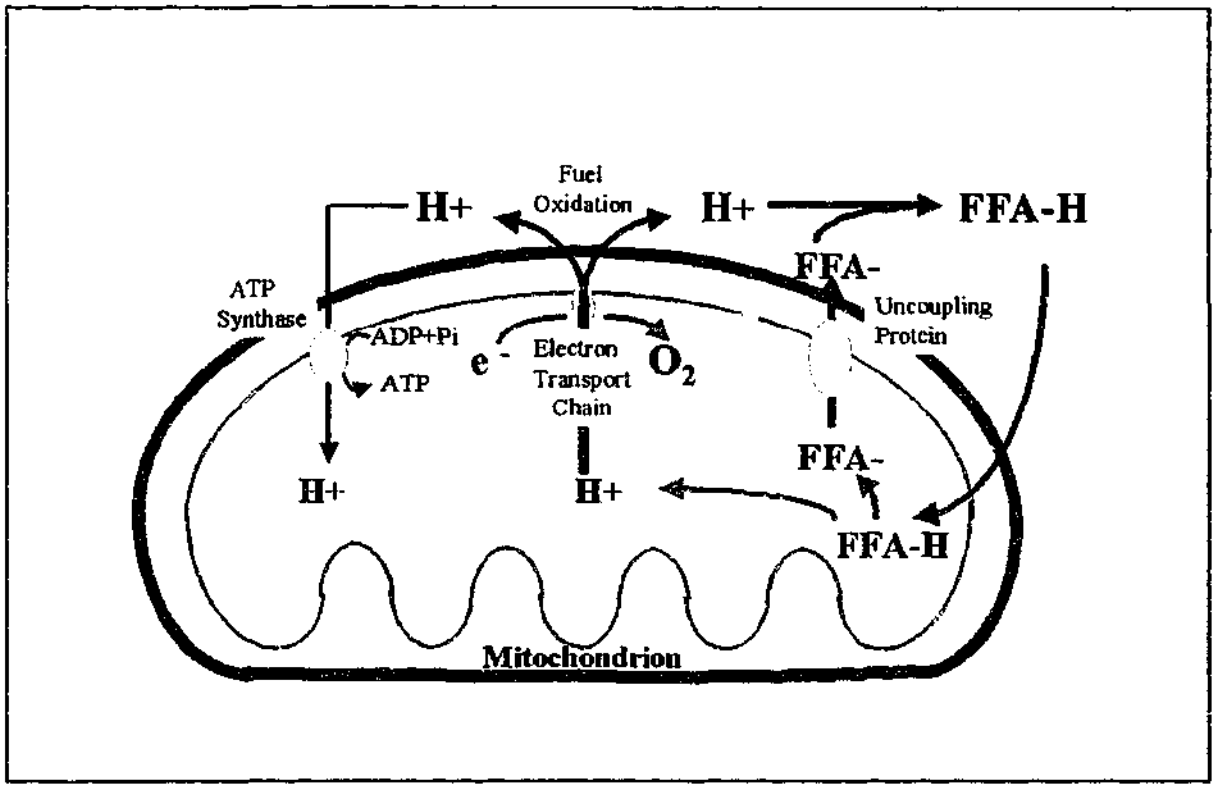


***Figure 5:8.*** *Mitochondrial mechanisms in BAT. Fuels are oxidised by the electron transport chain, creating a proton (H+) electrochemical potential gradient. Under normal circumstances, protons descend this gradient via ATP synthesis. Uncoupling protein causes this electrochemical gradient to collapse by exporting free fatty-acid (FFA), which act as proton carriers. As a result, in the uncoupled state, fuel oxidations does not result in ATP generation, but energy is expended as heat.*

Non-shivering thermogenesis is normally activated by release of endogenous noradrenaline from the abundant sympathetic nerve terminals present within BAT.[216] It can also be elicited by *exogenous* administration of catecholamines.[217,115,205] The rises in body temperature resulting from BAT activation are accompanied by substantial increases in systemic $O_2$ consumption.[218,205,216] The observation of a dose-dependent rise in body temperature and $O_2$ consumption without muscle shivering during infusions of dobutamine and dopamine in the present study was therefore consistent with the notion that both agents activated non-shivering thermogenesis in the newborn lamb.

In contrast to 1-2 day lambs, body temperature was unchanged and increases in systemic $O_2$ consumption during catecholamine infusion were substantially lower by the start of the second week of life, indicating that a striking maturational change in the thermogenic response occurred during early neonatal development. This observation is in keeping with our current understanding of the developmental characteristics of BAT and its mitochondrial uncoupling protein in sheep. Specifically, while BAT is the principal constituent of body fat deposits at birth,[219] a relatively rapid transformation to white adipose tissue subsequently occurs.[220,221,219] At a molecular level, this transformation is associated with disappearance of uncoupling protein mRNA from adipocytes in the first few days after birth[220,222] and a marked fall in the protein expression of adipocytes by the end of the first postnatal week.[222] Presumably therefore, rises in systemic $O_2$ consumption in the 7-10 day lambs were, as in the adult, principally related to increases in tissue metabolism secondary to factors such as the stimulation of substrate mobilization and intermediary metabolism.

In this study, rises in systemic $O_2$ delivery during dobutamine and dopamine were similar in 1-2 and 7-10 day old animals. The dramatic increase in $O_2$ consumption which accompanied these agents in the 1-2 day lambs therefore had major consequences for the balance between systemic $O_2$ delivery and consumption. Specifically, at the peak infusion rate, rises in systemic $O_2$ consumption in this age group accounted for ≈70-80% of the elevations in $O_2$ delivery, compared to <20% in the older lambs. Moreover, the increase in systemic $O_2$ consumption observed in 1-2 day lambs was associated with an appreciable rise in systemic $O_2$ extraction ratio, indicative of a utilization of tissue $O_2$ reserves. Our data therefore suggest that the

changes in systemic $O_2$ balance which accompany catecholamine infusions in newborn lambs are fundamentally different to those in older animals and that in the early neonatal period, catecholamines do not produce changes in systemic $O_2$ balance favourable to tissue oxygenation.

Previous studies suggest that the thermogenic stimulation of isolated brown adipocytes derived from lambs occurs mainly via activation of $\beta_1$-adrenoceptors and to a lesser extent via $\alpha_1$-adrenoceptors.[223] The observation in the present study that the dobutamine-related changes in body temperature or systemic $O_2$ consumption were unaltered by individual adrenoceptor blockade would suggest that in the intact animal, the thermogenic response was not due to a predominant effect of dobutamine on a single adrenoceptor. However, combined $\alpha_1$-, $\beta_1$-, and $\beta_2$-adrenoceptor blockade resulted in a marked attenuation of thermogenesis and rises in $O_2$ consumption in response to dobutamine. This finding implies that the thermogenic activation observed in newborn lambs was the result of an interaction between two or more of these adrenoceptor subtypes. Indeed, it is known that an interaction between $\alpha_1$- and $\beta_1$-adrenoceptors may occur *in vivo*, with $\alpha_1$-adrenoceptors having a potentiating effect on $\beta_1$- induced responses.[224] However, it has also been suggested that the increases in systemic $O_2$ consumption during non-shivering thermogenesis may be related to rises in blood flow to adipose tissue.[225] Therefore the possibility that attenuation of $O_2$ consumption and body temperature responses after combined adrenoceptor blockade may have been related to the concomitant reduction in cardiac output response cannot be excluded.

*5.4.2 Comparison of dobutamine and dopamine effects.* The dose-dependent changes in systemic $O_2$ delivery and consumption differed for animals given dopamine, compared to those given dobutamine. This observation is consistent with differences in adrenoreceptor profiles and affinities for the two agents. However, the similarity of the peak responses to the two agents is consistent with the conclusion of the adrenoreceptor blockade studies that multiple adrenoreceptor subtypes are involved in the in vivo response observed in newborn animals.

It might also be important to examine which agent might provide more favourable (or less adverse) effects on the relationship between systemic $O_2$ consumption and delivery in the newborn period. However, examination of this relationship in the 1-2 day lambs demonstrate similar levels of $O_2$ consumption at all levels of $O_2$ delivery in lambs given dopamine and dobutamine, suggesting that neither agent provides a more favourable dose profile over the other. ***(Figure 5:9).***

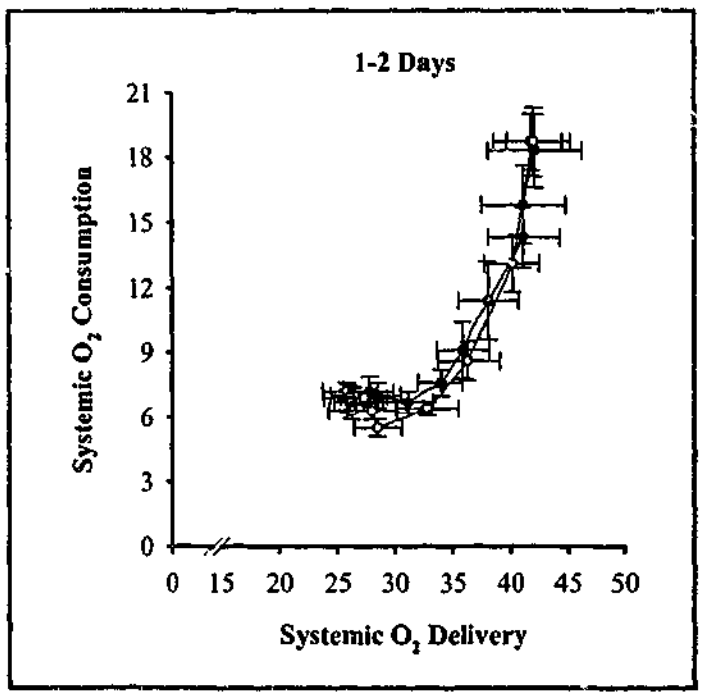


***Figure 5:9.*** *The relationship between systemic $O_2$ delivery and consumption in 1-2 day lambs during incremental infusions of dobutamine (•) and dopamine (•).*

***5.4.3 Changes in systemic $O_2$ delivery and consumption during inhibition of NO synthesis.*** The studies of NO synthesis inhibition provided further insights into the changing role of NO in the regulation of systemic $O_2$ consumption during postnatal development. In both 1-2 and 7-10 day old lambs, inhibition of NO synthesis with intravenous L-NNA at a dose known to profoundly reduce the enzymatic activity of NO synthase did not alter resting $O_2$ consumption. This is an intriquing finding we observed that the same L-NNA regime in anaesthetised adult sheep produced significant increases in systemic $O_2$ consumption of approximately 40%.[27] In that study, the increase in systemic O2 consumption after inhibition of NO synthesis was interpreted as evidence of an important inhibitory effect of NO on oxidative metabolism. This effect, which has also been observed in other studies[73] has been postulated to result from a number of actions, including inhibition of cytochrom. oxidase and a reduction in the mitochondrial membrane potential.[226,67,227]

Unfortunately, the present study cannot provide a definitive explanation for the lack of a rise in systemic $O_2$ consumption in newborn lambs after inhibition of NO synthesis. However, there are at least two areas of this phenomenon which warrant further investigation. The first is related to the changing role of NO in the regulation of oxidative metabolism during postnatal development. Specifically, postnatal changes in the regulation of cellular $O_2$ consumption[228] and rates of oxidative phosphorylation[229-231] and citric acid cycle activity[232] have been described. Further studies will be required to explore the potential changing role for NO in the modulation of these metabolic pathways during postnatal development.

The second is that the tonic release of NO may play an additional *stimulatory* role in the regulation of basal systemic $O_2$ consumption in the younger neonate through its

effects on BAT, probably through a combination of neurohumoral, vascular and intracellular influences. Thus, inhibition of NO synthesis with intraperitoneal injection of the L-arginine analogue L-NAME has been shown to reduce the firing rate of sympathetic nerves innervating interscapular BAT.[233] Furthermore, NO synthase has been demonstrated in the endothelium of arterioles supplying BAT and it has been demonstrated that the sensitivity of these vessels to NO inhibition is ten-fold greater that that of the coronary vasculature.[234] NO synthase has also been isolated from the adipocytes within the adipose tissue itself[235] where it is thought to modulate cation channel activity.[236] Thus, the absence of a measurable change in systemic $O_2$ consumption after inhibition of NO synthesis may result from the abolition of two counter-regulating effects of NO on $O_2$ metabolism, namely an inhibitory effect mediated through its effects on oxidative phosphorylation and a stimulatory effect mediated through its additional effects on BAT.

***5.4.4 Effect of systemic inhibition of NO synthesis on the responses to dobutamine.***

In order to examine the role of NO in modulating the responses to dobutamine, incremental infusions of dobutamine were studied before and after inhibition of NO synthesis with L-NNA. In these experiments a peak infusion rate of 10 $\mu g.kg^{-1}.min^{-1}$ was used because it was known from the studies employing higher infusion rates, that age-related differences in both temperature and systemic $O_2$ consumption response were evident at this level. It also appeared likely that it would be possible to restore animals to near- baseline conditions after the infusion was discontinued, which would allow the effects of NO synthesis inhibition to be studied within the same animal.

Although inhibition of NO synthesis did not alter basal systemic $O_2$ consumption in the 1-2 day old lambs, it abolished the increase in $O_2$ consumption associated with dobutamine infusion up to $10\mu g.kg^{-1}.min^{-1}$ in this group. While the precise mechanisms for this observation remain undefined, the data supports the possibility of an additional mechanism whereby NO modulates the response of BAT to catecholamine stimulation and is consistent with data which demonstrated that increases in blood flow to BAT and the thermogenic response within it during noradrenaline infusion were abolished by inhibition of NO synthesis.[125]

***5.4.5 Limitations.*** The present study had a number of potential limitations. First, experiments were performed under general anaesthesia, in large part because infusion of catecholamines into the conscious newborn may result in arousal, with the confounding effect of rises in systemic $O_2$ consumption due to an associated increase in muscle activity. General anaesthesia may have altered both resting variables and the magnitude of responses in systemic $O_2$ delivery, consumption and body temperature during catecholamine administration. It therefore remains unclear to what extent the findings of the present study can be extrapolated to the conscious newborn. Nonetheless, many critically ill infants in intensive care would be routinely sedated, thus the conditions of this model may in some ways be representative of the clinical setting. The second limitation was that systemic $O_2$ delivery and consumption were mathematically coupled because of the use of the shared variable cardiac output in their calculation.[237] This is unlikely to have detracted from the main conclusions of the study, however, particularly during dobutamine infusion because the marked increases in systemic $O_2$ consumption produced by dobutamine

in 1-2 day lambs were related predominantly to differences in a-v $O_2$ extraction rather than cardiac output.

***5.4.6 Clinical implications.*** BAT is present in the human fetus from around the 20th week of gestation onwards and is abundant in both the pre-term and full-term neonate.[238] Uncoupling protein is also present at birth in preterm and term infants.[239] Furthermore, prominent increases in systemic $O_2$ consumption have been reported after infusion of noradrenaline in newborn infants.[240] These observations suggest that the prominent thermogenic response to dobutamine and dopamine, observed in newborn lambs, as well as the associated rise in systemic $O_2$ consumption, and the adverse effects on the balance between systemic $O_2$ delivery and consumption may also occur with administration of these agents to the human neonate, particularly at higher infusion rates.

# CHAPTER 6 LEFT VENTRICULAR MYOCARDIAL OXYGEN DELIVERY AND CONSUMPTION

"It is quite clear that the regulation of coronary blood flow in the immature heart is so different from that of the adult that it warrants extensive research... These questions are an intellectual goldmine awaiting those with pans, sluice boxes , and a love of discovery."

Thornburg 1999[241]

The ability of the myocardium to maintain an optimal relationship between an increase in its $O_2$ consumption with equivalent elevations in blood flow and $O_2$ delivery is an important homeostatic mechanism, which may determine the degree of contractile reserve and protect the myocardium from the toxic effects of work-induced ischaemia. This chapter examines these relationships during inotropic stimulation in the young neonate and investigates the role of NO in modulating them.

Dobutamine-related increases in LV myocardial blood flow and $O_2$ delivery were blunted in the early neonatal period. As a result, while dobutamine-induced increases in $O_2$ consumption were closely matched by proportional increases in myocardial blood flow and $O_2$ delivery in the 7-10 day group, this close matching was not maintained in the 1-2 day neonates. However, in neither group, was the close coupling between increases in LV myocardial $O_2$ consumption and delivery evident during dopamine infusion. Although inhibition of NO synthesis altered both left ventricular myocardial $O_2$ delivery and consumption, it did not significantly modulate the delivery-consumption relationships during dobutamine infusion.

Fundamental changes occur in the ability of the myocardium to match inotrope-related increases in its $O_2$ consumption with equivalent changes in $O_2$ delivery in the early postnatal period. Dobutamine may have some advantages over dopamine in maintaining these relationships, particularly beyond the first week of life. While NO is an important modulator of LV myocardial vasodilator responses, it does not have a substantial role in maintaining an appropriate balance between LV $O_2$ supply and demand during β-adrenergic stimulation.

## *6.1 INTRODUCTION*

In addition to augmenting left ventricular function, inotropes such as dobutamine and dopamine also increase myocardial $O_2$ consumption. In the adult heart, this increase in $O_2$ consumption during inotropic stimulation is normally closely matched by an equivalent increase in LV myocardial blood flow and $O_2$ delivery.[21] This ability of the adult myocardium to fuel an increase in $O_2$ consumption through elevated blood flow and $O_2$ delivery is an extremely important homeostatic mechanism, which may not only determine the degree of contractile reserve,[25] but also protect the myocardium from the toxic effects of work-induced ischaemia.[26] The increase in myocardial $O_2$ delivery during inotropic stimulation appears to be mediated predominantly by coronary vasodilator metabolites, including adenosine,[21] although direct stimulation of vasodilator adrenoreceptors on the coronary vasculature may also play a role.[242,24]

There are currently no data, however, which assess the degree to which this vitally-important homeostatic mechanism for matching increases in myocardial $O_2$ consumption and delivery during inotropic stimulation is developed in the very young neonate. Nonetheless, it has been suggested that the coronary vasodilator response to adenosine may be blunted in the neonatal myocardium[110] and that the contribution of adenosine to the regulation of myocardial blood flow may be altered in the newborn compared to the adult heart.[243] Furthermore, data from our laboratory has shown that the substantial elevation in left ventricular myocardial $O_2$ consumption during the normal perinatal transition is dependent on an increase in $O_2$ extraction, as the increases in blood flow are only modest.[109] Potentially therefore, the important homeostatic mechanisms which fuel and meet the increased $O_2$ demand

with appropriate elevations in delivery may be poorly developed in the young neonate, presenting them with a precarious balance between aerobic metabolism and $O_2$ debt. This limitation may be of particular relevance to clinical practice where there are important concerns over potentially toxic myocardial effects of inotropes in the neonate, which may have a metabolic basis.[244-246]

An additional factor that needs to be considered in the regulation of myocardial blood flow and $O_2$ consumption in the neonate is NO. It has been suggested that NO production exerts a basal coronary vasodilator effect that does not appear to change significantly between late fetal and adult life.[241] However, while NO did not appear to modulate resting myocardial $O_2$ consumption in adult dogs[247,60,248] or alter the myocardial $O_2$ consumption-delivery relationship during dobutamine stimulation in adult sheep,[27] inhibition of NO synthesis reduced myocardial $O_2$ consumption in near term-fetal sheep by more than 50%.[241] The latter observation suggests a powerful developmental change in the contribution of NO to the regulation of myocardial blood flow and $O_2$ consumption. However, the potential importance of any such change for myocardial blood flow, $O_2$ delivery and consumption during inotropic stimulation in the neonate is unknown .

Accordingly, the aims of the studies in this chapter were to examine the changes in and interrelationships between LV myocardial blood flow, $O_2$ delivery and $O_2$ consumption during inotropic stimulation with dobutamine and dopamine in the early neonatal period. In addition, the modulatory role of endogenous NO on the effects of dobutamine on these responses and interrelationships were also evaluated.

## 6.2 METHODS

***Surgical preparation.*** Forty two lambs, of which 20 were aged 1-2 days and weighed 4.7±0.2kg and 22 were aged 7-10 days and weighed 6.2±0.3kg, were surgically prepared under general anaesthesia, as described in Chapter 2. As part of the preparation, cannulae were inserted through adventitial purse-string sutures in the descending thoracic aorta and left atrium for blood sampling and pressure measurement and into the coronary sinus for blood sampling. An ultrasonic, perivascular flow probe was placed around the circumflex coronary artery.

***Experimental protocol.*** Three protocols were used in this group of lambs. The first two protocols examined the changes in left ventricular myocardial blood flow and oxygenation variables during incremental inotropic stimulation with either dobutamine or dopamine. Dobutamine was infused at rates up to $40\mu g.kg^{-1}.min^{-1}$ in seven 1-2 day and eight 7-10 day old animals. Dopamine was infused at incremental rates up to $40\mu g.kg^{-1}.min^{-1}$ in another 13 animals, of which six were 1-2 day and seven, 7-10 days old. The third protocol addressed the effects of inhibition of NO synthesis on dobutamine-related responses in a separate groups of 14 animals, of which 7 were 1-2 days and 7 were 7-10 days old. In these, the responses to an incremental infusion of dobutamine at rates up to $10\mu g.kg^{-1}.min^{-1}$ were studied, before and after inhibition of NO synthesis, with an intravenous infusion of $\omega$-nitro-L-arginine (L-NNA) given at a dose of 25 mg/kg, as described in detail in Chapter 2.

***Physiologic measurements and calculations.*** All physiological measurements and calculations have been described in detail in Chapter 2. To summarise, left ventricular myocardial blood flow ($ml{\cdot}min^{-1}{\cdot}100gLV^{-1}$) was derived by normalising

circumflex coronary arterial blood flow to the weight of left ventricular myocardium which it perfused. Left ventricular coronary vascular resistance (mmHg/ml·min$^{-1}$·100gLV$^{-1}$) was calculated from the difference between aortic and left atrial pressure, divided by $Q_{LV}$. LV myocardial $O_2$ delivery (ml·min$^{-1}$·100gLV$^{-1}$) was computed as $Q_{LV} \cdot C_{Ao}O_2$ and LV myocardial $O_2$ consumption (ml·min$^{-1}$·100gLV$^{-1}$) as $Q_{LV} \cdot (C_{Ao}O_2 - C_{CS}O_2)$. Finally, the left ventricular myocardial $O_2$ extraction ratio was computed as the ratio between its $O_2$ consumption and delivery.

## *6.3 RESULTS*

### 6.3.1 Baseline variables.

Baseline variables for the twenty 1-2 day and the twenty-two 7-10 day animals used in the three protocols of this study are combined and presented in Table 6:1. Left ventricular myocardial blood flow, coronary vascular resistance and $O_2$ delivery was similar in both age groups. Oxygen consumption tended to be higher in the older animals, and that as a result, the baseline left ventricular myocardial $O_2$ extraction ratio was significantly greater in the 7-10 day old group ($p<0.01$).

| | Group | | |
|---|---|---|---|
| | **1-2 day (n=20)** | **7-10 day (n=22)** | **P Value** |
| **LV Myocardial Blood Flow ($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 117±11 | 146±13 | n.s. |
| **Coronary Vascular Resistance (mmHg/ $ml \cdot min^{-1} \cdot 100g^{-1}$)** | 0.56±0.06 | 0.52±0.06 | n.s. |
| **LV Myocardial $O_2$ Delivery ($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 17.1±1.5 | 17.3±1.5 | n.s. |
| **LV Myocardial $O_2$ Extraction Ratio** | 0.56±0.02 | 0.67±0.02 | <0.01 |
| **LV Myocardial $O_2$ Consumption ($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 9.5±0.9 | 11.3±1.0 | n.s. |

*Table 6:1. Baseline variables in 1-2 day and 7-10 day animals.*

### *6.3.2 Changes in LV Myocardial Blood Flow, $O_2$ Delivery and Consumption During Dobutamine Infusion.*

***Left ventricular myocardial blood flow, vascular resistance and $O_2$ delivery.*** Incremental infusion of dobutamine at rates up to 40 $\mu g.kg^{-1}.min^{-1}$ resulted in progressive increases in left ventricular myocardial blood flow to peak levels which were 180±6 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ (1-2 day) and 334±75 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ (7-10 day) above baseline at the peak infusion rate (both $p<0.001$). These increases at the peak infusion rate were associated with concomitant reductions in myocardial coronary vascular resistance of 0.40±0.05 mmHg/ $ml \cdot min^{-1} \cdot 100gLV^{-1}$ in 1-2 day animals, and of 0.26±0.05 mmHg/ $ml \cdot min^{-1} \cdot 100gLV^{-1}$ in 7-10 day group (both $p<0.001$). Left ventricular myocardial $O_2$ delivery increased by 21.0±0.9 and 29±7 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ in the 1-2 day and 7-10 day groups respectively (both $p<0.001$). As a result, the dobutamine-related increments in LV myocardial blood flow and $O_2$ delivery were significantly greater in the older animals (both $p<0.05$; ***Figure 6.1)***.

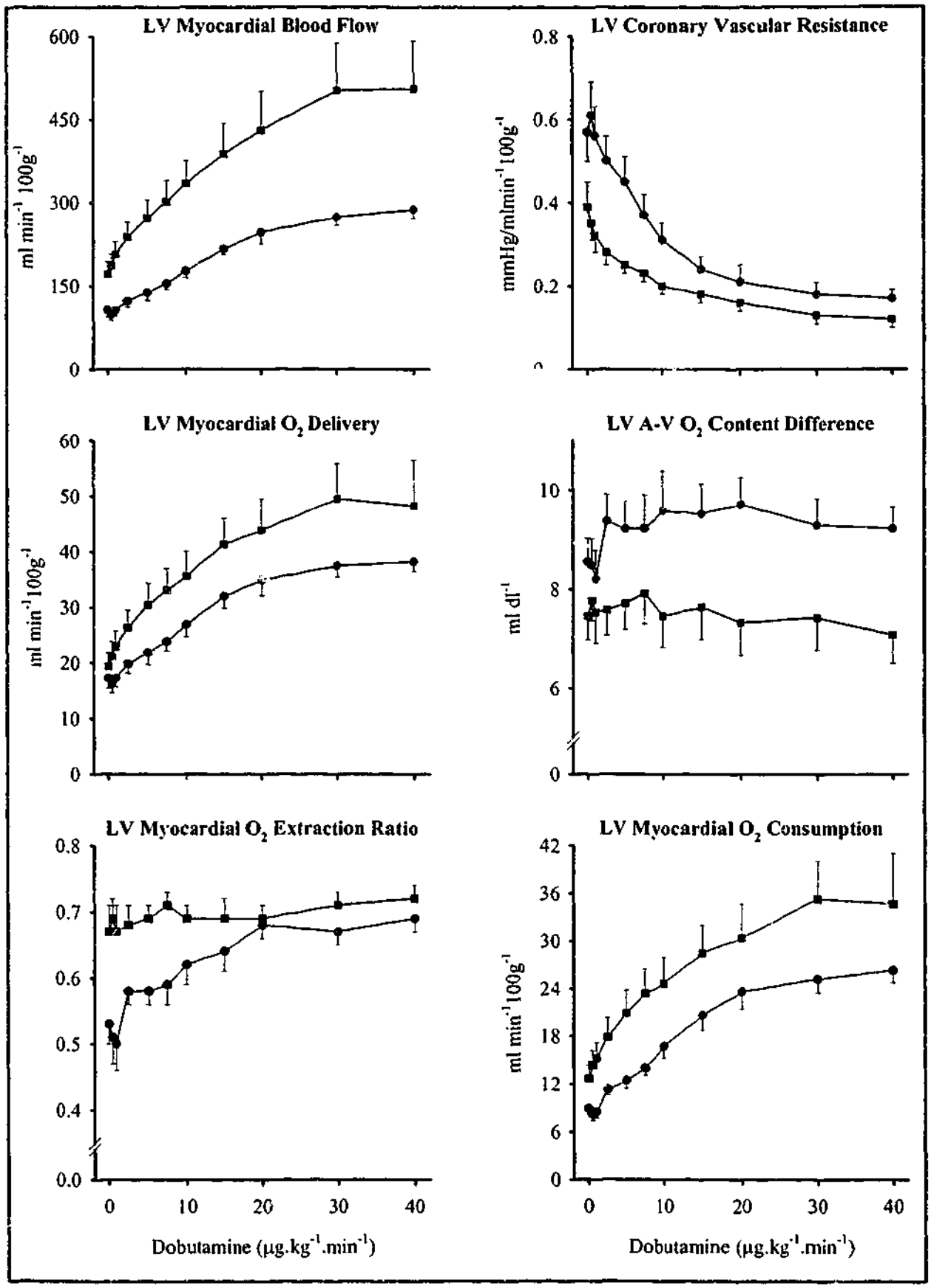


***Figure 6:1.*** *LV myocardial blood flow and oxygenation responses during incremental infusion of dobutamine in 1-2 day* (•) *and 7-10 day* (■) *lambs.*

***Left ventricular myocardial $O_2$ extraction ratio and $O_2$ consumption.*** LV myocardial $O_2$ extraction ratio, although unchanged in the 7-10 day group was

increased by 33±9% above baseline levels in the younger animals ($P<0.001$). Substantial increases in LV myocardial $O_2$ consumption of 17.4±1.2 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ and 22.0±5.3 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ were elicited by dobutamine in the 1-2 day and 7-10 day animals respectively (both $p<0.001$), with no significant difference between groups ***(Figure 6.1)***.

***Relationship between LV myocardial $O_2$ consumption and $O_2$ delivery during dobutamine infusion.*** In the 7-10 day animals, increases in left ventricular myocardial $O_2$ consumption were closely matched by proportional increases in myocardial $O_2$ delivery (slope of relationship = 0.99). By contrast, in the 1-2 day group, a close match between increases in myocardial $O_2$ consumption and $O_2$ delivery was **not** present (slope = 0.6; ***Table 6.2, Figure 6.2***).

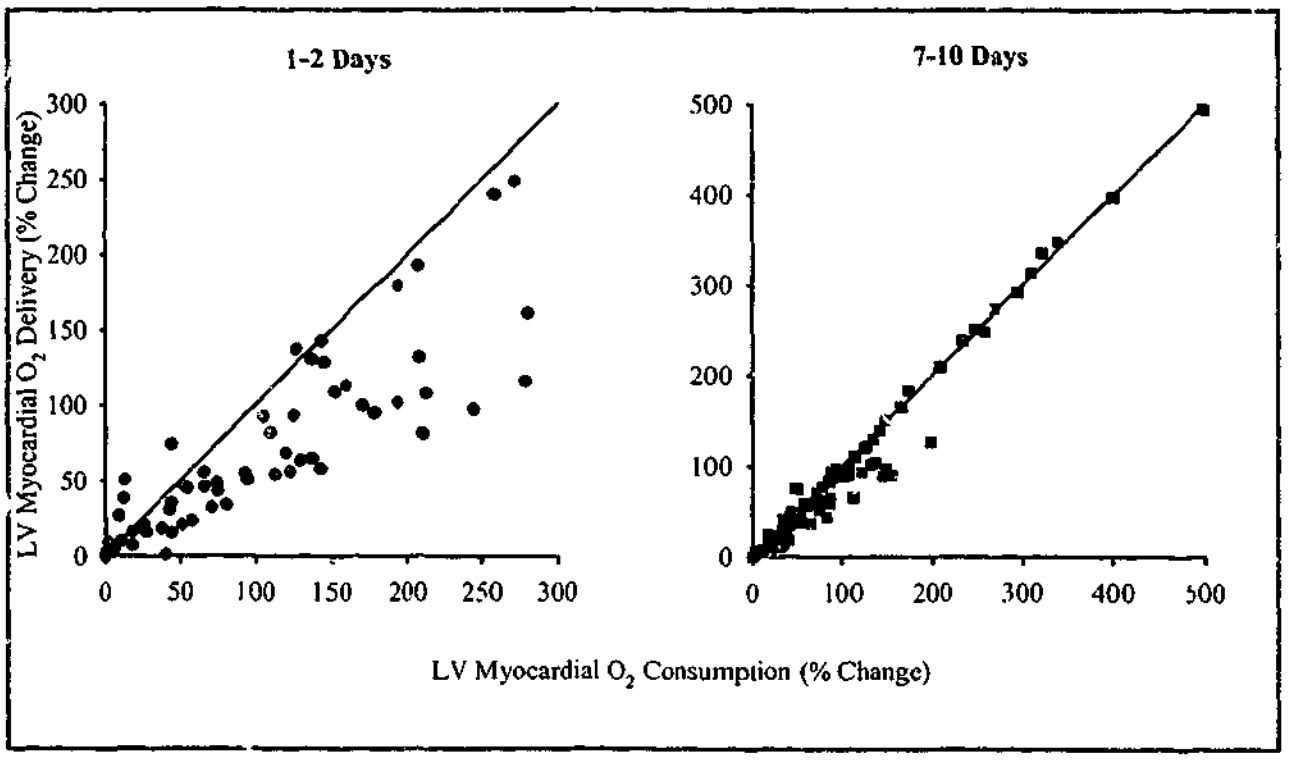


***Figure 6:2.*** *Proportional changes in LV myocardial $O_2$ consumption and delivery during incremental infusion of dobutamine in 1-2 day and 7-10 day lambs. The line plotted for each curve represents the line of unity for a 'perfect' relationship.*

| Y = MX+C | | | | |
|---|---|---|---|---|
| **Y** = % Change in Delivery. **M** = Slope Factor. **X** = % Change in Consumption. **C** = Y Intercept. **P** vs line of unity | | | | |
| | M | C | R | P |
| 1-2 day Animals | 0.61 | 3.97 | 0.90 | <0.05 |
| 7-10 day Animals | 0.99 | -6.79 | 0.99 | n.s. |

***Table 6:2.*** *Slope and intercept factors for the linear relationship between proportional changes in LV myocardial $O_2$ consumption (x-axis), and delivery (y-axis) during dobutamine infusion. The 'p' value, obtained by comparing the observed relationship with that for a perfect coupling between increases in $O_2$ consumption and delivery.*

### *6.3.3 Changes in LV myocardial blood flow, $O_2$ delivery and consumption during dopamine Infusion.*

***Changes in LV myocardial blood flow, vascular resistance and $O_2$ delivery.*** Incremental infusion of dopamine at rates up to 40μg.kg$^{-1}$.min$^{-1}$ resulted in progressive increases in LV myocardial blood flow to levels which were 183±31 (1-2 day) and 185±18 ml·min$^{-1}$·100gLV$^{-1}$ (7-10 day) above baseline at the peak infusion rate (both $p<0.001$). These increases were associated with reductions in LV myocardial coronary vascular resistance of 0.24±0.03 mmHg/ml·min$^{-1}$·100gLV$^{-1}$ in 1-2 day animals and of 0.28±0.08 mmHg/ ml·min$^{-1}$·100gLV$^{-1}$ in 7-10 day animals (both $p<0.001$), as well as increases in LV myocardial $O_2$ delivery of 19.0±5.0 and 17.5±2.2 ml·min$^{-1}$·100gLV$^{-1}$ in the younger and older groups respectively (both $p<0.001$) Increments in LV myocardial blood flow and $O_2$ delivery were similar in both age groups studied ***(Fig. 6.3)***.

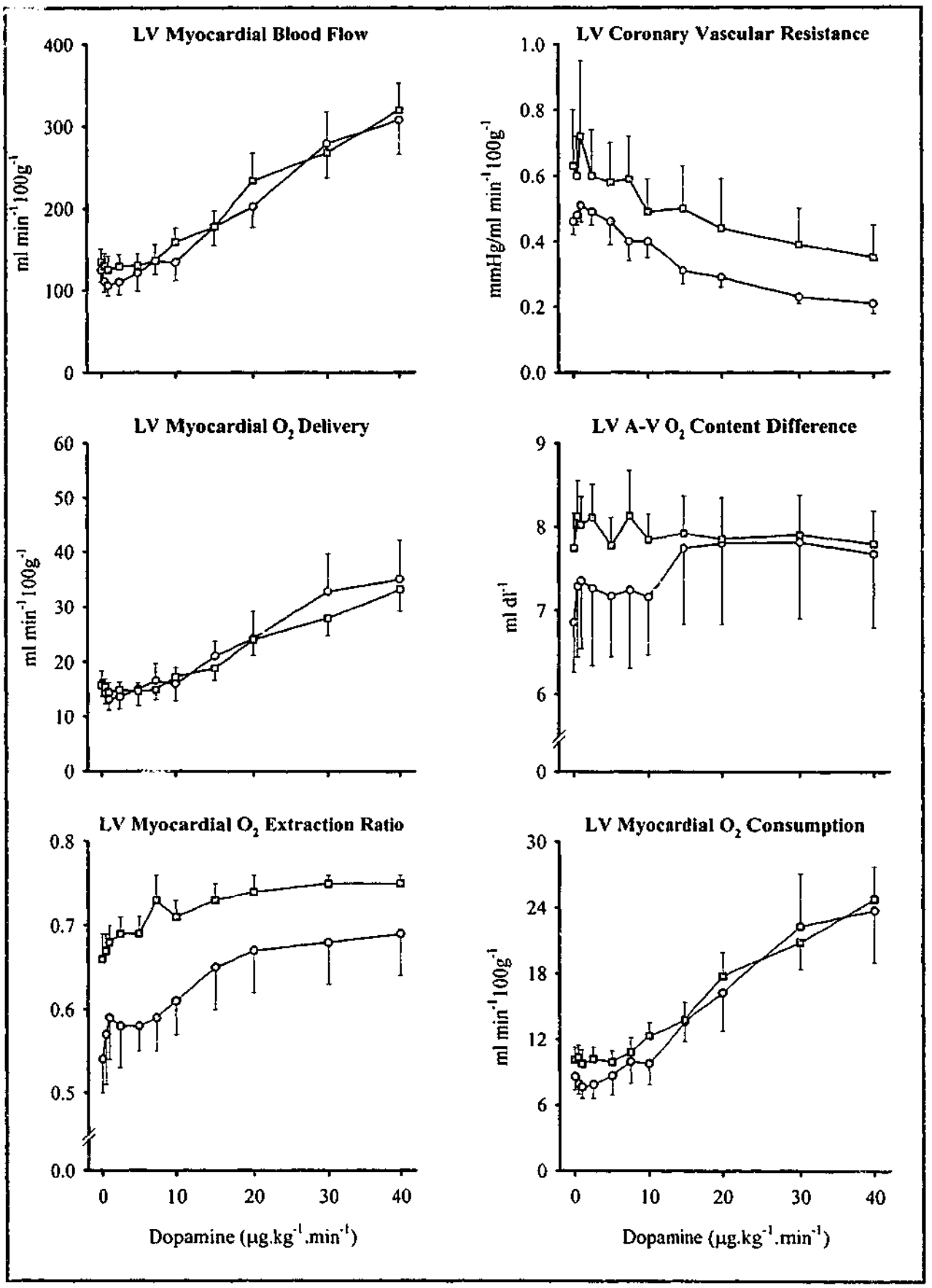


***Figure6:3.*** *LV myocardial blood flow and oxygenation responses during incremental infusion of dopamine in 1-2 day (●) and 7-10 day (▲) lambs.*

***LV myocardial $O_2$ extraction ratio and $O_2$ consumption.*** Significant increases in the LV myocardial $O_2$ extraction ratio (both $p<0.001$) were observed during dopamine infusion in both younger (28±4%) and older animals (15±5%). Similar increases in LV myocardial $O_2$ consumption of 15.2±3.7 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ and 14.7±2.0 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ were elicited by dopamine in the 1-2 day and 7-10 day animals respectively (both $p<0.001$; ***Figure 6.3***).

***Relationship between LV myocardial $O_2$ consumption and $O_2$ delivery during Dopamine Infusion.*** During infusion of dopamine, the increases in LV myocardial $O_2$ consumption were not matched by proportional increases in $O_2$ delivery in either the 1-2 day (slope = 0.69) or 7-10 day animals (slope = 0.80; ***Figure 6.4, Table 6:3***).

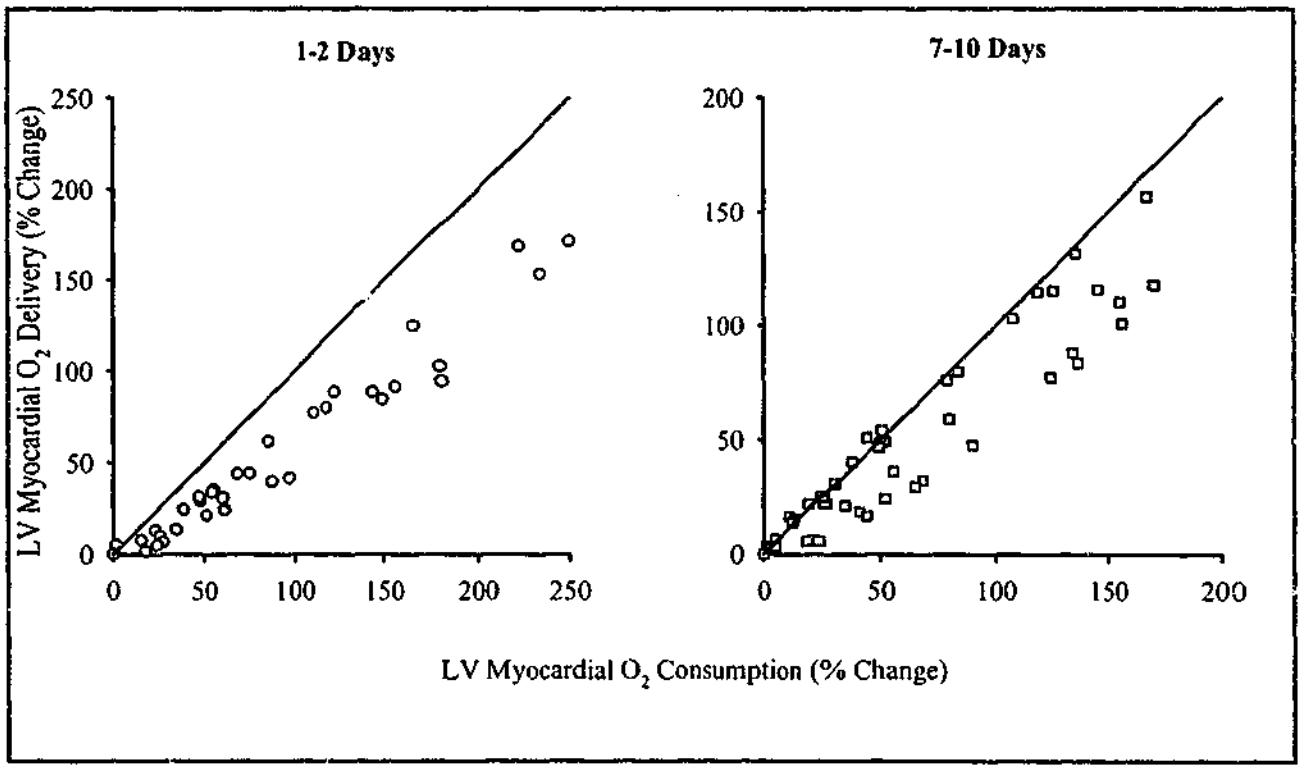


***Figure 6:4.*** *Proportional changes in LV myocardial $O_2$ consumption and delivery during incremental infusion of dopamine in 1-2day and 7-10 day lambs. The line plotted for each curve represents the line of unity for a 'perfect' relationship.*

| Y = MX+C | | | | |
|---|---|---|---|---|
| Y = % Change in Delivery. M = Slope Factor. X = % Change in Consumption. C = Y Intercept. P vs line of unity | | | | |
| | M | C | R | P |
| 1-2 day Animals | 0.69 | -7.6 | 0.96 | <0.05 |
| 7-10 day Animals | 0.80 | 0.81 | 0.93 | <0.05 |

***Table 6:3.*** *Slope and intercept factors for the linear relationship between proportional changes in LV myocardial $O_2$ consumption (x-axis), and delivery (y-axis) during dopamine infusion. The 'p' value, obtained by comparing the observed relationship with that for a perfect coupling between increases in $O_2$ consumption and delivery.*

### *6.3.4 Comparison of Dobutamine and Dopamine Responses.*

The peak reduction in LV coronary vascular resistance in the 1-2 day animals was significantly greater ($p<0.05$) in those receiving dobutamine. In the 7-10 day group, there was a tendency toward a greater peak increment in LV myocardial blood flow in response to dobutamine infusion, although this trend did not reach statistical significance ($p=0.09$). The peak changes in all other measures were similar during dobutamine and dopamine infusions. Nonetheless, despite this similarity in peak responses, rises in myocardial blood flow, $O_2$ delivery and consumption appeared to occur earlier in animals given dobutamine. ***(Figure 6:5 & 6:6, Table 6:4)***.

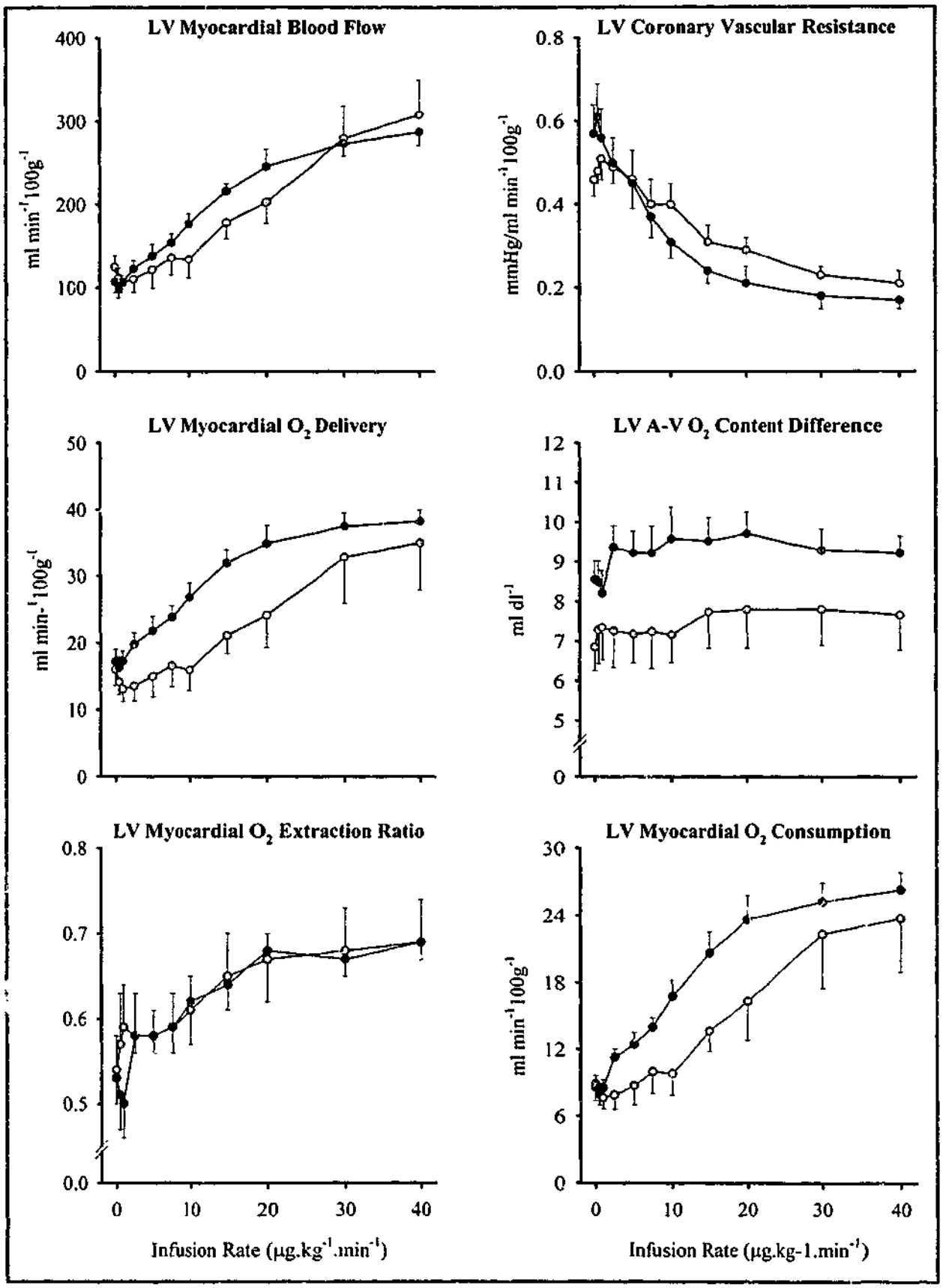


***Figure 6:5.*** *LV myocardial blood flow and oxygenation responses in 1-2 day animals during incremental infusions of dobutamine (•) and dopamine (•).*

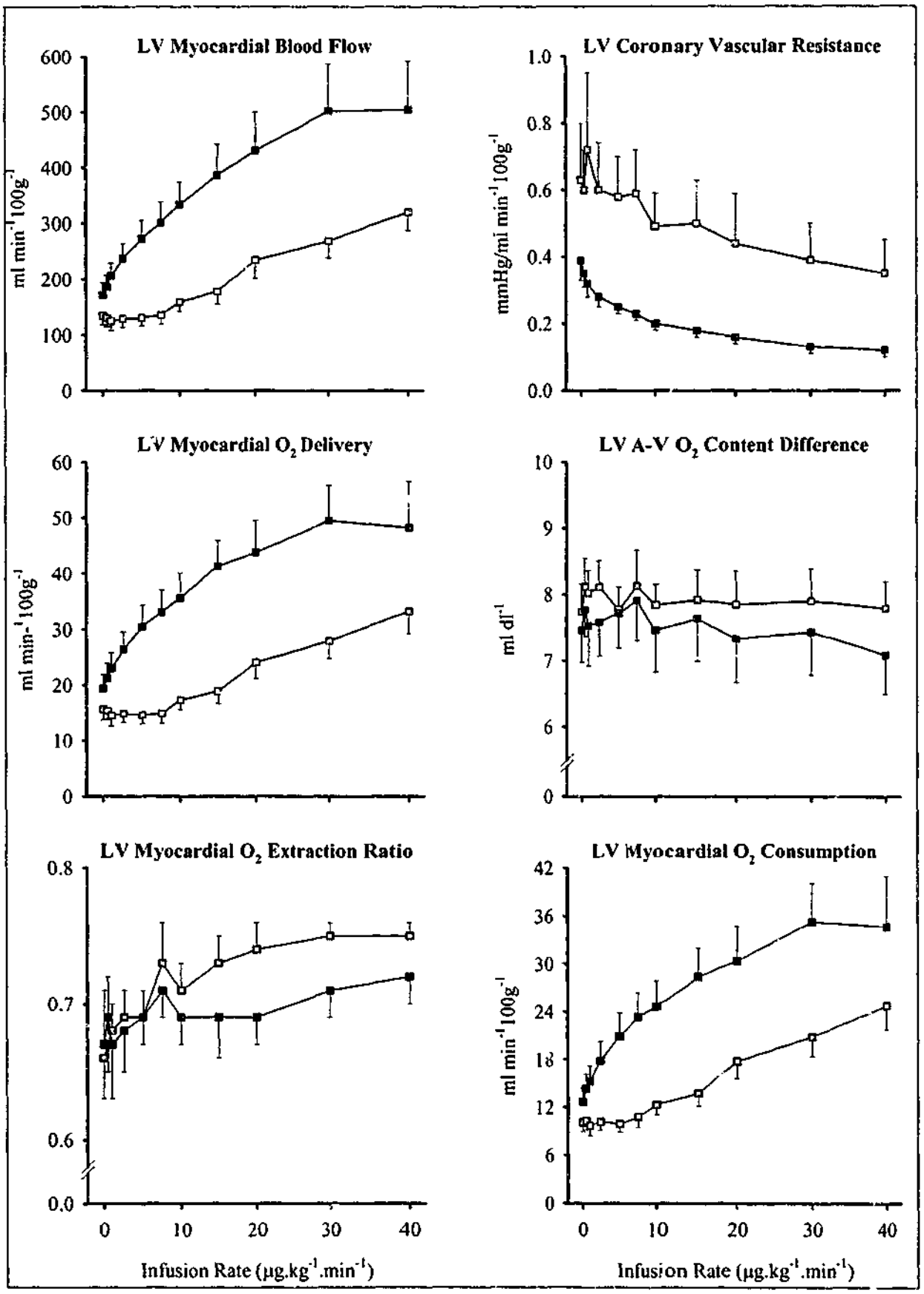


***Figure 6:6.*** *LV myocardial blood flow and oxygenation responses in 7-10 day animals during incremental infusions of dobutamine* (■) *and dopamine* (■).

| | | Dobutamine | Dopamine | P Value |
|---|---|---|---|---|
| **LV Myocardial Blood Flow** | 1-2 days | 180±6 | 183±31 | n.s. |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 334±76 | 185±18 | <0.1 |
| **Coronary Vascular Resistance** | 1-2 days | -0.40±0.05 | -0.24±0.03 | <0.05 |
| **($mmHg/ ml \cdot min^{1} \cdot 100g^{-1}$)** | 7-10 days | -0.26±0.05 | -0.28±.08 | n.s. |
| **LV Myocardial $O_2$ Delivery** | 1-2 days | 21.0±0.9 | 19.0±5.0 | n.s. |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 28.9±7.4 | 17.5±2.2 | n.s. |
| **LV Myocardial $O_2$ Extraction Ratio** | 1-2 days | 0.16±0.04 | 0.15±0.02 | n.s. |
| | 7-10 days | 0.05±0.02 | 0.09±0.03 | n.s. |
| **LV Myocardial $O_2$ Consumption** | 1-2 days | 17.4±1.2 | 15.2±3.7 | n.s. |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 22.0±5.3 | 14.7±1.9 | n.s. |

***Table 6:4.*** *Comparison of peak changes during incremental infusion of dobutamine and dopamine in 1-2 day lambs.*

### *6.3.5 Effects of Inhibition of NO Synthesis on Responses to Dobutamine.*

***Effect of NO synthase inhibition.*** Intravenous administration of L-NNA (25 mg/kg) increased left ventricular myocardial blood flow, coronary vascular resistance and left ventricular myocardial $O_2$ delivery in both groups (all $p<0.05$). L-NNA also increased the LV myocardial $O_2$ extraction ratio in the 1-2 day animals ($p<0.01$), as well as LV myocardial $O_2$ consumption in this group ($p<0.05$) and in the 7-10 day animals ($p<0.01$; ***Table 6:5***).

| | | Before L-NNA | After L-NNA | p Value |
|---|---|---|---|---|
| **LV Myocardial Blood Flow** | 1-2 days | 106±11 | 134±17 | <0.05 |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 129±23 | 163±29 | <0.01 |
| **Coronary Vascular Resistance** | 1-2 days | 0.42±0.06 | 0.57±0.08 | <0.01 |
| **($mmHg/ ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 0.48±0.07 | 0.58±0.08 | <0.05 |
| **LV Myocardial $O_2$ Delivery** | 1-2 days | 14.6±1.4 | 19.2±1.5 | <0.01 |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 14.5±2.5 | 18.5±3.1 | <0.01 |
| **LV Myocardial $O_2$ Extraction Ratio** | 1-2 days | 0.60±0.02 | 0.59±0.01 | n.s. |
| | 7-10 days | 0.67±0.03 | 0.71±0.03 | <0.01 |
| **LV Myocardial $O_2$ Consumption** | 1-2 days | 8.7±0.9 | 11.3±1.3 | <0.05 |
| **($ml \cdot min^{-1} \cdot 100g^{-1}$)** | 7-10 days | 9.6±1.6 | 12.9±2.0 | <0.001 |

***Table 6:5*** *Comparison of LV myocardial blood flow and oxygenation, before and after L-NNA in 1-2 and 7-10 day lambs.*

***Effect of NO inhibition on dobutamine-related changes in LV myocardial blood flow, vascular resistance and $O_2$ delivery.***

Prior to L-NNA administration, in the 1-2 day animals dobutamine infusion at rates up to $10\mu g.kg^{-1}.min^{-1}$ increased left ventricular myocardial blood flow by 77±11 $ml \cdot min^{-1} \cdot 100gLV^{-1}$, reduced left ventricular coronary vascular resistance by 0.34±0.09, increased left ventricular myocardial $O_2$ delivery by 9.5±1.3 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ and LV myocardial $O_2$ consumption by 7.8±1.5 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ (all $p<0.01$). In this group intravenous L-NNA significantly blunted the changes in LV myocardial blood flow ($p<0.05$), LV coronary vascular resistance ($p<0.005$), LV myocardial $O_2$ delivery ($p<0.05$) and LV myocardial $O_2$ consumption ($p<0.05$) during subsequent dobutamine infusion ***(Figure 6:7)***.

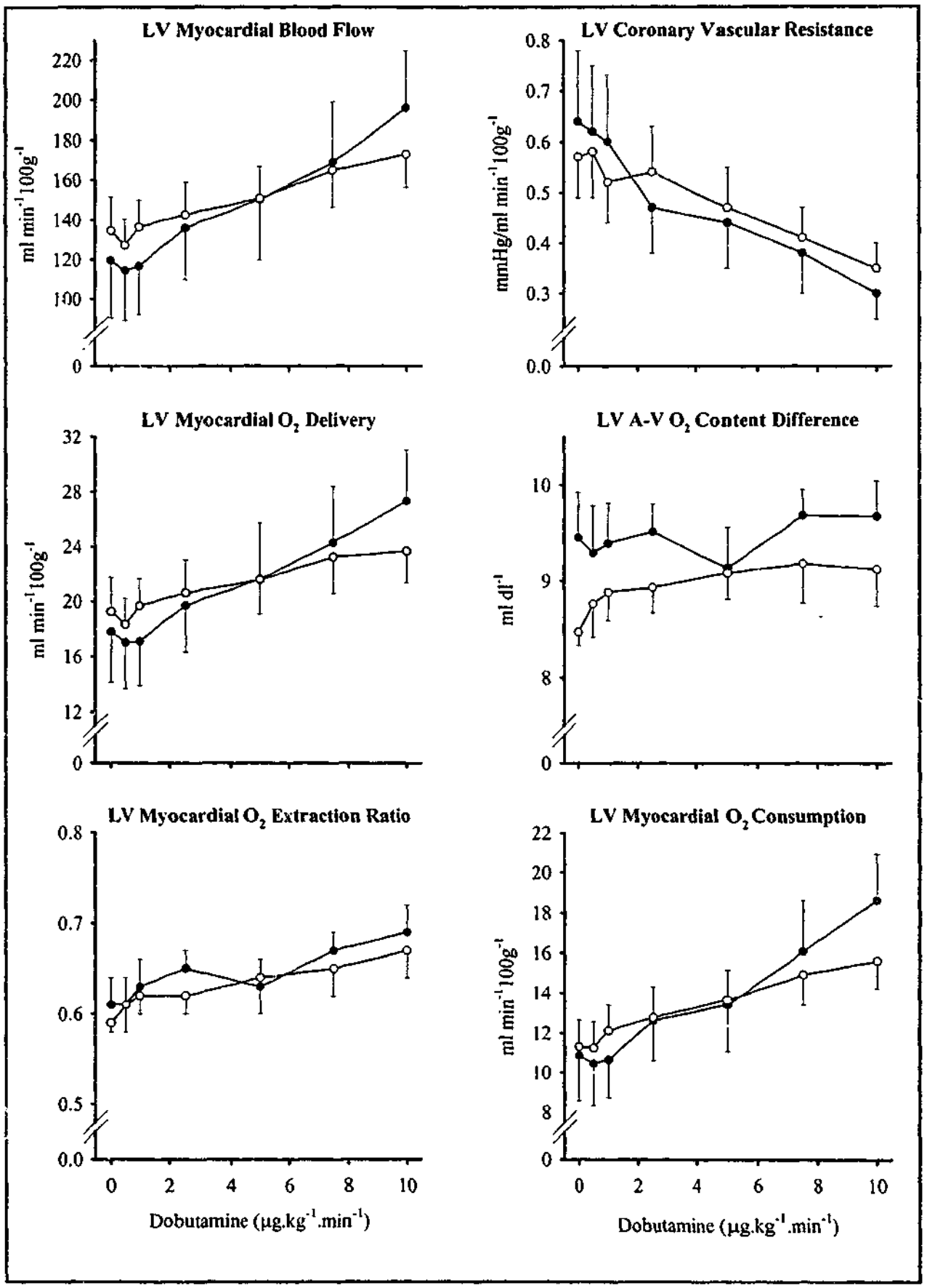


***Figure 6:7.*** *LV myocardial blood flow and oxygenation responses in 1-2 day animals during incremental infusions of dobutamine before* (•) *and after* (o) *L-NNA administration.*

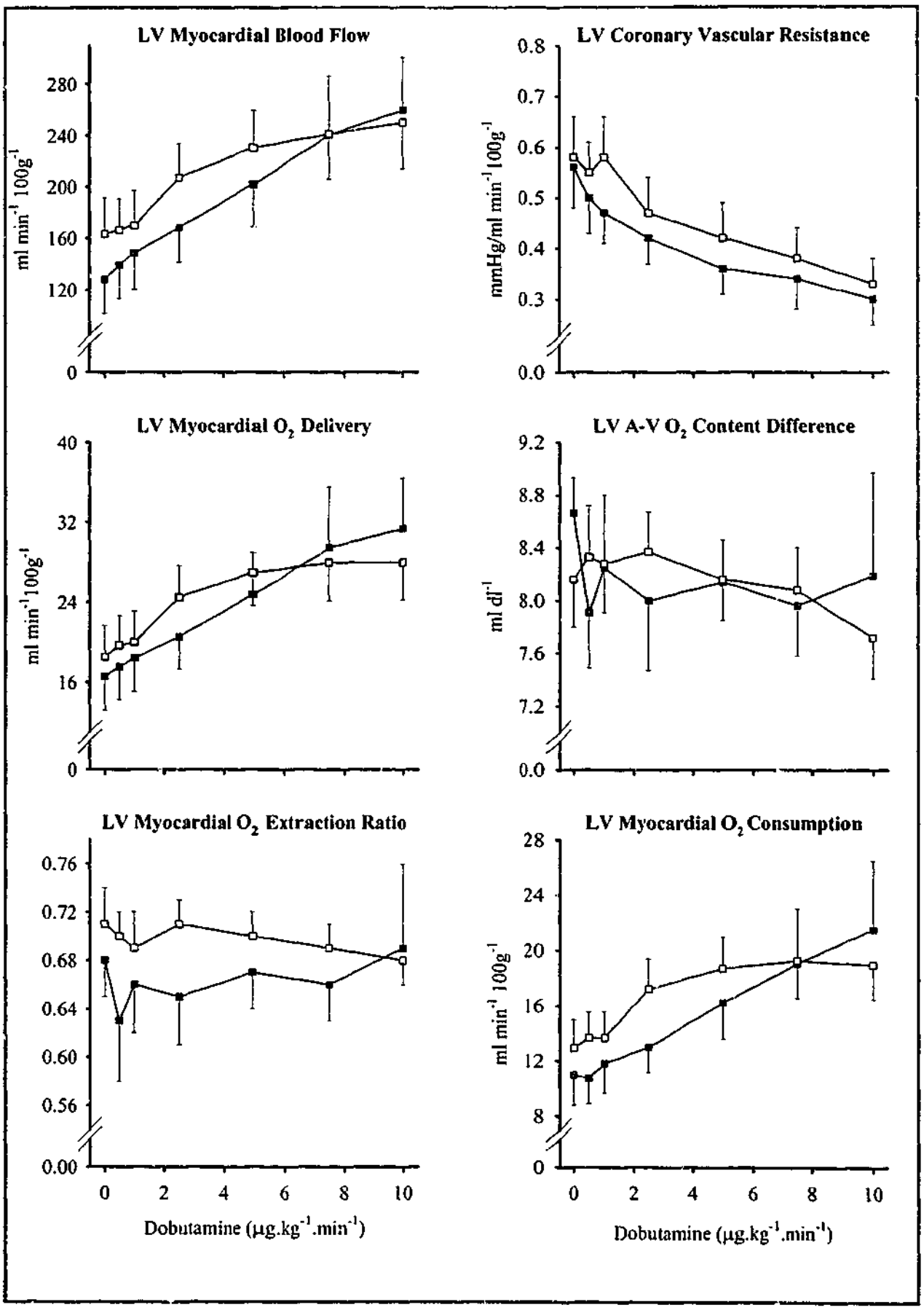


***Figure 6:8.*** *LV myocardial blood flow and oxygenation responses in 7-10 day animals during incremental infusions of dobutamine before* (■) *and after L-NNA administration* (□).

In the 7-10 day animals, dobutamine infusion increased LV myocardial blood flow by 132±22 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ ($p<0.01$), reduced LV coronary vascular resistance by 0.26±0.04 $mmHg/ml \cdot min^{-1} \cdot 100gLV^{-1}$ ($p<0.01$) and increased LV myocardial $O_2$ delivery by 14.8±2.8 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ and LV myocardial $O_2$ consumption by 10.6±3.3 $ml \cdot min^{-1} \cdot 100gLV^{-1}$ (both $p<0.01$). In this group, dobutamine-related changes in left ventricular myocardial blood flow and coronary vascular resistance were not altered by L-NNA administration, although dobutamine-related changes in LV myocardial $O_2$ delivery tended to be attenuated ($0.05<p<0.1$). However, as in the younger animals, dobutamine-related increases in LV myocardial $O_2$ consumption were significantly attenuated by prior L-NNA administration ($p<0.05$; ***Figure 6:8***).

***Effect of NO inhibition on the relationship between LV myocardial $O_2$ consumption and $O_2$ delivery during dobutamine infusion.***

Despite the effects of L-NNA on basal LV myocardial blood flow, $O_2$ delivery and $O_2$ consumption and its effects of dobutamine-related responses, there was no significant effect of NO synthase inhibition on the overall relationship between delivery and consumption during dobutamine infusion in either group ***(Figure 6:9)***.

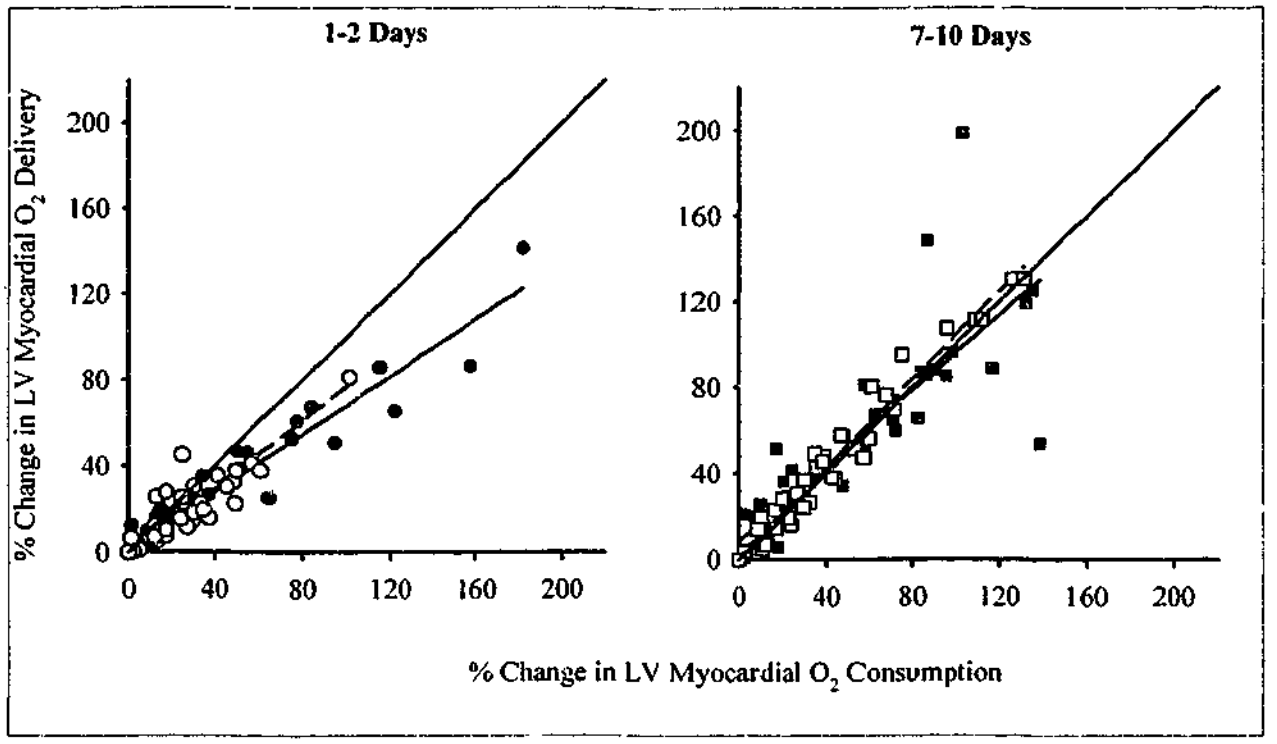


***Figure 6:9.*** *Relationship between changes in LV myocardial $O_2$ consumption and delivery during dobutamine infusion in 1-2 day (left-hand panel) and 7-10 day (right-hand panel) lambs during dobutamine infusion before (closed symbols) and after (open symbols) L-NNA. The line of unity is represented by the solid black line and the regression lines for the relationships before and after L-NNA are represented by the solid and dashed blue lines respectively.*

### *6.4 Discussion.*

Three main observations have arisen from this chapter, which has investigated the changes in left ventricular myocardial $O_2$ consumption, blood flow and $O_2$ delivery during inotropic stimulation and NO inhibition. First, in the 7-10 day lambs, the increase in myocardial $O_2$ consumption during dobutamine infusion was matched by proportional increase in $O_2$ delivery. This important homeostatic mechanism was not evident in the 1-2 day group, suggesting that the ability to couple increases in consumption with delivery was acquired during postnatal life. The second observation was that the close coupling between increases in LV myocardial $O_2$ consumption and delivery did not occur with dopamine in either group. Third,

although inhibition of NO synthesis altered left ventricular myocardial $O_2$ delivery and consumption, it did not significantly modulate the delivery-consumption relationship during dobutamine infusion.

### *6.4.1 Relationship Between Left Ventricular Myocardial $O_2$ Consumption and Delivery During Dobutamine Infusion.*

Dobutamine-related increases in LV myocardial $O_2$ consumption were closely matched by proportional increases in myocardial blood flow and $O_2$ delivery in the 7-10 day group, a phenomenon which has been demonstrated in the adult heart[21]. However, this close relationship between $O_2$ consumption and delivery was not evident in the 1-2 day neonates. As a result, the increased metabolic demand in this group could only be met by an increase in left ventricular $O_2$ extraction.

This finding is consistent with other studies from our laboratory in which it was demonstrated that the increase in LV myocardial $O_2$ consumption at birth occurs in despite only minimal changes in LV myocardial blood flow[109]. These observations together suggest that the mechanisms present in the adult myocardium which provide the necessary increases in blood flow to meet rises in $O_2$ consumption, are poorly developed in the young neonate. The relative lack of this mechanism in the young neonate appears at least in part, to be compensated by a less desirable increase in myocardial $O_2$ extraction.

A recognition of the importance of the adult heart's ability to maintain an optimal relationship between its $O_2$ demand and delivery was followed an intense interest in the possible molecular mechanisms underpinning this phenomenon. Berne and coworkers,[249,250] have produced evidence which strongly supports the role of

adenosine in mediating the coupling between $O_2$ consumption and delivery during interventions such as inotropic stimulation and increased arterial load or during exercise. It has been suggested that adenosine is released by the cardiac myocyte into the interstitial compartment in amounts proportional to the level of applied stress. Adenosine acts on specific receptors located on coronary vascular smooth muscle to mediate profound coronary vasodilation.[249]

Our data is in keeping with other studies, which suggest that this important adenosine-mediated homeostasis is poorly developed in the young neonate. First, it has been demonstrated that the increase in myocardial blood flow in response to adenosine in infants after surgery for congenital heart disease is only 37% of that of adults.[110] Second, it has been shown in the neonate, that increases in coronary blood flow during elevations in myocardial work are unaltered by the adenosine antagonist aminophylline. This would suggest that there must be other mechanisms whereby coronary flow can be increased during periods of increased $O_2$ requirements.[243] Possible mechanisms which have been proposed include hydrogen ion concentration, $CO_2$ tension,[243] or potentially NO.[251-253] However, our observation of an increase in myocardial $O_2$ extraction in the younger neonate during dobutamine infusion would suggest that the vasodilator impact of these possible adaptive mechanisms of the immature myocardium is inferior to those mediated by adenosine in the older heart.

***6.4.2 Comparison of Dobutamine and Dopamine.*** While in the 7-10 day group the increase in LV myocardial $O_2$ consumption during dobutamine infusion was closely matched by proportional increases in $O_2$ delivery, this close coupling was not present during infusions of dopamine in either group. Thus in all animals, dopamine infusion was associated with an increase in the left ventricular $O_2$ extraction ratio. This

observation is consistent with studies in patients after cardiac surgery[254] and during cardiac catheterisation,[255] in whom both dobutamine and dopamine increased myocardial $O_2$ consumption. With dobutamine this increase was matched by a similar increase in coronary blood flow; however, failure of the expected increase in coronary blood flow with dopamine suggested coronary constriction. It was therefore suggested that although dobutamine and dopamine have similar haemodynamic effects, dobutamine may offer the important advantage of not limiting the increase in coronary blood flow associated with increased $O_2$ demand.

These fundamental differences between dobutamine and dopamine may be interpreted alongside recent information concerning the regulation of myocardial blood flow during catecholamine stimulation. While, as described above, it had been assumed that the increase in coronary blood flow during catecholamine infusions is mediated by local metabolic feedback secondary to cardiac beta-receptor activation, recent data suggest that additional 'feedforward' coronary vasodilation, mediated through interaction of the catecholamine with adrenoreceptors on the coronary vasculature may also play a role.[242,24] It has also been demonstrated that catecholamines with high-affinity for postjunctional α-receptors on the coronary vasculature may paradoxically mediate coronary vasoconstriction, against a background of increased myocardial work.[256] Our observation of a blunted myocardial blood flow response to dopamine would support this observation. To assess the contribution of post-junctional coronary α-adrenoceptors stimulation by dopamine, this agent was given before and after the administration of the α-adrenergic antagonist prazosin. In the control state, dopamine increased coronary vascular conductance by approximately 50%, compared to the much greater increase

in myocardial $O_2$ consumption of around 250%. After α-adrenoceptor blockade, dopamine's effects on left ventricular $O_2$ consumption were unchanged, although it produced a doubling in coronary vascular conductance compared to control levels.[257] These findings suggested that during dopamine infusion, postjunctional α-adrenoceptor-dependent coronary vasoconstrictor influences compete with metabolically coupled vasodilation.

***6.4.3 Role of NO.*** The increase in LV myocardial blood flow which occurred in animals receiving the NO synthase blocker, L-NNA., contrasted with other studies which reported that blood flow was either unchanged[56,57] or reduced[58,59,61] during this intervention. However, our finding was still consistent with the notion that endogenous NO plays a significant role in the coronary vasodilatation for the following reason. The magnitude of the increase in LV myocardial blood flow (50%) was only about half of the rise in systemic arterial pressure (89%), signifying that an increase in LV coronary vascular resistance occurred after L-NNA administration. Left ventricular myocardial $O_2$ consumption also increased with NO synthase inhibition in the present study, a finding which contrasts with the lack of change[56-59] or the reduction[61] in this variable reported after intracoronary administration of a NO synthase inhibitor. This observed rise in LV myocardial $O_2$ consumption was most likely related to a predominance of the metabolic cost of an augmentation in LV external work[65] and a rise in LV wall stress accompanying elevations in aortic blood pressure, although other direct myocardial effects of NO synthase inhibition cannot be entirely discounted.

Consistent with the evidence pointing to a substantial coronary vasodilator role for NO during β-adrenergic stimulation,[258] inhibition of NO synthesis blunted subsequent dobutamine-induced increases in LV myocardial blood flow in our study. An important consequence of this blunting of myocardial blood flow was a reduction in LV myocardial $O_2$ delivery responses to dobutamine. However, the latter was paralleled by an attenuation of rises in LV $O_2$ consumption, with the result that the relationship between LV myocardial $O_2$ delivery and $O_2$ consumption was unaffected by NO synthase inhibition. Taken together, these findings suggest that, while NO is an important modulator of LV myocardial vasodilator responses, it does not have a substantial physiological role in the maintenance of an appropriate balance between LV $O_2$ supply and demand during β-adrenergic stimulation.

***6.4.4 Clinical Implications.*** Most clinical studies performed in the immediate neonatal period have concentrated on the quantifiable, and often 'desirable' end-points such as changes in systemic haemodynamics and left ventricular function during inotropic stimulation. However, a few studies suggest potentially concerning effects of inotropic stimulation on metabolic function and myocyte integrity in the neonatal heart. Indeed, myocardial necrosis has been demonstrated in animals after the prolonged administration of inotropes.[244,246] Studies of myocardial metabolism, using magnetic resonance spectroscopy have demonstrated marked reductions in the concentrations of high-energy phosphate during inotropic stimulation in neonatal, but not in adult myocardium.[259] Our data suggest that these reductions in high-energy phosphates during inotropic stimulation may be due to impaired vasodilator response.

*6.4.5 Conclusions.* This chapter demonstrates that the close coupling between LV myocardial $O_2$ consumption and delivery during inotropic stimulation appears to be a maturational phenomenon, which is demonstrable in the older age groups, but is absent in the young neonatal lamb. The relationship between this observation and the known toxic effects of inotropes on the neonatal myocardium requires further clarification.

# CHAPTER 7. GENERAL DISCUSSION & CONCLUSIONS

"I do not like having graduate students. I do not like to suggest a problem and suggest a method for its solution and feel responsible after the student is unable to work out the problem by the suggested method by the time his wife is going to have a baby so that he cannot get a job. I find the old saying that 'A PhD thesis is research done by a professor under particularly trying circumstances' is for me the *dead* truth."

Richard, Feynman

### *7.1 Effects of Inotropes On Integrated Cardiovascular Physiology*

These studies began against a background of increasing knowledge of the effects of inotropes on integrated cardiovascular physiology in the adult, as well as increasing application of these insights to the treatment of critical illness. The introductory sections of the preceding chapters of this thesis have highlighted the relative abundance of work in the adult literature relating to the effects of inotropic agents such as dobutamine and dopamine on central haemodynamics and left ventricular performance (Chapter 3), systemic and pulmonary circulations (Chapter 4), systemic oxygen delivery and consumption (Chapter 5) and left ventricular myocardial oxygen delivery and consumption (Chapter 6). Viewed collectively, these findings are consistent with the notion that administration of an inotropic agent in the adult is associated with responses which are, in general, coordinated and complementary.

Illustrative of this broad concept, haemodynamic changes within major vascular compartments frequently display qualitative similarities. Thus, administration of dobutamine in the adult results in pronounced increases in systemic, pulmonary and myocardial blood flows, which are associated with commensurate reductions in systemic, pulmonary and coronary vascular resistances. Furthermore, in the adult, beneficial effects within one body system are generally translated into a corresponding beneficial effect within a downstream system. For example, rises in cardiac output produced by dobutamine in the adult are associated with rises in systemic oxygen delivery. As accompanying elevations in systemic oxygen requirements are fairly minor, these rises in systemic oxygen delivery serve to

promote a maintenance or improvement in the tissue oxygen environment. Finally, a number of mechanisms may come into play to maintain homeostasis in many organs in the adult during inotropic exposure. Within the heart, for example, the maintenance of homeostasis is facilitated by the tight coupling that exist between rises in oxygen delivery and increases in tissue oxygen uptake.

There are numerous mechanisms whereby fundamental differences in the actions of inotropes, between the neonate and adult might occur. Thus, it is widely recognised that considerable changes occur during postnatal development not only in cardiac structure and function, but also within the pulmonary and systemic vasculature and in the regulation of metabolic function. However, the introductory sections of the preceding chapters of this thesis have also emphasized that, compared to the adult, there is a relative paucity of information (and at times considerable controversy) in the neonate about the effects of inotropic agents such as dobutamine and dopamine on aspects of central haemodynamics and left ventricular performance (Chapter 3), systemic and pulmonary circulations (Chapter 4), systemic oxygen delivery and consumption (Chapter 5) and left ventricular myocardial oxygen delivery and consumption (Chapter 6). Moreover, even less information is available about the global and regional effect of inotropic agents on integrated cardiovascular physiology in the neonatal circulation.

The studies described in Chapters 3-6 of this thesis have provided several areas of new information about the effect of inotropic agents within the various physiological systems of the neonate that were examined. Of note, pulmonary vasodilator responses to dobutamine and dopamine were blunted in the initial days after birth. In the heart, a tight coupling between rises in oxygen delivery and

consumption was not evident in the immediate period after birth. In systemic tissues, the beneficial effects of rises in oxygen delivery were largely offset by very substantial increases in oxygen consumption related to thermogenic activity in brown adipose tissue. Viewed on a system by system basis, these findings point to important differences in the type or magnitude of responses in body systems to inotropic agents in the neonate. Viewed together, however, these findings indicate that the integrated cardiovascular response to inotropic agents in the neonate is more heterogeneous and less coordinated than the adult. Indeed, responses to these agents in the neonate may be associated with potentially adverse physiological sequelae. Thus, the blunting of pulmonary (but not systemic) vasodilator responses to dobutamine in newborn lambs was accompanied by significant elevations in pulmonary blood pressures. Furthermore, as exemplified by the exaggerated increase in systemic oxygen consumption accompanying rises in systemic oxygen delivery observed with dobutamine or dopamine infusion in newborn lambs, beneficial effects of inotropes in one physiological area may not necessarily translated be into a corresponding effect within another area.

A major factor which is likely to be implicated in the differing nature of the integrated cardiovascular response to inotropic agents observed in the neonate is the striking structural and metabolic changes which are a fundamental accompaniment of postnatal development. The likely contribution of pulmonary vascular structural features to pulmonary vascular responses to dobutamine in the initial days after birth has been discussed in Chapter 4. The pivotal contribution of transitory brown adipose tissue deposits to the exaggerated thermogenic reponses occurring in response to both dobutamine and dopamine in the initial

days after birth has been discussed in Chapter 5. The likely contribution of immature vasodilator mechanism to the lack of tight coupling between myocardial oxygen delivery and consumption observed during dobutamine administration in the early neonate has been discussed in Chapter 6. A considerable challenge for the future, in both the experimental and clinical setting, would therefore appear to be the optimisation of the physiological effects of inotropic agents in the milieu created by these normal developmental hurdles.

### *7:2 Future Directions.*

The purpose of this thesis was to form the beginning of an ongoing contribution in this area. It now appears that there are more studies to be done than when it started.

While the thesis considers the effects of the more commonly used inotropes, dobutamine and dopamine on integrated, neonatal cardiovascular physiology, other agents may be of equal interest. One such group are the Phosphodiesterase-III inhibitors,[260-262] including milrinone and enoximone which act by inhibiting the breakdown of cAMP by phosphodiesterase (PDE) within the myocardial cell and vascular smooth muscle. While rarely used in the preterm neonate, these agents are gaining increasing popularity for the prevention and management of the low-output state after surgery in infants with congenital heart disease.[263-266] There is evidence from adult data that they provide favourable effects on myocardial supply-demand relationships,[267-269] and their potent systemic vasodilator effects[270] may distribute blood flow to vital organs and enhance ventriculovascular coupling to a greater extent than dobutamine.[271,272] In one study, a contractile response to

amrinone and milrinone was demonstrated in isolated papillary muscles from 14-to-16 day-old immature rabbits, which was greater than in those from adults.[273] However, given the profound developmental changes in PDE expression in the myocardium, which appears to be species-dependent,[274-278] further studies of the effects of these agents on myocardial function in the developing circulation will be required.

Another potential important clinical advance in the treatment of low-cardiac output and hypotension in the young neonate, which might be addressed in future studies, relates to the use of steroids to enhance the effects of conventional inotropic agents. In some critically-ill, hypotensive premature infants, treatment with catecholamine-related agents is not always effective. It has been suggested that this phenomenon reflects a downregulation of cardiovascular adrenergic receptors, combined with a degree of adrenal insufficiency.[279] In these patients, a brief course of steroid treatment may be successful in stabilizing the cardiovascular status and decreasing the requirement for pressor/inotrope support.[280] In one study, mean blood pressure increased in hypotensive neonates after administration of the steroid, hydrocortisone.[281] Another randomised study[282] compared the efficacy of hydrocortisone with dopamine for the treatment of hypotensive, very low birthweight infants, demonstrating that all 19 infants randomised to dopamine and 17 of 21 randomised to the hydrocortisone group 'responded', with no statistically significant difference in efficacy between dopamine and hydrocortisone noted. However, further, well-designed randomized and controlled clinical trials, which examine, not only the changes in arterial blood pressure during administration of these agents are needed to determine the

potential short- and long-term side effects of steroid administration on integrated cardiovascular performance in preterm infants with pressor-resistant hypotension.

The aim of this work was to examine the effects of cardiovascular agents on integrated cardiovascular physiology in a newborn model. While the experiments presented demonstrate important developmental effects of inotropic agents on integrated cardiovascular performance, significant areas of this integration have not been addressed. These include an assessment of the implications of developmental changes in ventricular diastolic performance for the overall effects of adrenergic agents. Important developmental changes in ventricular relaxation and compliance characteristics have been described[283-285] and it is likely that they will play an important modulating effects of inotropes in the developing circulation.

Another area of interest which has not been addressed relates to the specific assessment of ventriculovascular coupling. The intricate coupling between the ventricle and the vasculature is an extremely important clinical determinant of cardiovascular function. While many treatments in the child with heart failure are aimed at augmenting ventricular systolic performance, it is clear that without the ability of the vasculature to convert within itself the increased pressure-work of the ventricle into flow-work, these therapeutic strategies would be of little benefit. A measure of this interaction between the ventricle and the vasculature would clearly provide us with a valuable additional tool in the assessment of cardiovascular performance.

Another important area which has not been examined is the distribution of increased cardiac output to regional organ systems of the body. In this thesis, my

examination of blood flow was restricted, in this heavily-instrumented model to overall cardiac output and flow to the myocardium itself. It is likely that any changes in cardiac output which I observed in my studies represented the cumulative effect of increases in flow to some organs, coupled with decreases to others.[286,287] Potentially future studies, which address regional blood flow to, particularly the vital organs, including the brain, kidneys and bowel would make significant contributions to our understanding of overall cardiovascular integration during inotropic infusions. Finally if the ultimate therapeutic aim of inotropic treatments is to optimise metabolic function, the examination of their effects cannot be considered to be complete, without an assessment of their effects on cellular metabolism. In the current studies, I have 'lumped' metabolism into a single measure of systemic $O_2$ consumption. However, more precise examination of regional metabolic function would provide considerably more insights into the ultimate end-organ effects of inotropic treatments. Techniques, for examining cellular metabolism in vivo, including magnetic resonance spectroscopy have already been used in the examination of inotropic effects in the developing animal.[259]

This research was stimulated by a desire to understand more about how these potent therapeutic agents influence the patients for whom I care. To this end, another obvious limitation of this work, is that I employed 'normal' animals for my studies. It is clear that my results cannot be directly applied to, for example the sick preterm infant, in whom there will be further immaturity of cardiovascular and metabolic function, or the sick neonate with 'persistent fetal circulation' in whom baseline pulmonary vascular resistance will be considerably elevated. They cannot be directly applied to the infant after cardiac surgery, in

whom baseline levels of systemic $O_2$ delivery will be considerably reduced and pulmonary vascular resistance elevated, or to the patient with complex congenital heart disease, in whom the systemic circulation may be supported by a subaortic right ventricle. However, what is important is that these studies have provided a foundation on which further more clinically relevant investigations can be based. Already, we have investigated the effects of vasoactive agents in a model of neonatal pulmonary hypertension[288] and in infants with pulmonary hypertension after cardiac surgery.[289] We have examined the determinants of systemic $O_2$ delivery-consumption relationships in the infant after cardiac surgery[290] and in an animal models of cardiopulmonary bypass.[291] The integrated approach has allowed us to investigate the effects of inotropic stimulation on cardiovascular performance in patients with complex congenital heart disease[292] and to examine the relationships between changes in central haemodynamics and blood flow to vital organs in the sick, preterm infant.[83] Further studies are underway and it is likely that they will continue into the future.

***7.3 Closing Remarks.*** This thesis describes a series of experiments which have provided me with insights into the effects of postnatal development on cardiovascular integration. It has shown me the potential of an 'integrated approach' to examine the effects of interventions on a complex system, in this case, the cardiovascular system. It has demonstrated to me that it is not possible to directly extrapolate data from adult studies to the immature circulation and that any investigation must pay sufficient attention to the important developmental backdrop against which therapeutic interventions occur. Finally, and potentially most importantly, this thesis has provided important foundations for my own

'development' as a cardiovascular scientist and clinician, foundations which I hope I can use in the years ahead.

*"To the physician particularly, a scientific discipline is an incalculable gift, which leavens his whole life, giving exactness to habits of thought and tempering the mind with that judicious faculty of distrust which can alone, amid the uncertainties of practice, make him wise unto salvation".*

William Osler [293]

# APPENDICES

## Appendix A Anaesthesia with α-Chloralose

Studies were performed in an acute open-chest lamb model, using general anaesthesia. This was for a number of reasons. First, it was considered that it would have been difficult to reliably maintain the complex instrumentation in a chronic, conscious preparation. Second, the need to study the very early neonatal period would have required fetal surgery, thus increaasing the complexity of the experimental studies and therefore reducing the chances of success. Third, infusion of high-dose catecholamines into the conscious newborn may have resulted in arousal, with confounding effects on, for example systemic $O_2$ consumption due to an associated increase in muscle activity. Finally, many critically ill infants in intensive care would be routinely sedated, thus the conditions of this model may in some ways be representative of the clinical setting.

α-Chloralose was chosen because it has been demonstrated to have a much lower myocardial depressant effect than other anaesthetic drugs commonly used for experimental purposes.[154,153] It is known that even it may alter endogenous catecholamine release[155] and have interactions with β-adrenergic receptors.[156] However, these effects have been demonstrated be transient and to occur after high-dose intravenous boluses.[156,294] Furthermore even large intravenous boluses of α-chloralose did not alter systemic or pulmonary vascular resistances,[294] or systemic $O_2$ delivery or consumption in newborn lambs.[155]

## Appendix B Dobutamine

Dobutamine is a synthetic catecholamine, originally developed in 1975 by Tuttle and Mills.[10] Its pharmacological effects are due to direct interactions with α- and β- adrenoreceptors and do not appear to rely on the release of endogenous catecholamine stores.[295,10] The compound which is used clinically and experimentally is a mixture of (-) and (+) isomers,[296] with the (-) isomer being a potent $\alpha_1$-adrenoreceptor agonist and the (+) isomer being about 10 times as potent a β-adrenoreceptor agonist as the (-) isomer.[173]

The cardiovascular actions of dobutamine infusions are a composite of the distinct pharmacological properties of the (-) and (+) stereoisomers of dobutamine. Infusions at rates exceeding approximately 2.5$\mu g \cdot kg^{-1} \cdot min^{-1}$ usually increase myocardial contractility and result in an increase in heart rate which is less marked than with isoprenaline. Dobutamine has a rapid onset of action and a half-life in the dog and in humans of approximately 2 minutes,[297,142] being metabolised in the liver by conjugation with glucoronic acid.[297] Its major metabolites are 3-O-methyldobutamine and dobutamine glucoronide, both of which are thought to be biologically inactive.

## Appendix C Selective Adrenoreceptor Blockade

In order to characterise the specific role of $\alpha_1, \alpha_2, \beta_1$ and $\beta_2$ adrenoceptor activation on dobutamine responses in 1-2 day old lambs, incremental infusions of dobutamine were administered after selective adrenoreceptor blockade with one of the following agents:

***1) Prazosin,*** an $\alpha_1$ adrenoreceptor antagonist prazosin (Sigma), given as an intravenous bolus, 0.2 $mg.kg^{-1}$, followed by an intravenous infusion at a rate of 1 $mg.kg^{-1}.hr^{-1}$. Its affinity for $\alpha_1$-adrenoreceptors in about 1000-fold greater than for $\alpha_2$ receptors.[298] In a previous study, an infusion of 0.12 $mg.kg^{-1}.hr^{-1}$ was administered to achieve $\alpha_1$ blockade in sheep.[299]

***2) Yohimbine,*** an $\alpha_2$ adrenoceptor antagonist (Sigma), given as an intravenous bolus, $1mg.kg^{-1}$, followed by an intravenous infusion at a rate of 1 $mg.kg^{-1}.hr^{-1}$. Functional and radioligand studies of yohimbine demonstrate that it has an $\alpha_2/\alpha_1$ sensitivity ratio of 45.[300] In previous studies between 0.4 and 1.2 $mg.kg^{-1}$ was administered in sheep[301] and $1mg.kg^{-1}$ in lambs[302] in order to achieve $\alpha_2$-blockade.

***3) CGP 20712A*** a $\beta_1$ adrenoreceptor antagonist, given as an intravenous bolus 50 μg/kg, followed by an infusion at a rate of 50 $\mu g.kg^{-1}.hr^{-1}$. This agent has been demonstrated to be extremely selective for the $\beta_1$-adrenoreceptor, with studies from rat brain demonstrating a $\beta_1/\beta_2$ sensitivity ratio of 10,000.[303] Intravenous infusions of 30 $\mu g\ kg^{-1}\ hour^{-1}$ were administered to dogs to achieve blockade of the $\beta_1$-adrenoreceptor.[304]

*4) ICI 118551,* a $\beta_2$ adrenoreceptor antagonist, given as an intravenous bolus of 0.2 $mg.kg^{-1}$, followed by a continuous infusion of 0.2 $mg.kg^{-1}.hr^{-1}$ . This agent has an in vitro $\beta_2/\beta_1$-selectivity ratio, of 123, compared to the equivalent ratio of 2.2 for propranolol.[305] The potency and selectivity of ICI 118,551 for antagonism of vascular versus atrial actions of isoproterenol in anaesthetised dogs was greater than 250:1.[305] An intravenous bolus of ICI 118551, 0.2 $mg.kg^{-1}$ bolus followed by an infusion of 0.2$mg.kg^{-1}hour^{-1}$ significantly blunted the haemodynamic responses to the β adrenoceptor agonist dopexamine in anaesthetised dogs.[306]

## Appendix D Dopamine

Dopamine is a naturally-occurring neurotransmitter, with high amounts present in the brain, particularly the basal ganglia. It acts on dopaminergic receptors, as well as α- and β-adrenoreceptors.[307] There is evidence that the dopamine has differential dose-related effects, which reflect its relative affinity for the different receptor subtypes. It has been suggested that at low infusion rates its renal vasodilator response predominate,[308-311] due to its actions on vasodilator dopaminergic receptors in the renal arterioles. However, more recent data questions the specificity of this low-dose effect.[312-314] At intermediate infusion ranges, β-adrenergic effects becoming evident, with α-adrenergic actions predominating at higher doses[307]. In addition, an important contribution to the actions of dopamine is made by the release of endogenous noradrenaline and adrenaline.[315-317] Thus plasma levels of these catecholamines were increased by dopamine infusion in sick preterm infants.[318]

Dopamine is rapidly metabolised by both monoamine oxidase and catechol-O-methyl transferase, present in circulating blood to form 3,4-dihydroxyphenylacetic acid and homovanillic acid, both of which are conjugated and excreted in the sulphate or glucoronide form.[319] Its plasma half-life in older children and adults is approximately 2 minutes.[307] However, in critically-ill preterm infants its plasma half-life is prolonged to approximately 7 minutes.[320]

## Appendix E Systemic Inhibition of NO Synthesis with Intravenous N$^{\omega}$-nitro-L-arginine (L-NNA)

The dose of intravenous N$^{\omega}$-nitro-L-arginine (L-NNA) (25mg kg$^{-1}$) which was used in the experiments, presented in this thesis was based on pilot experiments in 3 lambs, in which cumulative intravenous doses of L-NNA were administered in the presence of a background infusion of dobutamine (5μg kg$^{-1}$min$^{-1}$). The haemodynamic response plateaued over the range 20-30 mg kg$^{-1}$ ***(Figure E:1)***

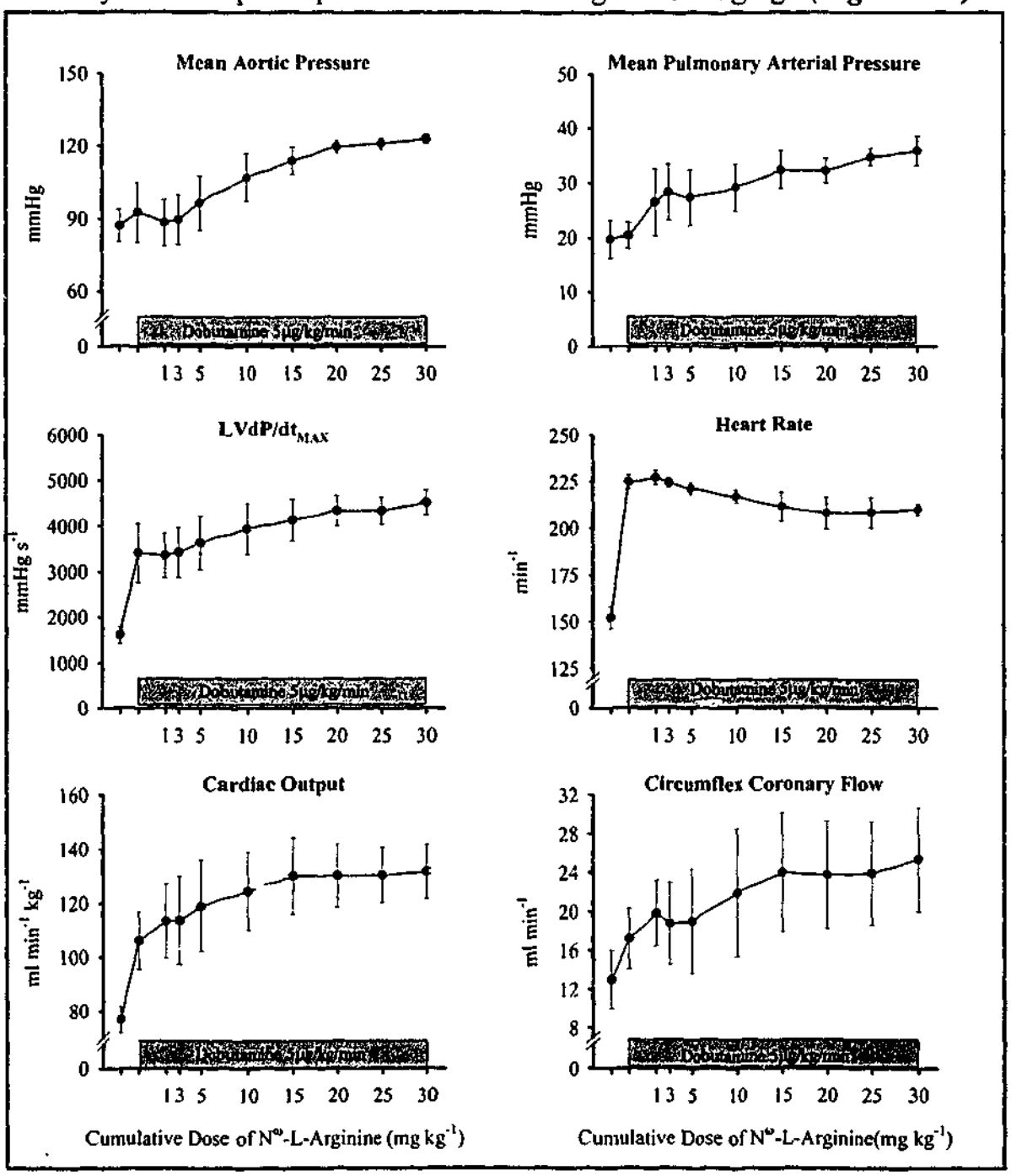


*Figure E:1. Effects of a cumulative dose of L-NNA against a background infusion of dobutamine 5μg kg$^{-1}$ min$^{-1}$. The haemodynamic response plateaued at an L-NNA dose of 20-30mg kg$^{-1}$*

## Appendix F Reproducibility of Sequential Dobutamine Infusions.

In three animals an incremental infusion of dobutamine up to 10 $\mu g.kg^{-1}.min^{-1}$ was administered. The infusion was then weaned and a period of 30 minutes allowed to elapse, after which the incremental infusion was repeated. While some of the variables were not restored to baseline levels by the time, that the second infusion was commenced, apart from minor differences, the overall pattern of the responses to the sequential infusions were similar ***(Figure F:1)***.

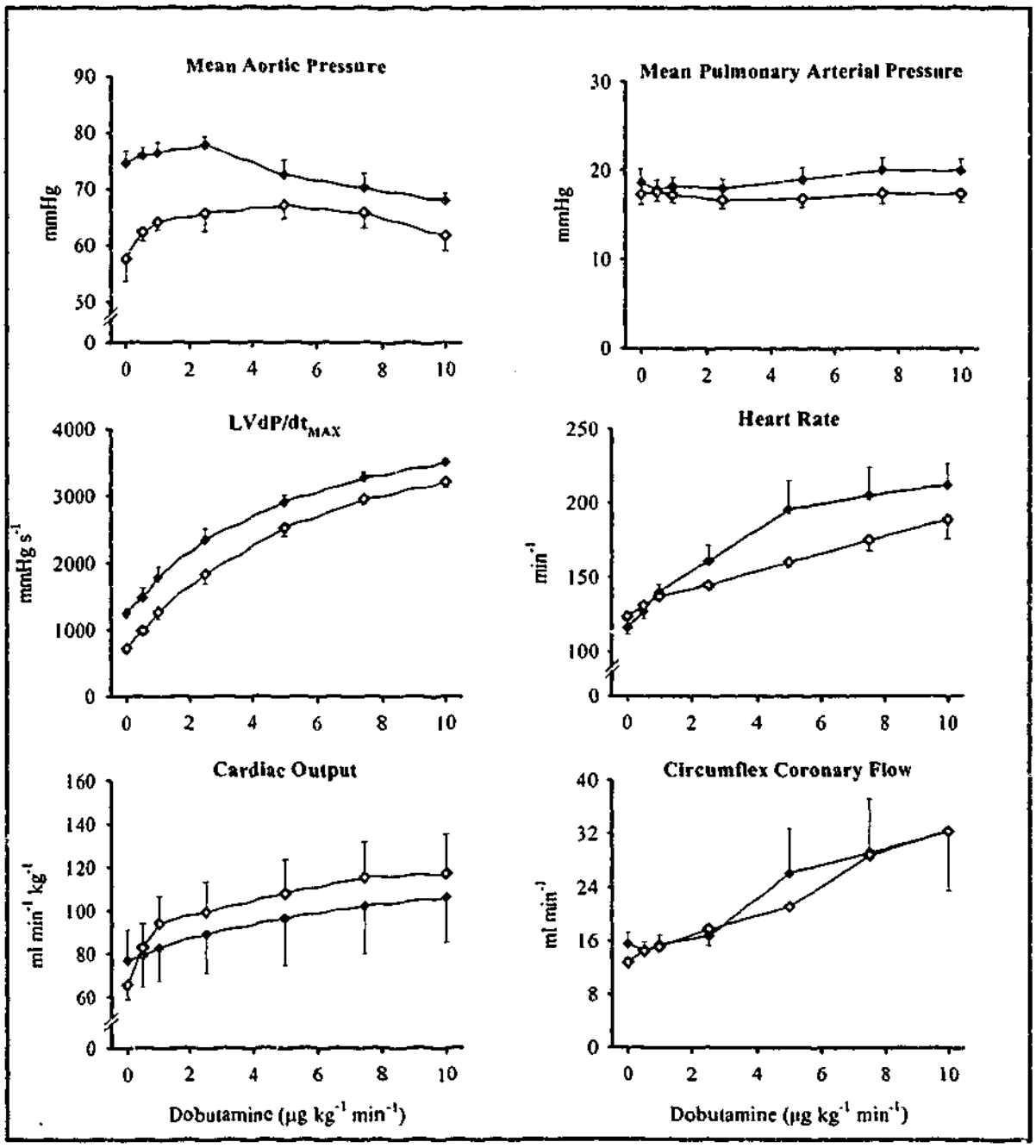


***Figure F:1.*** *Effects of sequential incremental infusions of dobutamine up to 10 $\mu g.kg^{-1}.min^{-1}$. The haemodynamic response to the first (♦) and second (◊) infusions were similar.*

Appendix G Measurement of Aortic and Circumflex Coronary Flow Using The Transonic® System.

Transonic flow probes were used to measure blood flow in the ascending aorta and in the circumflex coronary artery. The Transonic® technique is ultrasound-based. An ultrasound signal is directed through the vessel of interest during which the signal is accelerated or decelerated depending on the directional movement of the blood and the orientation of the transmitting transducer. If the ultrasonic signal consists of a wide, constant intensity field that insonicates the full cross-section of the vessel, then the degree of acceleratory change of the signal is a function of the volume flow of blood intersecting the beam of ultrasound.

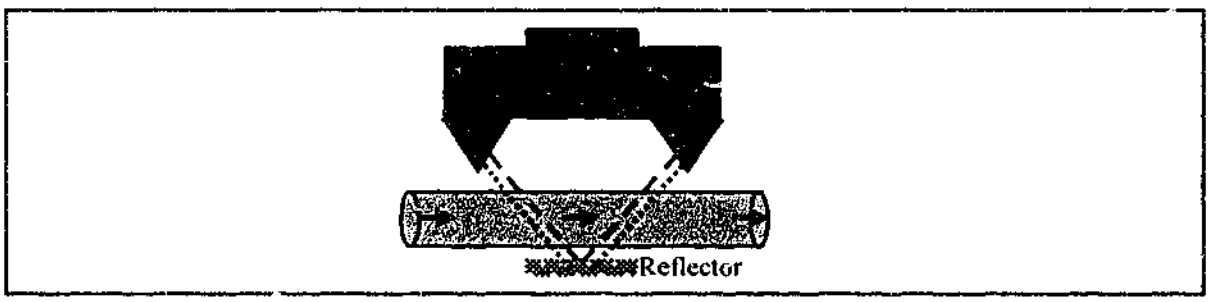


***Figure G:1. Schematic view of the perivascular Transonic ultrasonic volume flowsensor.*** *Using wide beam illumination, two transducers pass ultrasonic signals back and forth, alternately intersecting the flowing liquid in upstream and downstream directions. The flowmeter derives an accurate measure of the "transit time" it took for the wave of ultrasound to travel from one transducer to the other. The difference between the upstream and downstream integrated transit times is a measure of volume flow.*

In this way the technique differs from Doppler ultrasound, which derives volume flow from separate estimates of average velocity inside a vessel cross-sectional area. In the 'S' series and 'R' series transducers which were used in these experiments each flow probe has two ultrasound transducers, which considerably reduces error sue to vessel-probe malalignment. The transducers are positioned

on one side of the vessel under study and a reflector is positioned midway between the two transducers on the opposite side of the vessel. ***(Figure G:1)***

Important additional features of the transonic flow system are that the technique is insensitive to haematocrit and that the transducers do not have to be constrictive, an important disadvantage of electromagnetic flow systems.[321] The Characteristics of the flow transducers which were used are presented in ***Table G:1.***

| | **Relative Accuracy (%)** | **Resolution (ml $min^{-1}$)** | **Range (L)** |
|---|---|---|---|
| **2 'R'** | ± 2 | 0.1 | 0-0.5 |
| **10 'S'** | ± 2 | 8 | 0-10 |
| **12 'S'** | ± 2 | 8 | 0-20 |

***Table G:1 Characteristics of the transonic flow probes used in this thesis*** *(Data from manufacturer).*

In vivo validation studies demonstrate that there was no significant difference between blood flow measured using the transit-time technique and either electromagnetic flow probes or pump withdrawal methods.[322] Studies in conscious sheep demonstrated that cardiac output measured with the transit-time method agreed closely with either thermodilution or timed collection of blood when the transducer is placed around the ascending aorta (as employed in this thesis). However, when placed around the pulmonary trunk, the technique consistently underestimates flow.[323]

## Appendix H Measurement of $LVdP/dt_{MAX}$ with a Micromanometer-Tipped Catheter as an Indicator of LV Contractility.

The measurement of left ventricular pressure for the accurate assessment of changes in ventricular function cannot rely on the use of fluid-filled systems which have insufficient frequency response and excessive phase and amplitude errors. Micromanometer-tipped systems have been developed to overcome these limitations. The pressure sensor, which has a high frequency response is located at the tip of the catheter, so that high-fidelity pressure measurements can be made at source ***(Figure H:1)***.

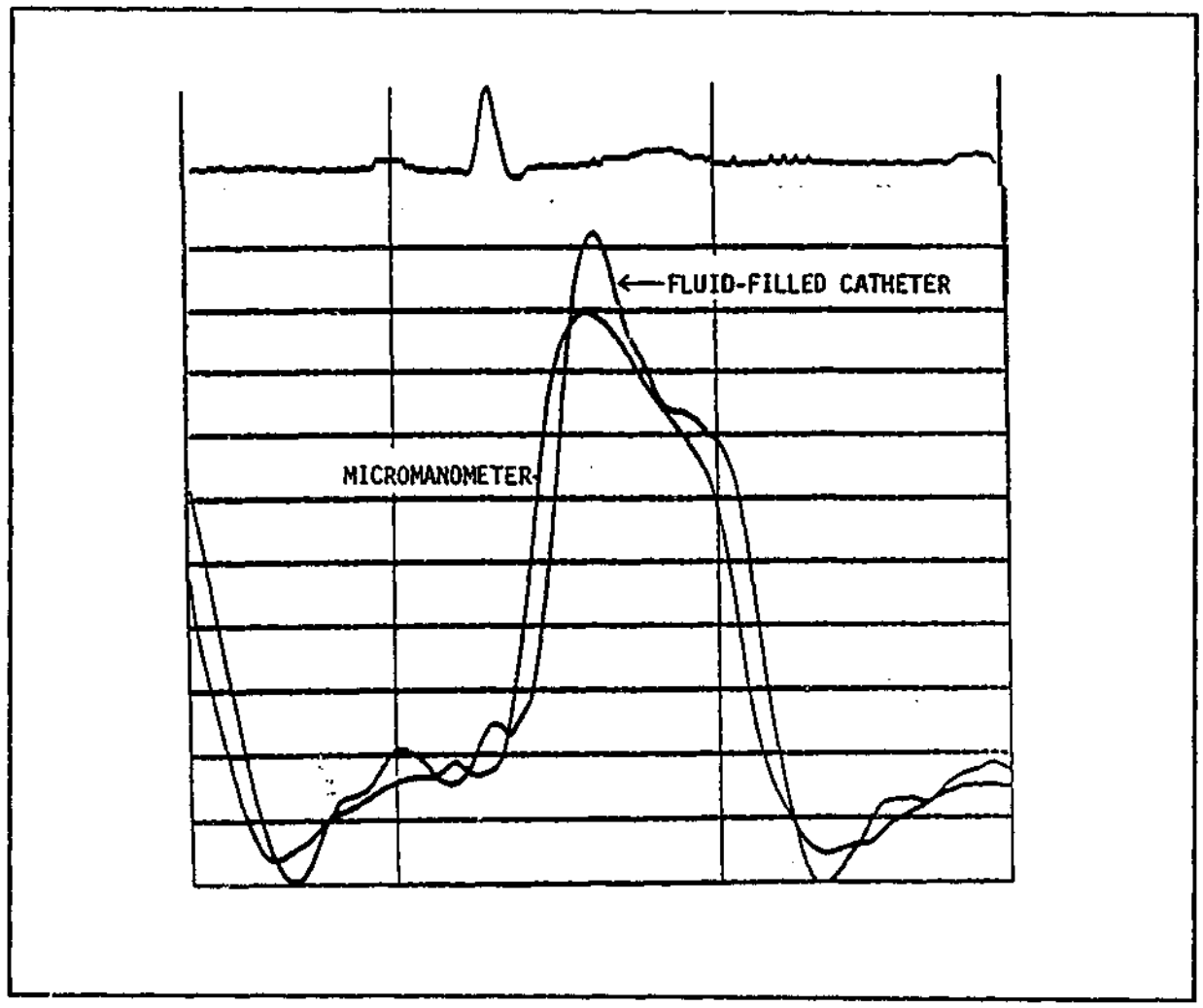


***Figure H:1.*** *Simultaneous recording of left ventricular pressure with a fluid-filled and micromanometer-tipped catheter. The trace from the fluid-filled catheter is delayed and exhibits overshoot at the peak of left ventricular pressure.*

In the experiments presented in this thesis, measurement of left ventricular pressure was performed with a 2F micromanometer-tipped catheter (Millar Instruments). The ultraminiature pressure transducer mounted on the tip of these catheters has a linear response, with an output accuracy of ±0.5% between –50 and +300 mmHg and a frequency response of up to 20kHz. Temperature drift is minimal at physiological temperatures, but in order to minimise this, the catheter was placed in the circulation for at least 30 minutes prior to final calibration. It was then calibrated using an electrical reference for 0 (atmospheric) and 100mmHg.

The left ventricular signal was differentiated on-line with a customised differentiator (Baker Institute, Melbourne), the output of which was proportional to frequency up to 100 Hz (±5%).[324] It has been demonstrated that this frequency characteristic enables reliable recordings of LV dP/dt at levels up to 9000 mmHgs$^{-1}$.[325] The differentiated LV pressure signal was used in turn, to derive LV $dP/dt_{MAX}$(the maximal rate of rise of LV pressure), which was employed as the index of ventricular performance.

There are a number of isvolumic, ejection and end-systolic indexes of ventricular performance now available for research and clinical purposes. I have reviewed the advantages and disadvantages of the indexes in previous publications[326,327]. The sensitivity of each to load and inotropic state of the ventricle was examined by Kass and coworkers[328]. In general, it appeared that indexes which are most sensitive to changes in inotropic state are also most affected by changes in ventricular load . Thus while the end-systolic index, $E_{MAX}$ is much less sensitive to alterations in ventricular load than $dP/dt_{MAX}$, it is also less sensitive to changes

in contractility. Ideally, therefore, we would have employed both an isovolumic and end-systolic measure, if the techniques had been available .

It is important to note that a number of mathematical techniques have been developed to reduce the load sensitivity of $dP/dt_{MAX}$. These included, normalisation of the dP/dt signal to either developed pressure or to ventricular end-diastolic volume. However, the literature suggests that these techniques are associated with their own disadvantages. Thus, $dP/dt_{MAX}$, normalised to developed pressure was very insensitive in separating normal from abnormal in a groups of patients with obvious and advanced left ventricular disease, defined in terms of reduced ejection fraction, raised end-diastolic pressure[329]. Studies from isolated canine heart-lung preparations in which load and inotropic state was carefully regulated led the authors to conclude that that none of these normalisation methods have any advantage over $dP/dt_{MAX}$ for evaluating changes in contractility[330].

## Appendix I Measurement of Pulmonary Blood Flow With Thermodilution

The measurement of pulmonary blood flow by thermodilution was first described by Fegler,[331] who based the technique on the mathematical formulation used for indicator 'dye-dilution' methods, using temperature as the 'indicator'. A known volume of injectate is injected into the central venous system and the exponential decay in temperature of the blood in the pulmonary artery is measured with a thermistor during successive cardiac cycles. The cardiac output is inversely proportional to the rate of cooling of the blood and is computed according to the equation:-

$$CO = \frac{Vi \times Ci \times Si\,(Tb-Ti) \times 60}{Sb \times Cb \int_{0} Tb(t)dt}$$

where Vi is the known volume of injectate with specific heat of Ci, specific gravity of Si and temperature Ti. Cb, Sb and Tb, represents the same for the blood. If the specific heat and the specific gravity for the injectate and for blood are known, the equation can be represented as:-

$$CO = \frac{1.08 \times Vi(Tb-Ti)}{\int_{0} Tb(t)dt}$$

Thermodilution is widely used in the clinical and research setting for measuring pulmonary blood flow. However, measurements are unreliable in the presence of an intracardiac shunt, tricuspid or pulmonary regurgitation and mechanical ventilation.[332] In order to minimise the effect of ventilation in the present studies, all measurements of pulmonary blood flow were performed during expiration, when intrathoracic pressure was at its lowest level.

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