
UNIVERSITY OF NORTH TEXAS
HEALTH SCIENCE CENTER AT FORT WORTH
1970

Yi, Kun Don. Dobutamine increases mechanical function and cytosolic phosphorylation potential during moderate right ventricular hypoperfusion Master of Science (Biomedical Sciences), August, 2000, 101 pp, 4 tables, 18 figures, references, 108 titles.

This study was conducted to investigate the functional and metabolic effects of regional inotropic stimulation with dobutamine during right ventricular (RV) hypoperfusion. Right coronary perfusion pressure was incrementally lowered to 40 mmHg from 100 mmHg, and two-doses of dobutamine (0.01 and 0.06 μg/kg/min) were continuously infused for 15 min into the right coronary artery in pentobarbital sodium-anesthetized mongrel dogs of either sex. Myocardial energy metabolites, glycolytic intermediates, glycogen, and phosphorylation potential were measured in freeze-clamped RV biopsies. RV hypoperfusion caused a 54% decrease in right coronary blood flow, a decrease in lactate uptake, and an increase in glucose uptake. Systolic segment shortening, isometric force, MVO_2, and oxygen utilization efficiency (O_2UE: power/MVO_2) decreased significantly. Energy reserves were unaffected by the hypoperfusion. Low-dose dobutamine during hypoperfusion improved regional mechanical function without increasing MVO_2, and thus, improved O_2UE. Remarkably, low-dose dobutamine markedly increased phosphocreatine content and phosphorylation potential. In contrast, high-dose dobutamine produced only transient improvements in function and efficiency and sharp decreases in energy reserves. Analysis of glycolytic intermediates showed a sustained augmentation of glycolysis during low-dose dobutamine, but glycolysis was limited by high-dose

dobutamine at the level of glyceraldehyde-3-phosphate dehydrogenase. Dobutamine is capable of improving both contractile function and cellular energetics in underperfused RV myocardium at low but not high dose dobutamine. Therefore, dosage should be carefully selected.

DOBUTAMINE INCREASES MECHANICAL FUNCTION AND

CYTOSOLIC PHOSPHORYLATION POTENTIAL

DURING MODERATE RIGHT VENTRICULAR HYPOPERFUSION

Kun Don Yi, B.S.

APPROVED:

H. Fred Downey

Major Professor, H. Fred Downey, PhD.

Committee Member, Robert T. Mallet, PhD.

Committee Member, Eugene E. Quist, PhD.

Chair, Department of Integrative Physiology, Peter B. Raven, PhD.

Dean, Graduate School of Biomedical Sciences, Thomas Yorio, PhD.

DOBUTAMINE INCREASES MECHANICAL FUNCTION AND CYTOSOLIC PHOSPHORYLATION POTENTIAL DURING MODERATE RIGHT VENTRICULAR HYPOPERFUSION

THESIS

Presented to the Graduate Council of the
University of North Texas Health Science Center at Fort Worth
In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Kun Don Yi, B.S.

Fort Worth, TX

August, 2000

ACKNOWLEDGEMENTS

This work was supported by National Heart, Lung, and Blood Institute grants HL 35027 and HL 50441.

I wish to thank those who have been instrumental in my education and research, Dr. H. Fred Downey and Dr. Robert T. Mallet. I greatly appreciate their guidance, leadership, encouragement, and patience over the past three years. In addition, I would also like to thank Dr. Eugene E. Quist, who served on my committee, and Dr. Xiaoming Bian. I also gratefully acknowledge the expert technical assistance of Arthur Williams, B.S., Min Fu, B.S., Jie Sun, B.S., Bradley Hart, M.S., and Srinath Setty, M.B.B.S.

Last but not least, I would like to thank my family, friends, and God, not necessarily in that order, for their support and patience.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

Figure

CHAPTER II

Figure

APPENDIX

Figure

CHAPTER I

INTRODUCTION

A balance between the myocardial oxygen supply and demand must be maintained for the working myocardium to function properly. The normal heart is able to vary its output, and thus, coronary blood flow must be able to vary in response to the heart's metabolic requirements. If coronary blood flow to a region of the myocardium is insufficient in relation to regional oxygen and metabolic requirements, oxygen delivery to the mitochondria is reduced, resulting in myocardial ischemia (22), as in obstructive and/or dynamic coronary arterial flow reduction. Myocardial ischemia can also be caused by an increase in oxygen demand as seen during tachycardia, exercise, or left ventricular hypertrophy if coronary blood flow cannot increase oxygen supply sufficiently (23, 29, 47, 48, 61, 70). When myocardial blood perfusion is compromised, the degree of imbalance between the myocardial oxygen supply and demand may vary greatly, depending on a variety of factors that influence oxygen supply and demand (Figure 1; 42).

Myocardial oxygen demand is determined mainly by heart rate, wall tension, and contractile state, while myocardial oxygen supply is determined by coronary blood flow and oxygen extraction. Heart rate is one of the major determinants of myocardial oxygen uptake. Wall tension also determines oxygen uptake and is increased when either preload or afterload increases. Myocardial

contractile work accounts for about 80% of the resting myocardial oxygen consumption. The remaining 20% is accounted for by the non-contractile functions of the heart, i.e., basal metabolic requirements. When myocardial activity increases above resting levels, the fraction of oxygen consumption associated with contractile work increases proportionately (5, 7, 57).

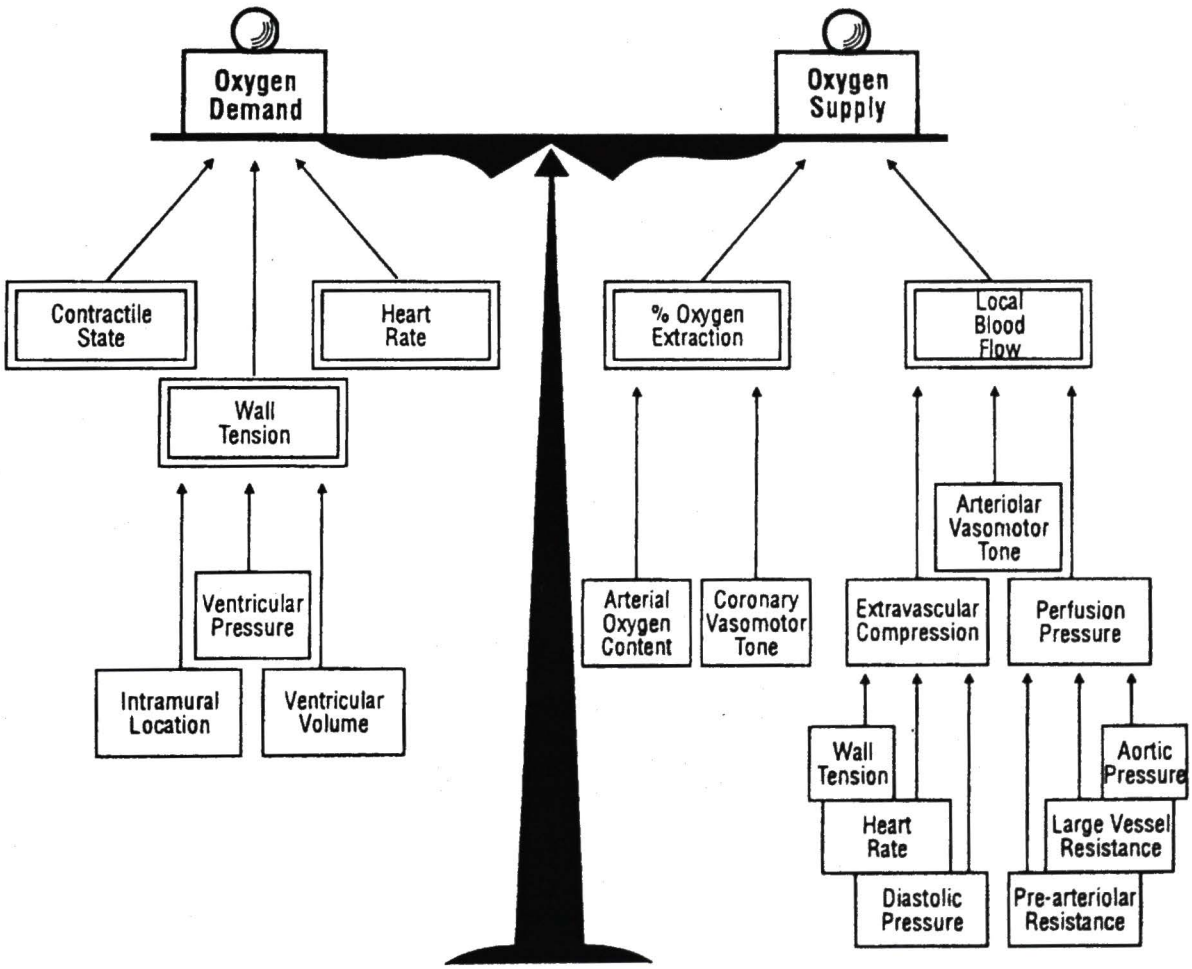


Figure 1. Determinants of the balance between myocardial oxygen consumption and coronary blood flow. (From Maseri, A. *Ischemic heart disease : a rational basis for clinical practise and clinical research.* New York: Churchill Livingstone, p. 166, 1995.)

Therefore, a decrease in oxygen supply or an increase in oxygen demand can induce an imbalance between the myocardial oxygen supply and demand ratio, which may release byproducts of anaerobic glycolysis, including lactate. When the oxygen supply reserve is depleted as in obstructive coronary artery disease, the imbalance can be extreme and prolonged, resulting in irreversible damage to the myocardial tissue (20, 22, 56). Under these conditions, the myocardial oxygen supply and demand ratio can only be corrected by a concomitant reduction in myocardial oxygen demand. A reduction in demand can be accomplished by either decreasing myocardial power, i.e. contractile function, (6) or increasing oxygen utilization efficiency (39). The mechanisms for this protective downregulation of myocardial oxygen demand have not yet been established.

Myocardial ischemia may clinically manifested as contractile failure that can result in heart failure, pulmonary congestion, and shortness of breath (27, 51). Pulmonary congestion is associated with left ventricular dysfunction, which will ultimately lead to pulmonary edema. Chest pain and arrhythmias are also associated with ischemia (Figure 2; 42, 47). Changes in ECG are also seen, usually ST segment changes accompanied by an inverted T wave (12, 43, 69). ST segment is usually elevated in epicardial or transmural ischemia (69); however, in effort angina, which indicates endocardial ischemia, the ST segment is depressed because the direction of current flow is away from the electrode (12). The inverted T-wave is thought to reflect ischemia induced variations in the rate of repolarization throughout the myocardium, which results from variations in the action potential duration, possibly due to the inadequate ATP production at

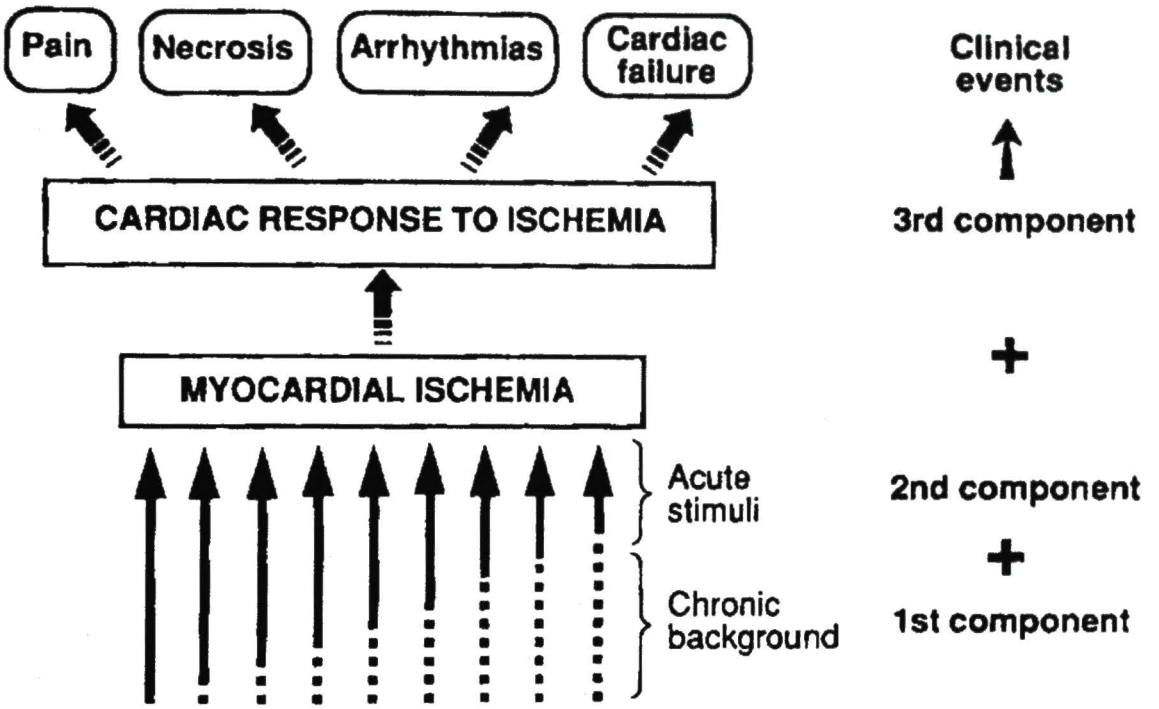


Figure 2. Consequences of ischemia. Chronic background represents coronary obstruction such as atherosclerosis. Acute stimuli represent sudden increases in myocardial oxygen demand. (From Maseri, A. *Ischemic heart disease : a rational basis for clinical practise and clinical research.* New York: Churchill Livingstone, p. 166, 1995.)

the cell membrane (51). If ischemia is extreme and prolonged, it will progress to irreversible infarction (20, 22, 56). The severity of damage sustained by the myocardium is determined by the severity of the ischemia. Several studies have shown that in experimental animals, myocardial cell death begins within 15-40 min of total occlusion of the coronary artery; after about 6 hours, few viable cells remain (56). Irreversible cell death begins in the endocardium, where energy requirements are greatest and spreads to the epicardium (56).

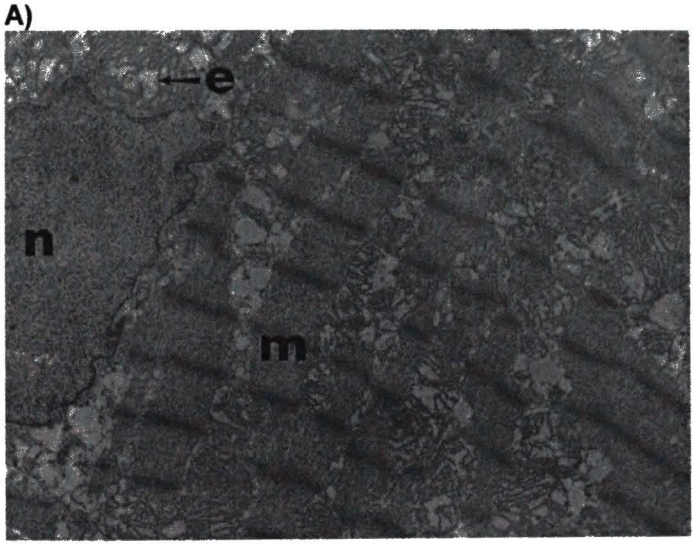
Functional and Metabolic Consequences of Ischemia

Important mechanical and metabolic differences exist between mild and severe ischemia. The degree and duration of ischemia determine the extent of contractile and metabolic damage to the myocardium. Mild ischemia is characterized by a moderate decrease in systolic function, stimulation of anaerobic glycolysis, and little changes in energy metabolites (51). Thus, mild ischemia produces a potentially compensating effect by increasing the rate of anaerobic production of ATP. In contrast, severely ischemic myocardium is characterized by irreversible impairment of myocardial tissue, leading to necrosis, due to extremely low or no oxygen delivery to the myocardium (51). Like mild ischemia, the immediate sequelae of coronary occlusion are the initial loss of contractile function and arrhythmias, followed by significant decreases in high energy phosphates and inhibition of glycolysis due to acidosis and the build-up of NADH (51). The consequent decrease in the rate of oxygen-independent production of ATP has potentially lethal effects on the ischemic myocardium. The ultimate consequence of severe ischemia is the subsequent death of ischemic myocardial cells that represents an acute myocardial infarction (Figure 3; 47).

Studies in large animals have shown that a 20–50% decrease in coronary blood flow results in immediate reduction of contractile function and decreased myocardial ATP and phosphocreatine contents (17, 64); however, with continued ischemia, high energy phosphate levels, as well as lactate, return to baseline values with no improvement in mechanical function (3, 45, 49, 53). In this phenomenon of myocardial hibernation, a hypoperfused condition characterized

by reversible but persistent impairment of myocardial and ventricular function, the ischemic myocardium actively downregulates contractile function to achieve energy supply and demand balance such that the tissue maintains its integrity and viability during oxygen supply restriction (54). Thus, downregulation of myocardial oxygen demand safeguards the myocardium during decreased oxygen supply without the metabolic and pathophysiological consequences of myocardial ischemia.

A)



B)

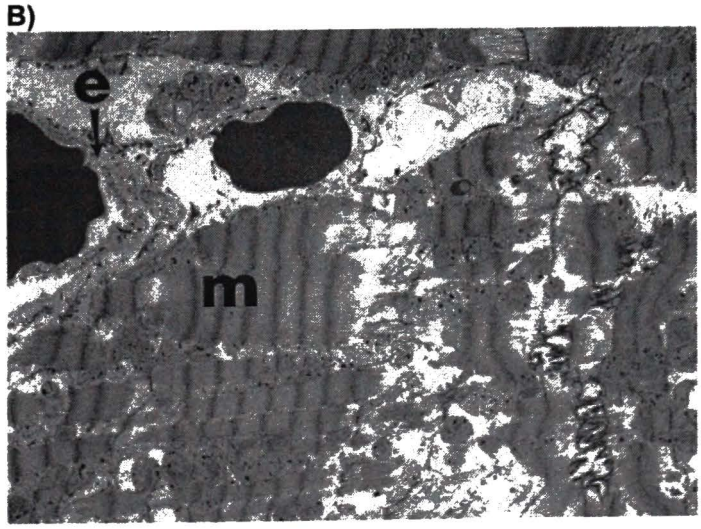


Figure 3. A) Electron micrograph obtained from reversibly, previous ischemic subendocardium of an anesthetized dog. The intramitochondrial edema (e) is evident, but myofilaments (m) and nucleus (n) appear to be intact. B) Previously ischemic myocardium that under-went 2 hr of left coronary de-scending coronary artery occlusion followed by 4 hr reperfusion. Note both myocytes (m) and vascular entdothelial cell (e) damage. (From Lazar, HL. Current therapy in acute coronary ischemia. Mount Kisco, NY: Futura Publishing CO., Inc., p.3, 1993.

Aerobic and Anaerobic Metabolism in Ischemia

Under normal conditions, i.e. oxygen supply/demand balance, free fatty acids are the preferred oxidative substrates and are transported to the heart bound to albumin. However, myocardial substrate metabolism during ischemia is dependent upon the severity of the ischemia (Figure 4;(51).

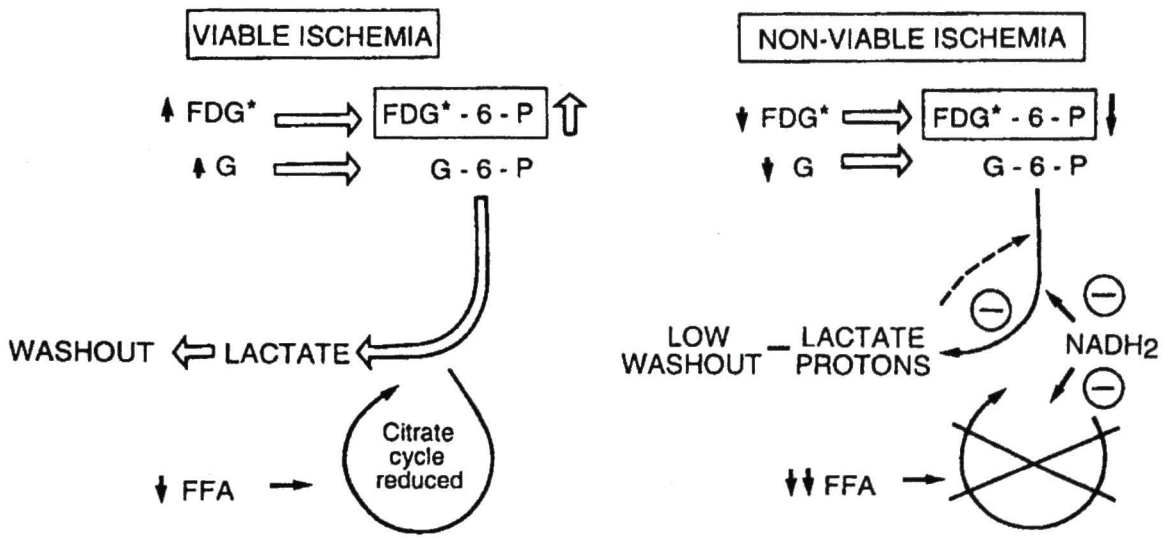


Figure 5. Glucose imaging and viability. Proposed use of F18-fluorodeoxyglucose(FDG*) in acting as a marker for glucose uptake. FDG* is not metabolized. Hence, the intensity of extraction of FDG* by the myocardium as detected by position emission tomography is an index of viability. If "mismatch," glucose extraction is increased in relation to coronary blood flow, measured by NH_3. In very severe ischemia or infarction, glucose delivery is decreased, glycolysis is inhibited, and extraction of FDG* decreases. (From Opie, L. H. *The heart : physiology, from cell to circulation*. Philadelphia: Lippincott-Raven, p. 334, 1998.

During severe ischemia, i.e. reduction of coronary blood flow > 70%, there is a transient increase in anaerobic glycolysis, followed by inhibition due to the accumulation of lactate and protons with severe or complete contractile dysfunction. As blood flow and oxygen delivery decrease, the rate of contractile work also decreases, concomitantly with glycogen stores, and lactate

accumulates. With severe reduction in blood flow, there is an inadequate washout of detrimental byproducts of glycolysis and a decrease in intracellular pH. Eventually, inhibition of glycolysis due to H^+ inhibition of phosphofructokinase (PFK), a key regulatory enzyme, occurs. Furthermore, the glycolytic flux through glyceraldehyde-3-phosphate dehydrogenase may also be rate limiting due to a low cytosolic NAD^+/ NADH ratio (50).

On the other hand, a modest decreases in flow causes a decrease in myocardial oxygen consumption, resulting in enhanced utilization of glucose and lactate (Figure 5; 47). This, in turn, results in the reduced rate of free fatty acid oxidation since the breakdown of fatty acids requires more oxygen than either glucose and lactate for the release of a given amount of energy. However, despite the reduction in contractile function and transient lactate production during mild to moderate ischemia, the primary oxidative substrate utilized is free fatty acids (40-42). Studies in pigs with ~60% reduction in coronary blood flow, exogenous free fatty acid oxidation provided most of the energy for ATP synthesis during ischemia even under conditions of severely impaired contractile function, reduced myocardial oxygen consumption, and lactate production (18, 40-42, 67). Therefore, the energy that is produced during mild to moderate ischemia is derived from both anaerobic glycolysis and fatty acid ß-oxidation.

Possible Mechanisms of Contractile Failure in Ischemia

Within seconds after the onset of myocardial ischemia, an immediate reduction of contractile function occurs: active segment shortening is converted to paradoxical segment lengthening, or passive bulging. The mechanisms

responsible for the rapid decline in contractile function in acute myocardial ischemia have been extensively studied but are poorly understood. Suggested mediators of regional contractile dysfunction during acute myocardial ischemia include: a) decreased cytosolic phosphorylation potential (55), b) depletion of high energy phosphate (21), c) decreased free energy from ATP hydrolysis (26, 31), d) disruption of sarcoplasmic Ca^{2+} transport (9, 11), e) lactate accumulation (4, 52, 58, 68, 71), f) decreased intracellular pH (2, 10, 28, 32), g) vascular collapse (33), h) increased cytosolic inorganic phosphate (P_I) (19, 30, 49).

The effects of poor oxygen delivery result in depletion of high energy phosphates, including ATP and phosphocreatine. Researchers have shown that contractile failure occurs before a major depletion of ATP, which is initially conserved due to the buffering function of phosphocreatine (55). The initial decrease in cytosolic phosphocreatine appears to be sufficiently severe to suggest that the problem may lie with energy transfer from the mitochondria. However, studies have shown that phosphocreatine content, as well as cytosolic phosphorylation potential, return to baseline levels with prolonged ischemia even though contractile function remain significantly depressed (3, 45, 49, 53).

Much research has been devoted to determining whether accumulation of products of ischemia is responsible for the mechanical failure. Tennant, in 1935, was the first to relate a buildup of lactic acid to early contractile failure (68). It has also been proposed that intracellular acidosis is responsible for early contractile failure due to the protons displacing calcium from binding sites on the thin contractile filaments (28). Cobbe and Poole-Wilson introduced a similar

proposal in 1980 that stressed the retention of carbon dioxide, acting by the production of intracellular acidosis (10). Lee and Allen introduced direct evidence in 1991 that acidosis causes contractile failure without decreasing cytosolic calcium levels (38). However, intracellular acidosis accounts for only half the total decrease in mechanical function during ischemia (25). Other studies have shown that mechanical failure occurs before a fall in pH ((19, 33, 62), indicating that other mechanisms are responsible for the initial, rapid fall in contractile function during ischemia.

Recently, an alternative hypothesis was introduced: that the buildup of inorganic phosphate is an important factor in the early contractile failure seen in ischemia (34, 38). He et al. demonstrated that inorganic phosphate apparently plays an important role since inorganic phosphate rose dramatically before a decline in contractile function was detected (19). The mechanisms by which inorganic phosphate mediates contractile function during ischemia have been extensively studied. Evidence indicates that inorganic phosphate may modulate Ca^{2+} handling in the sarcoplasmic reticulum (72). Xiang et al. showed that an increase in inorganic phosphate decreased Ca^{2+} transient, which would result in a reduction in mechanical function (72). Inorganic phosphate may also decrease the sensitivity of myofibrils to Ca^{2+} (36), decrease the activity of myofibril ATPase (63), and affect myocardial oxygen consumption and utilization by regulating enzymatic oxidation (8).

It was also suggested that vascular collapse may contribute to the initial decrease in contractile failure during ischemia (19, 33). This idea has only been

supported by indirect evidence or by exclusion. Koretsune et al. microembolized the coronary circulation at a constant perfusion pressure to produce tissue-level ischemia while maintaining coronary vascular tone. The results demonstrated that during the first 30 seconds of ischemia, intracellular pH, inorganic phosphate, and ATP did not change despite the decrease in mechanical function. During the microembolization, contractile function declined at a slower rate while the metabolites did not change, suggesting that vascular collapse makes a significant contribution to early contractile failure (33). The mechanisms continue to be in question.

Effects of Dobutamine During Ischemia

The effects of positive inotropic stimulation on ischemic myocardium have been studied for some time due to the frequent use of inotropic agents in the treatment of patients with cardiac failure associated with coronary artery disease, congestive heart failure, myocardial infarction, or post cardiac surgery. Many inotropic agents act directly on β_1 – adrenergic receptors to activate adenylate cyclase, which increases cAMP. The accumulation of cAMP increases myocardial contractility by increasing the influx of Ca^{2+} through voltage gated channels (14). Sympathomimetic amines such as norepinephrine, epinephrine, and isoproterenol increase myocardial contractile function by directly stimulating β_1 – adrenergic receptor sites in the myocardium (66). However, their clinical application has generally been limited due to their positive chronotropic effects and by their action either to increase or decrease peripheral vascular resistance and thus, to change arterial pressure. For example, isoproterenol significantly increases myocardial contractility and cardiac output but also induces

tachycardia and decrease peripheral resistance; increases in heart rate and decrease in peripheral resistance is not desirable when myocardial ischemia is a problem. Increased heart rate further increases myocardial oxygen demands, while decreased peripheral arterial resistance decreases arterial pressure and coronary blood flow. These effects will worsen the ischemia.

Dobutamine, a synthetic cathecolamine derived from isoproterenol, was developed to be selective for β_1 – adrenergic receptors. . However, it also has a weak capacity to stimulate β_2 and α-adrenoreceptors in the cardiovascular system (37). It acts via its (-)-isomer on the α -adrenergic receptors and via its (+)-isomer on β_1 and β_2 -adrenergic receptors (1, 60). In the heart, the racemic mixture of dobutamine used clinically results in a relatively strong additive inotropic effects mediated by the (-) and (+)-enantiomer stimulation of α_1 and β_1-adrenoreceptors, respectively. Also, there is a relatively weak chronotropic effect of the (+)-isomer (59). Therefore, dobutamine directly increases myocardial contractility without a marked increase in heart rate or a change in peripheral arterial resistance (66). The absence of marked blood pressure changes is due to the physiologic antagonism that occurs in the vasculature between the α_1 vasoconstrictive and β_2 vasodilatory adrenergic receptor activities.

Studies of inotropic stimulation during ischemia of the left ventricle have shown wide variability on regional contractile function and metabolism depending on the specific inotropic agent, the degree of ischemia, and the experimental model (3, 13, 15, 64, 73). Several previous experimental studies have found that dobutamine stimulation during ischemia did not increase infarct size, despite an

increase in myocardial oxygen consumption and contractile function, if coronary blood flow to the ischemic region also increased (13). Goodlett et al found that isoproterenol did not further impair regional high energy phosphate stores of an ischemic region of canine left ventricle (15). Yanagi et al also reported that when marked tachycardia was avoided, intravenous dobutamine increased coronary blood flow and improved contractile function without depletion of high energy phosphates in the moderately ischemic canine left ventricle (73). However, Shulz et al and Arai et al demonstrated that intracoronary dobutamine infusion during prolonged, moderate ischemia improved regional systolic contractile function and increased myocardial oxygen consumption, but also caused an energy supply and demand imbalance by depleting the myocardial high energy phosphates (3, 64).

Relatively few studies have focused on the effects of dobutamine stimulation on the right ventricle and its energy metabolism; however, as in the left ventricular studies, data on the right ventricle have shown the same type of contradictions. Intravenous infusion of dobutamine significantly increased cardiac and stroke volume indexes by a direct augmentation of right ventricular systolic performance in patients with acute right ventricular infarction, but there was also a significant increase in heart rate and mean arterial pressure. The increase myocardial oxygen demands of dobutamine were not offset by a decrease in ventricular size and wall tension (13). Therefore, the use of dobutamine in patients with predominant right ventricular dysfunction could worsen ischemia. On the other hand, Greyson et al reported that intravenous dobutamine increased global but not regional right ventricular contractile function

during prolonged, moderate ischemia in swine without a reduction in regional myocardial high energy phosphate levels. However, dobutamine stimulation caused a further decrease in coronary venous pH and continued lactate production, indicating exacerbation of ischemia (16).

Comparison of Left and Right Ventricles

The left and right ventricles have very different workloads, wall tension and structure, coronary flow patterns, perfusion, and metabolic rates (35, 44, 65), The right ventricular wall is much thinner than that of the left ventricle, reflecting the lower workload of the right ventricle. While the left ventricle must pump the blood to various organs of the body against a higher vascular resistance, the right ventricle only has to drive the blood into the lungs with a much lower resistance. Therefore, the right ventricle has to generate less pressure than the left ventricle (49). Coronary blood flow per gram tissue in the right ventricle is about two-thirds of that in the left ventricle (44). The perfusion territory of the right coronary artery is also much smaller than that of the left (44). Coronary blood flow and oxygen reserves are greater in the right ventricle than in the left ventricle (74, 35). The resting oxygen extraction in the left ventricle is ~75% compared to that of the right ventricle which is ~40-50% (35). The control of blood flow and perfusion pressure of the two ventricles also differs. Autoregulation in the right ventricle is not as effective as that of the left ventricle. In the left ventricle, changes in coronary perfusion pressure from 60 to 130 mm

Hg have little affect on left coronary blood flow, whereas, in the right ventricle, coronary blood flow changes proportionately with changes in coronary perfusion pressure (74). Therefore, right ventricular responses to inotropic stimulation should not be assumed solely by extrapolation from left ventricular responses.

Rationale for This Study

Positive inotropic stimulation increases oxygen demands, and the myocardium must be able to meet this increased demand by either increasing oxygen supply or by more efficiently utilizing the oxygen available to avoid depleting energy reserves. In left ventricular experimental studies, Lee and Downey proposed that when the myocardial oxygen supply is limited, the myocardium improves oxygen supply-demand balance by increasing its oxygen utilization efficiency during inotropic stimulation (39). The implication of this study is that the myocardium can downregulate its oxygen demand without a proportional decrease in function to avoid ischemia during inotropic stimulation of hypoperfused myocardium. In right ventricular myocardium, energy metabolites including ATP, phosphocreatine, creatine and inorganic phosphate remained unaltered in the face of moderate hypoperfusion in the absence of β-adrenergic stimulation (24). Does the right myocardium have a similar downregulatory mechanism in the face of inotropic challenge during restricted oxygen supply to avoid ischemia without depleting energy sources? If it does, could the beneficial effects be more pronounced with dobutamine rather than isoproterenol?

To date, experimental studies with regional dobutamine infusion in the right ventricle have not been reported. To eliminate the systemic effects of dobutamine given intravenously, it is important to explore the effects of regional inotropic stimulation on the right ventricle. Dobutamine is widely used in the clinical setting and may have detrimental effects on right ventricular energy reserves. Regional inotropic stimulation is expected to have similar results found by Greyson et al with i.v. dobutamine treatment (16). However, regional systolic contractile function is also expected to increase. Itoya et al reported that during right coronary hypoperfusion, the right ventricle increases its energetic efficiency without significant changes to high energy phosphate levels (24). However, the effects of regional inotropic stimulation during hypoperfusion on high energy phosphates is unknown. Therefore, these experiments were designed to determine the effects of regional positive inotropic stimulation on myocardial energy metabolism, contractile function, high energy phosphates, and phosphocreatine phosphorylation potential in moderately underperfused right ventricle of *in situ* blood perfused myocardium

Specific Aims

The first aim of the investigation was to determine the effects of right ventricular regional inotropic stimulation on contractile function (i.e. percent segment shortening and right ventricular isometric force) and myocardial oxygen consumption during regional right ventricular coronary hypoperfusion. Moderate regional coronary hypoperfusion was produced by incrementally decreasing right ventricular coronary perfusion pressure from 100 mmHg (baseline) to ~40 mm Hg. The final perfusion pressure was achieved by decreasing the perfusion

pressure to a produce ~37% decrease in percent segment shortening with ~54% reduction in blood flow. We hypothesized that during continued coronary flow, despite the reduction, regional dobutamine infusion will increase myocardial oxygen consumption and improve regional contractile function. We found that inotropic stimulation indeed improve the contractile function of the myocardium without a significant increase in MVO_2. Therefore, we concluded that inotropic stimulation significantly increased contractile function by increasing myocardial oxygen utilization efficiency.

The second aim of this investigation was to determine if regional low-dose dobutamine infusion during coronary hypoperfusion that produced ~37% decrease in percent segment shortening with ~54% reduction in blood flow, increased regional contractile function by utilizing high energy phosphate reserves. A portion of RV free wall in the RCA perfusion territory was biopsied at CPP = 41 ± 2 mm Hg during low-dose dobutamine infusion for 15 min. We found that even though inotropic stimulation increased regional function, it did not result in a reduction of high energy phosphates. Surprisingly, there was an increased concentration of CrP with dobutamine infusion.

The third aim of this investigation was to determine if regional high-dose dobutamine infusion during coronary hypoperfusion increased regional contractile function by utilizing high energy phosphate reserves. A portion of RV free wall in the RCA perfusion territory was biopsied at CPP = 38 ± 3 mm Hg during high-dose dobutamine infusion for 15 min. We found that high-dose

dobutamine only improved regional function transiently and caused a dramatic reduction of high energy phosphates.

The fourth aim of this study was to delineate the mechanism by which low-dose dobutamine increased energy reserves during right ventricular hypoperfusion. Measurements of glycolytic intermediates indicate that there is an increase in glycolytic flux during low-dose dobutamine treatment, but glycolysis became limited at glyceraldehyde 3-phosphate dehydrogenase during high dobutamine treatment. Therefore, it appears that low-dose dobutamine, in addition to its inotropic effect, also has a beneficial effect on the energetics of the myocardium.

Significance

Our findings suggest that low-dose dobutamine may have a potentially beneficial effect on myocardial high energy phosphates during right coronary moderate ischemia. The right myocardium becomes more efficient during low-dose dobutamine stimulation. The myocardium downregulates its oxygen demands by increasing its oxygen utilization efficiency, in addition to improving the energetics of the myocardium. In contrast, administration of high-dose dobutamine may be detrimental to the myocardium during oxygen supply restriction. The myocardium is less efficient, possibly due to the increased heart rate, and depletes energy reserves in an attempt to sustain contractile function. Our findings have also shown that during low-dose dobutamine administration, glycolytic flux is increased, whereas the opposite effect occurs with high-dose dobutamine. Therefore, dosage should be considered carefully.

CHAPTER II

Parallel Enhancement by Dobutamine of Contractile Function and Energy Reserves in Hypoperfused Canine Right Ventricular Myocardium

Kun Don Yi, H. Fred Downey, Xiaoming Bian, Min Fu, and Robert T. Mallet

American Journal of Physiology:

Heart and Circulation Physiology

(to be submitted)

ABSTRACT

Although the $ß_1$ adrenergic agent dobutamine is used clinically to provide inotropic support to failing myocardium, it reportedly depletes myocardial energy reserves and, thus, could exacerbate cardiac injury. This investigation delineated the contractile and energetic effects of low *vs.* high dobutamine doses in hypoperfused right ventricular (RV) myocardium. The right coronary artery (RCA) of anesthetized dogs was cannulated for controlled perfusion with arterial blood, and regional RV contractile function was measured. RCA pressure was lowered from 100 mm Hg baseline to 40 mm Hg, and flow fell by 54%. At 15 min hypoperfusion, dobutamine was infused into the RCA at either 0.01 (low dobutamine) or 0.06 μg/kg/min (high dobutamine) for 15 min. Regional power (systolic segment shortening · isometric developed force · heart rate) stabilized at 63% of baseline during hypoperfusion. Low dobutamine restored power to the baseline value, but did not increase heart rate or RV myocardial O_2 consumption (MVO_2) and, thus, increased myocardial O_2 utilization efficiency (O_2UE: power/MVO_2). At 5 min, high dobutamine enhancement of power was similar to that of low dobutamine, but by 15 min power and O_2UE fell to untreated levels. Remarkably, low dobutamine sharply increased phosphocreatine and lowered creatine contents, and increased phosphorylation potential threefold *vs.* untreated hypoperfused myocardium. In contrast, high dobutamine lowered phosphocreatine, increased creatine and inorganic phosphate, and lowered

phosphorylation potential to 45% of the untreated value. Analyses of glucose uptake and glycolytic intermediates revealed sustained enhancement of glycolysis by low dobutamine, but glycolysis became limited at glyceraldehyde 3-phosphate dehydrogenase during high dobutamine treatment. In summary, low dobutamine improved mechanical performance and efficiency of hypoperfused RV myocardium while increasing myocardial energy reserves, but higher dobutamine failed to sustain improved function and depleted energy reserves. Dobutamine is capable of improving both contractile function and cellular energetics in hypoperfused RV myocardium, but dosage should be carefully selected.

KEY WORDS

Phosphorylation potential, phosphocreatine, glycogen, glycolysis, oxygen utilization efficiency

ABBREVIATIONS

AoP: aortic pressure; Cr: creatine; DAP: dihydroxyacetone phosphate; dP/dt: rate of intraventricular pressure change; F6P: fructose 6-phosphate; FDP: fructose 1,6-*bis*-phosphate; GAP: glyceraldehyde 3-phosphate; G6P: glucose 6-phosphate; HR: heart rate; MVO_2: myocardial O_2 consumption; O_2UE: oxygen utilization efficiency; PCr: phosphocreatine; PEP: phosphoenolpyruvate; 2PG: 2-phosphoglycerate; 3PG: 3-phosphoglycerate; P_i: inorganic phosphate; Po_2, Pco_2:

partial pressures of O_2 and CO_2; RCBF: right coronary blood flow; RCP: right coronary arterial perfusion pressure; RV: right ventricle; RVP: right ventricular pressure

ENZYMES

glyceraldehyde 3-phosphate dehydrogenase (EC 1.2.1.12), lactate dehydrogenase (EC 1.1.1.27), phosphoglycerate kinase (EC 2.7.2.3)

INTRODUCTION

Dobutamine and other β-adrenergic agents powerfully stimulate myocardial contractile function and, thus, are potentially effective treatments for cardiac insufficiency. Unfortunately, β-adrenergic stimulation of myocardium incurs a cost: by increasing energy demand without a commensurate increase in energy supply, β-adrenergic agents deplete the myocardium of its critically important energy reserves (36, 46). This energy depletion can produce an array of undesirable sequelae, including arrhythmias (22), oxygen wasting (13, 32), and myocardial necrosis (23). Moreover, β-adrenergic agents stimulate formation of harmful oxyradicals (9, 28, 36). These problems have limited the clinical application of β-adrenergic agents, and the potential utility of these drugs to provide inotropic support for failing myocardium has not been fully realized.

The effects of dobutamine on contractile function and energy reserves in left ventricular myocardium have been studied extensively. In general, dobutamine increased function but depleted high energy phosphate compounds in hypertrophied (1, 21, 45) or hypoperfused (8, 33, 44) left ventricular myocardium of dogs and pigs. Comparatively few studies have examined the hemodynamic and energetic effects of β-adrenergic stimulation in right ventricular myocardium. The left and right ventricles have very different

workloads, wall tension and structure, coronary flow patterns, perfusion, and metabolic rates (16, 20, 37), so right ventricular responses to inotropic stimulation should not be assumed solely by extrapolation from left ventricular responses. Recently, Greyson *et al.* (7) reported that intravenous dobutamine increased global but not regional right ventricular contractile function during prolonged right coronary hypoperfusion in pigs without a concordant reduction in myocardial high energy phosphate content. In contrast, Schwartz *et al.* (35) recently reported an increase in high energy phosphates in porcine right ventricle during isoproterenol infusion in the absence of coronary flow limitation.

We recently demonstrated that β-adrenergically stimulated, hypoperfused left ventricular myocardium can restore its oxygen supply:demand balance and maintain its contractile function by increasing its oxygen utilization efficiency (17). In right ventricular myocardium, energy metabolites including ATP, phosphocreatine, creatine and inorganic phosphate remained unaltered in the face of moderately severe right coronary hypoperfusion in the absence of β-adrenergic stimulation (10). If the mechanisms that increase oxygen utilization efficiency during β-adrenergic stimulation of left ventricular myocardium also operate in right ventricle, it seems possible that energy reserves of hypoperfused right ventricular myocardium might be maintained or even increased by dobutamine stimulation. This possibility was examined in anesthetized open-chest dogs by lowering right coronary perfusion pressure sufficiently to partially

compromise regional contractile function, then infusing dobutamine at two different intracoronary concentrations. Global and regional contractile function, regional myocardial consumption of oxygen, glucose and lactate, and oxygen utilization efficiency were monitored. Myocardium was sampled for measurement of energy metabolites, glycolytic intermediates and glycogen. At the lower concentration, dobutamine produced sustained increases in regional contractile performance and oxygen utilization efficiency, and markedly enhanced cytosolic phosphorylation potential of the hypoperfused right ventricular myocardium. In contrast, the higher dobutamine concentration produced only transient improvements in function, and phosphorylation potential fell sharply. Thus, the inotropic and energetic effects of dobutamine in right ventricular myocardium appear to be heavily dose-dependent.

METHODS

Surgical preparation

Animal experimentation was approved by the Institutional Animal Care and Use Committee of the University of North Texas Health Science Center at Fort Worth and was conducted in accordance with the *Guide to the Care and Use of Laboratory Animals* (NIH 85-23, revised 1996). Thirty-eight mongrel dogs of either gender were anesthetized initially with pentobarbital sodium (30 mg/kg body weight, i.v.). Supplemental pentobarbital sodium and fentanyl (10 μg/kg, i.v.) were administered as needed to maintain a surgical plane of anesthesia. The dogs were intubated by tracheotomy and ventilated with room air by a Harvard respirator. Arterial blood was frequently sampled and analyzed for Po_2, Pco_2, and pH; ventilation was adjusted to maintain these variables within limits of 100 – 140 mm Hg, 35 – 45 mm Hg, and 7.35 – 7.45, respectively. Sodium bicarbonate was administered i.v. to maintain normal arterial pH when Pco_2 was within normal limits. Body temperature was measured with a rectal thermometer and maintained at 36-37°C by a water-circulating heating pad.

A fluid-filled vinyl catheter connected to a pressure transducer was inserted into the thoracic aorta via the right femoral artery to measure systemic arterial blood pressure (AoP). Another vinyl catheter was inserted into the right femoral vein to administer supplemental anesthetic, sodium bicarbonate, heparin, and donor blood. The donor blood was infused as required to maintain arterial

blood pressure. A third catheter was placed in the left femoral artery to withdraw blood for an extracorporeal coronary arterial perfusion system (10, 17).

The myocardium was exposed via a right thoracotomy in the fourth intercostal space. The pericardium was incised, and the heart was suspended in a pericardial cradle. A Millar catheter-tip transducer was inserted through the right atrial appendage and advanced across the tricuspid valve to measure right ventricular pressure. The first derivative of right ventricular pressure (dP/dt) was computed electronically by a Grass model 7P20C differentiator.

The right coronary artery (RCA) was isolated near its origin and, after heparin administration (500 units/kg, i.v.), cannulated with a stainless steel cannula connected to the extracorporeal perfusion system. Right coronary perfusion pressure (RCP) was controlled by a pressurized reservoir supplied with blood withdrawn from the left femoral artery. A fluid-filled catheter was advanced to the cannula orifice and connected to a pressure transducer for monitoring RCP. Right coronary blood flow (RCBF) was measured electromagnetically with a Carolina Medical Electronics flowmeter and an in-line flow transducer.

Regional myocardial function

Within the perfusion territory of the RCA, a pair of piezoelectric crystals was implanted in the midwall of the right ventricle to measure segment length

(10, 17). The crystals were placed approximately 1 cm apart and positioned parallel to the principal axis of shortening in the perfusion territory of the RCA. End-diastolic length (EDL) and end-systolic length (ESL) were measured at the beginning of the positive deflection of the dP/dt record and 20 ms before the peak negative deflection, respectively. Myocardial segment shortening during systole (SS) was expressed as a fraction of EDL; thus, SS = [(EDL - ESL)/EDL]. An isometric force transducer was placed 10 mm toward the base of the heart, parallel to the position of the piezoelectric crystals in the perfusion territory of the RCA, to measure right ventricular isometric force. AoP, heart rate (HR), RCBF, RCP, right ventricular pressure (RVP) and dP/dt, right ventricular segment shortening, and right ventricular isometric force were recorded on a Grass Model 7D eight-channel polygraph. A vein draining the RCA perfusion territory was cannulated to collect venous samples (10).

Myocardial oxygen consumption and lactate and glucose uptakes

Coronary arterial and venous samples were collected anaerobically and chilled on ice until analysis. Po_2, Pco_2, and pH of each sample were measured with an Instrument Laboratory model 1730 blood gas analyzer, oxygen content with an Instrument Laboratory Model 682 Co-Oximeter, and blood glucose and lactate concentrations with a Yellow Springs Instruments Model 2300 L-lactate analyzer. Myocardial oxygen consumption (MVO_2) and lactate and glucose uptakes were determined from the product of arteriovenous difference and RCBF

(10, 17). An index of contractile power (PI) generated in the RCA perfusion territory was computed as the product of heart rate, SS, and isometric force (12). Oxygen utilization efficiency (O_2UE) was defined as the ratio of PI to MVO_2.

Right coronary perfusion territory

Since the RCA perfusion territory was biopsied during the protocols, it was not possible to directly measure its mass. Accordingly, the mass of the RCA perfused myocardium was estimated from the baseline RCBF at 100 mm Hg perfusion pressure by assuming flow to equal 0.5 ml/min/g (2, 25, 39, 43). With this approach, mean mass of the right coronary perfusion territory was calculated to be 21 ± 2 g.

Experimental protocols

Group 1, Untreated Hypoperfusion (n = 9). The RCA was perfused at a pressure of 100 mm Hg for approximately 30 min to allow hemodynamic variables and regional function to stabilize after the surgical preparation. During this period, blood gases were monitored and adjusted if necessary. After stabilization, baseline measurements were obtained during 15 min at 100 mm Hg RCP. RCP was then incrementally decreased to 60 and 50 mm Hg and held at each level for 15 min. Blood samples and hemodynamic data were collected at 5 and 15 min for each perfusion pressure. Data from these two RCP levels are not presented in the figures and tables for clarity. RCP was then lowered to 40 ± 2

mm Hg for 30 min to produce a significant decrease in SS but not paradoxical shortening. SS fell by ~37% at this perfusion pressure (Figure 1). Blood samples and hemodynamic data were collected at 5, 15, 20, and 30 min. Sucrose was continuously infused into the RCA at 25-30 min, and coronary venous samples were collected each minute of sucrose infusion. Within 15 s after the last venous blood sample was collected, a transmural portion of the right ventricular free wall within the RCA perfusion territory was biopsied with aluminum Wollenberger tongs pre-cooled in liquid nitrogen.

Group 2, Low Dobutamine Treatment (n = 13). The protocol for Group 2 was similar to that of Group 1 except that 15 min after RCP was lowered to 41 ± 2 mm Hg, dobutamine was continuously infused into the RCA for an additional 15 min at a rate of 0.01 μg/kg body mass/min. A portion of the RCA perfusion territory was biopsied as described in Group 1.

Group 3, High Dobutamine Treatment (n = 6). The protocol for Group 3 was the same as that of Group 2, except that 15 min after RCP was lowered to 38 ± 3 mm Hg dobutamine was infused at a higher rate (0.06 μg/kg body mass/min). A portion of the RCA perfusion territory was biopsied as described in Group 1.

Group 4, Baseline Control (n = 11). This experiment served as time control for the hypoperfusion protocols. After a post-surgical stabilization period of 30 min, RCA was perfused at baseline pressure of 100 mm Hg for 75 min. Blood samples and hemodynamic data were collected at the same time-points described in the Group 1 protocol. A portion of RCA perfusion territory was biopsied as described in Group 1.

Myocardial metabolite analyses

Myocardium in the center of the RCA perfusion territory was biopsied at the completion of each protocol. Immediately after biopsy, the frozen myocardium was quickly immersed in liquid nitrogen and subsequently stored at –90°C until metabolite extraction. Only frozen myocardium compressed between the clamps was used for metabolite assays. Frozen myocardium was ground to a fine power in a mortar under liquid nitrogen, and energy metabolites, glycolytic intermediates, and sucrose were extracted in 4 vol ice-cold 0.3 N $HClO_4$ as described previously (10, 39). ATP, phosphocreatine (PCr), creatine (Cr), inorganic phosphate (P_i), glycolytic intermediates, glycogen and sucrose were measured by colorimetric assays (10, 39). An aliquot of powdered tissue was desiccated to constant mass at 100°C for determination of dry mass. The appropriate correction factors for dilution and tissue mass were applied. Samples from all four groups were extracted on the same day to prevent artifactual differences in measured metabolites.

Determination of P_i and phosphorylation state of phosphocreatine

Phosphocreatine phosphorylation potential ({PCr}/({Cr}[P_i])) was determined as an index of the cytosolic ATP phosphorylation potential (10, 39, 41). Intracellular P_i (mM) was calculated from the following equation:

$$\text{Intracellular } P_i = [\text{tissue } P_i - (\text{plasma } P_i \times E_c)] / [1 - (E_c + R_{dw})]$$

where tissue P_i is the total myocardial P_i content (μmol/g wet wt), plasma P_i is the concentration of P_i in coronary venous plasma (mM), E_c is the myocardial extracellular space (ml/g wet wt), and R_{dw} is the myocardial dry mass to wet mass ratio. Extracellular space was determined as the sucrose distribution space (10).

Statistical analyses

All data are expressed as means ± SEM. Hemodynamic, functional, and metabolite data were analyzed by one-way analysis of variance (ANOVA) to determine the differences between groups and by one-way ANOVA for repeated measures to determine the differences between experimental conditions within each group. When significance was found with ANOVA, Student-Newman-Keuls multiple comparison tests were performed. Statistical significance was assumed at $p < 0.05$.

RESULTS

Right ventricular hemodynamics and blood gases

Hemodynamic variables of Groups 1, 2, and 3 are presented in Table 1. Data are presented for the baseline conditions with RCP at 100 mm Hg and after RCP reduction to 38-41 mm Hg, which caused right coronary blood flow (RCBF) to fall by 54% *vs.* baseline. Although coronary flow fell in lockstep with RCP, hemodynamic variables at 60 and 50 mm Hg did not differ among Groups 1-3 or from baseline values within each group (data not presented). At *c.* 40 mm Hg RCP, AoP, HR, maximum rate of relaxation ($-dP/dt_{min}$), and RVP did not differ among the three groups, nor from the respective baseline values. During low- and high-dose dobutamine stimulation, RCBF increased by 15-20%, but did not differ between Groups 2 and 3. Neither low- nor high-dose dobutamine treatment altered AoP, RVP, and $-dP/dt_{min}$. Low-dose dobutamine (Group 2) did not alter HR, while high-dose dobutamine (Group 3) significantly increased HR throughout the treatment period. The maximum rate of RV pressure development ($+dP/dt_{max}$) fell 13% from baseline during pre-dobutamine hypoperfusion (Table 1). Low-dose dobutamine treatment did not alter $+dP/dt_{max}$; however, in Group 3, high-dose dobutamine increased $+dP/dt_{max}$ ~19% vs. pre-treatment, to significantly higher levels than in Groups 1 and 2 during the same period. Hemodynamic variables of the time-matched normoperfusion Group 4 did not differ from baseline values in Groups 1, 2, and 3.

Table 2 presents coronary venous Po_2 and Pco_2. During moderate hypoperfusion in Groups 1, 2, and 3, Pvo_2 fell ~21%, while $Pvco_2$ increased 12%. Initially, low-dose dobutamine stimulation in Group 2 reduced Pvo_2 slightly; however, $Pvco_2$ was not altered, and with continued treatment, Pvo_2 and $Pvco_2$ remained stable. At 5 min high-dose dobutamine treatment in Group 3, Pvo_2 decreased significantly, while $Pvco_2$ increased compared to the untreated hypoperfused condition. At 15 min high-dose dobutamine stimulation, Pvo_2 continued to fall well below the 5 min value, while $Pvco_2$ continued to increase, in contrast to low-dose dobutamine treatment.

Lactate and glucose uptakes

Right ventricular uptakes of lactate and glucose are presented in Table 2. Lactate uptake fell markedly in each group during pre-dobutamine hypoperfusion. Low-dose dobutamine elicited a modest net lactate release. At 5 min, high-dose dobutamine produced a similar, modest lactate release; however, by 15 min lactate release had increased by seven-fold. Glucose uptake increased 33% *vs.* baseline after RCP was lowered to 40 mm Hg in Groups 1, 2, and 3. Both doses of dobutamine increased glucose uptake another 30% to roughly twice the baseline values, and glucose uptake in the two dobutamine groups did not differ.

Regional myocardial function

Contractile function, myocardial lactate and glucose uptakes, and myocardial O_2 consumption (MVO_2) were unaltered at 60 or 50 mm Hg *vs.* 100 mm Hg baseline (data not shown). With the reduction of RCP to ~40 mm Hg, segment shortening in all groups fell by ~37% compared to baseline values (Figure 1). At 5 min low-dose dobutamine treatment, SS recovered to baseline values, although there was no concomitant increase in RCBF (Table 1) and MVO_2 (Figure 4). The increase in SS was maintained for 15 min. Likewise, high-dose dobutamine initially restored SS to baseline levels; however, as treatment continued, SS fell to pre-treatment values. Changes in right ventricular isometric force and power index (PI) paralleled those of SS during moderate hypoperfusion and dobutamine infusion (Figure 2 and 3). Thus, isometric force and PI fell during right coronary hypoperfusion. Low-dose dobutamine produced sustained increases in isometric force and PI, but high-dose dobutamine elicited a biphasic response, wherein the initial enhancements of force and PI were lost by 15 min stimulation.

Myocardial oxygen consumption and oxygen utilization efficiency

Myocardial O_2 consumption fell 40% from baseline during pre-treatment hypoperfusion (Figure 4). Low-dose dobutamine did not significantly increase MVO_2 throughout the treatment period, despite increased regional contractile function. In contrast, high-dose dobutamine produced a sustained increase in

MVO_2 to the baseline range, although contractile function was increased only transiently. During pretreatment hypoperfusion, O_2UE fell significantly in all three groups (Figure 5). During low-dose dobutamine stimulation, the RV myocardium increased its O_2UE above baseline values and sustained this increase for 15 min. High-dose dobutamine treatment increased O_2UE similarly during the first 5 min but O_2UE later fell to the untreated level due to the decline in PI.

Energy metabolism and phosphorylation potential

Right ventricular myocardial contents of ATP, PCr, Cr, and P_i are presented in Figure 6. Right coronary hypoperfusion in the absence of dobutamine did not alter any of these energy metabolites, compared to normally perfused Group 4 time controls. When low-dose dobutamine (Group 2) was administered during moderate hypoperfusion, PCr content unexpectedly increased by 55%, Cr content fell concomitantly, and intracellular P_i concentration tended to decrease. In contrast, high-dose dobutamine (Group 3) depleted PCr and increased Cr content and P_i concentration. Thus, the two concentrations of dobutamine produced opposite effects on RV myocardial energy metabolites. To further define the effects of dobutamine on myocardial energy reserves, the energy metabolite ratios {PCr}/{Cr} and {PCr}/{ATP}, and PCr phosphorylation potential were determined (Figure 7). Low-dose dobutamine increased {PCr}/{ATP} well above the corresponding ratios in the other groups. Low-dose dobutamine also increased {PCr}/{Cr} more than two-

fold vs. the time control and non-treated hypoperfused groups, and three-fold vs. myocardium treated with the higher dobutamine dose. Moreover, low-dose dobutamine increased PCr phosphorylation potential, an index of cytosolic ATP phosphorylation potential (23), 2.5-fold above that of time control and untreated hypoperfusion groups. In striking contrast, the higher dobutamine infusion sharply lowered PCr phosphorylation potential to 25-30% of the respective values of groups 1 and 4. Thus, the two dobutamine doses produced remarkably different effects on cytosolic energy reserves of hypoperfused canine right ventricular myocardium.

Glycolytic intermediates and glycogen

The status of the glycolytic pathway was analyzed in an attempt to delineate mechanisms producing the disparate contractile and metabolic responses to low- *vs.* high-dose dobutamine. Figure 8 presents glycolytic intermediates as crossover plots, in which metabolite contents in the low- and high-dose dobutamine groups are normalized to the respective contents in the untreated hypoperfused group, and plotted in the glycolytic sequence. Both low- and high-dose dobutamine significantly increased all three hexose phosphate intermediates as well as dihydroxyacetone and glyceraldehyde-3-phosphate. However, the effect of the different dobutamine dosages differed beyond the glyceraldehyde-3-phosphate dehydrogenase/ phosphoglycerate kinase (GAPDH/PGK) enzyme couple. Low-dose dobutamine treatment did not alter 3-

phosphoglycerate, 2-phosphoglycerate, nor phosphoenolpyruvate contents. High-dose dobutamine stimulation significantly lowered all intermediates beyond glyceraldehyde-3-phosphate, indicating inhibition of glycolysis at the level of GAPDH/PGK. To further demonstrate the differing effects on glycolysis of the two dobutamine doses, Figure 9 presents a crossover plot in which intermediate contents in the high-dose dobutamine treatment group are normalized to the respective contents in the low-dose dobutamine treated group. All glycolytic intermediates beyond GAPDH/PGK were sharply lowered in the high-dose dobutamine treated group, relative to the low-dose dobutamine group.

Myocardial glycogen content (μmol/g dry weight) did not fall significantly in hypoperfused Group 1 (264 ± 23) *vs.* normally perfused Group 4 myocardium (315 ± 31). Glycogen mobilization by dobutamine was dose dependent: low-dose dobutamine (213 ± 13, $p < 0.05$ vs. Groups 3 and 4) only tended to deplete myocardial glycogen reserves relative to Group 1, but high-dose dobutamine (171 ± 11, $p < 0.05$ *vs.* Groups 2 and 4) further depleted myocardial glycogen reserves by 35% *vs.* untreated Group 1.

DISCUSSION

This study investigated the effects of dobutamine on regional contractile function, oxygen demand, and cytosolic energy reserves of canine right ventricular myocardium during coronary hypoperfusion. Functional and metabolic responses of this hypoperfused myocardium to dobutamine depended heavily on the applied concentration of the β-adrenergic agent. Inotropic stimulation with low-dose dobutamine significantly increased RV regional contractile function without a concomitant increase in MVO_2. Remarkably, low-dose dobutamine stimulation did not deplete but instead sharply increased high energy phosphate reserves and cytosolic phosphorylation potential. On the other hand, a six-fold higher dose of dobutamine produced a biphasic contractile response: RV regional systolic function initially increased but later fell to pretreatment values despite continued dobutamine infusion. High-dose dobutamine also depleted high energy phosphates, indicating a renewed metabolic supply-demand imbalance. Both dobutamine doses stimulated glucose metabolism, but glycolysis became limited at the level of glyceraldehyde 3-phosphate dehydrogenase during high-dose dobutamine stimulation.

Effects of ischemia and dobutamine on regional function and O_2 demand.

When RCP was lowered to approximately 40 mm Hg, regional contractile function fell by approximately 37%, with a concomitant decrease in MVO_2. This acute response is typical of hibernating myocardium, in which contractile function

is persistently but reversibly lowered (5, 30), enabling the chronically underperfused myocardium to remain viable despite restriction of its oxygen supply (17). Downregulation of myocardial oxygen demand during decreased oxygen supply without apparent metabolic and pathophysiological consequences of ischemia was observed in this study during pre-dobutamine hypoperfusion. Our findings of decreased RV function during moderate hypoperfusion are consistent with our previous study (10), in which contractile function was maintained during reductions in RCP until a critical level was reached between 30 and 40 mm Hg. In the left ventricle (6, 11, 17), the critical perfusion pressure is much nearer the normal resting level, resulting in a linear relationship between left ventricular function and coronary flow as perfusion pressure is lowered. Since the increased RV contractile function produced by low-dose dobutamine was not accompanied by a concomitant increase in MVO_2, downregulation of myocardial oxygen demand persisted despite inotropic stimulation. At the higher dobutamine dose, increased RV contractile function was accompanied by an increase in MVO_2; however, the myocardium could not sustain increased contractile function for 15 min, indicating renewed oxygen supply-demand mismatch due to β-adrenergic stimulation.

Effects of dobutamine on myocardial O_2 utilization efficiency and energy reserves

In the absence of dobutamine, RV myocardial ATP, PCr, and Cr contents, intracellular P_i concentration, and PCr phosphorylation potential were maintained at the respective baselines during RV hypoperfusion, despite a 54% reduction in RCBF and a 31% decline in O_2 utilization efficiency (O_2UE). This energetic stability is most likely due to the concomitant reduction of regional contractile function, which lowers energy demand. O_2UE was increased throughout treatment with low-dose dobutamine. The increase in regional contractile function, without a concomitant increase in MVO_2, may be explained by an increase in the efficiency of energy transfer from total to external mechanical work, as recently demonstrated by Krams *et al.* in stunned porcine myocardium treated with a low dose of dobutamine (15).

In the high-dose dobutamine group, O_2UE increased during the initial 5 min of treatment; however, this increase in O_2UE was not sustained. Although myocardial oxygen extraction increased in this group, oxidative metabolism was not sufficient to sustain regional contractile function, nor cytosolic phosphorylation potential. This unfavorable situation, which may have been exacerbated by the marked chronotropic effect of high-dose dobutamine, was not observed at the lower dose. Yanagi *et al.* (42) tested the effects of intravenous dobutamine on hypoperfused (coronary flow 50% of baseline) left ventricular

myocardium in open chest dogs. When dobutamine did not elicit tachycardia, the cytosolic P_i/PCr ratio did not change, indicating that myocardial energy reserves were maintained. When tachycardia occurred, P_i/PCr increased, indicating depletion of energy reserves. Thus, the chronotropic response to dobutamine may be the main determinant of myocardial energy supply:demand balance during coronary hypoperfusion.

The decreases in PCr and phosphorylation potential elicited by high-dose dobutamine are in accord with studies in moderately ischemic left ventricular myocardium by Zhang *et al.*, (44) who reported reductions in PCr/ATP in open chest dogs, and Schulz *et al.*, (34) who reported depletion of high energy phosphates in open chest pigs. Cytosolic phosphorylation potential has been found to be directly related to myocardial function (3, 18, 19), and it seems likely that the increased phosphorylation potential may have contributed to the sustained improvement in regional contractile performance by low-dose dobutamine. Conversely, during high dobutamine treatment, the myocardium could not adequately meet the increased energy demand imposed by inotropic stimulation, and, consequently, phosphorylation potential fell.

Dobutamine enhanced glycolysis: a mechanism for increased phosphorylation potential?

The myocardium gradually shifts from fatty acid to glucose as its principal energy source during moderate ischemia (38, 40). Under these conditions, glycolytic flux and glucose uptake are accelerated through the stimulation of glucose uptake and of phosphofructokinase activity (26, 27). Similarly, in this study glucose uptake increased as RCP was lowered, and increased even further during low dobutamine treatment. To examine dobutamine's effects on glycolysis, myocardial glycolytic intermediates were analyzed by crossover plots (14). Low dose dobutamine elevated the contents of several glycolytic intermediates, indicating increased entry of hexose into the glycolytic pathway. It thus appears that low-dose dobutamine further enhances glucose metabolism in hypoperfused RV myocardium.

High dose dobutamine produced a somewhat different glycolytic pattern. All five measured intermediates 'upstream' of GAPDH/PGK accumulated, but those intermediates beyond GAPDH/PGK fell sharply compared to the untreated hypoperfused group. During ischemia (24, 31) or in conditions of near-maximal glycolysis (14), NADH accumulates in the cytosol and limits GAPDH flux, which in turn constrains the overall glycolytic rate. Limitation of GAPDH causes intermediates in the first half of the glycolytic sequence to accumulate, and depletes intermediates beyond this reaction, producing a "crossover" in the

glycolytic plot (Figure 8). A sharp increase in lactate release, reflecting an NADH-dependent shift in lactate dehydrogenase equilibrium toward lactate formation, occurred as high-dose dobutamine treatment was extended beyond 5 min. Thus, it appears that NADH accumulated in the cytosol of cardiomyocytes during high- but not low-dose dobutamine stimulation, and served to constrain glycolytic flux. This glycolytic limitation may have contributed to the depletion of energy reserves during high-dose dobutamine treatment. On the other hand, enhancement of energy reserves of low-dose dobutamine treated myocardium may be due in part to a sustained increase in glycolysis. Hence, it appears that carefully selected, low doses of dobutamine can produce favorable increases in both myocardial performance and energy reserves, but higher concentrations of dobutamine are energetically costly and cannot sustain improved performance.

Limitations of the investigation

In this study, the RCA perfusion territory was biopsied during the experiments; thus, the mass of the perfusion area could not be measured directly. We have previously demonstrated (2, 25, 39, 43) that right coronary blood flow is approximately 0.50 ml/min/g of tissue at 100 mm Hg RCP. Our assumption that baseline RCBF was 0.50 ml/min/g for each heart produced an estimated RCA perfusion territory mass of 21 ± 2 g, in good agreement with directly measured values in dogs of similar size (2, 25, 39, 43). Since RCBF varies from dog to dog, this assumption must have produced errors in values

normalized per gram myocardium. These errors, however, are likely to be random rather than systematic. Indeed, baseline hemodynamic values and regional contractile function at 100 mm Hg RCP were not significantly different among the four groups; thus, there was no reason to expect systematic differences in the actual baseline flows.

The possibility must also be considered that reductions in RCP altered the mass of the RCA perfusion territory. We recently demonstrated that total occlusion of the left anterior descending coronary artery caused encroachment of normal perfusion into its territory by only ~2 mm (4). In the absence of total occlusion in the present study, such encroachment was probably even more limited. Hence, the reductions in RCP would have produced only modest, inconsequential decreases in the mass of the myocardium perfused by the right coronary artery.

During low-dose dobutamine treatment, PCr content and cytosolic phosphorylation potential increased appreciably, without the expected fall in intracellular P_i concentration. The inequality between the increase in PCr and decrease in P_i may reflect glycogen mobilization during low-dose dobutamine treatment. P_i is liberated when the hexose monophosphate components of glycogen are oxidized, which may offset P_i sequestration in the expanded PCr reserve. Conversely, PCr and glycogen degradation during high dobutamine

stimulation produced a less than expected accumulation of P_i. Under these conditions, some of the P_i may have effluxed into the extracellular space (29) and thereby limited intracellular P_i accumulation due to degradation of PCr and glycogen.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the expert technical assistance of Arthur Williams, B.S., Jie Sun, B.S., Bradley Hart, M.S., and Srinath Setty, M.B.B.S. This study was completed by Kun Don Yi in partial fulfillment of the requirements for the Master of Science degree at University of North Texas Health Science Center. This study was supported by NIH Grants HL-35027 and HL-50441.

REFERENCES

1. **Bache, R. J., J. Zhang, G. Path, H. Merkle, K. Hendrich, A. H. From, and K. Ugurbil.** High-energy phosphate responses to tachycardia and inotropic stimulation in left ventricular hypertrophy. *Am J Physiol* 266: H1959-1970, 1994.

2. **Bian, X., A. G. Williams, Jr., P. A. Gwirtz, and H. F. Downey.** Right coronary autoregulation in conscious, chronically instrumented dogs. *Am J Physiol* 275: H169-175, 1998.

3. **Bünger, R., R. T. Mallet, and D. A. Hartman.** Pyruvate-enhanced phosphorylation potential and inotropism in normoxic and postischemic isolated working heart. Near-complete prevention of reperfusion contractile failure. *Eur J Biochem* 180: 221-233, 1989.

4. **Cicutti, N., K. Rakusan, and H. F. Downey.** Coronary artery occlusion extends perfusion territory boundaries through microvascular collaterals. *Basic Res Cardiol* 89: 427-437, 1994.

5. **Coetzee, A., and P. Fourie.** Regional myocardial function in the presence of coronary artery stenosis and inotropic intervention: a case for myocardial hibernation? *Anesth Analg* 71: 327-333, 1990.

6. **Feigl, E. O.** Coronary physiology. *Physiol Rev* 63: 1-205, 1983.

7. **Greyson, C., J. Garcia, M. Mayr, and G. G. Schwartz.** Effects of inotropic stimulation on energy metabolism and systolic function of ischemic right ventricle. *Am J Physiol* 268: H1821-1828, 1995.

8. **Guth, B. D., R. Schulz, and G. Heusch.** Time course and mechanisms of contractile dysfunction during acute myocardial ischemia. *Circulation* 87: IV35-IV42, 1993.

9. **Haggendal, J., L. Jonsson, G. Johansson, S. Bjurstrom, J. Carlsten, and K. Thoren-Tolling.** Catecholamine-induced free radicals in myocardial cell necrosis on experimental stress in pigs. *Acta Physiol Scand* 131: 447-452, 1987.

10. **Itoya, M., R. T. Mallet, Z. P. Gao, A. G. Williams, Jr., and H. F. Downey.** Stability of high-energy phosphates in right ventricle: myocardial energetics during right coronary hypotension. *Am J Physiol* 271: H320-328, 1996.

11. **Iwamoto, T., X. J. Bai, and H. F. Downey.** Coronary perfusion related changes in myocardial contractile force and systolic ventricular stiffness. *Cardiovasc Res* 28: 1331-1336, 1994.

12. **Kedem, J., J. Sonn, M. Scheinowitz, and H. R. Weiss.** Relationship between local oxygen consumption and local and external cardiac work: effect of tachycardia. *Cardiovasc Res* 23: 1043-1052, 1989.

13. **Ko, W., J. A. Zelano, A. L. Fahey, K. Berman, D. Lang, O. W. Isom, and K. H. Krieger.** The effects of amrinone versus dobutamine on myocardial mechanics and energetics after hypothermic global ischemia. *J Thorac Cardiovasc Surg* 105: 1015-1024, 1993.

14. **Kobayashi, K., and J. R. Neely.** Control of maximum rates of glycolysis in rat cardiac muscle. *Circ Res* 44: 166-175, 1979.

15. **Krams, R., D. J. Duncker, E. O. McFalls, A. Hogendoorn, and P. D. Verdouw.** Dobutamine restores the reduced efficiency of energy transfer from total mechanical work to external mechanical work in stunned porcine myocardium. *Cardiovasc Res* 27: 740-747, 1993.

16. **Kusachi, S., O. Nishiyama, K. Yasuhara, D. Saito, S. Haraoka, and H. Nagashima.** Right and left ventricular oxygen metabolism in open-chest dogs. *Am J Physiol* 243: H761-7666, 1982.

17. **Lee, S. C., and H. F. Downey.** Downregulation of oxygen demand in isoprenaline stimulated canine myocardium. *Cardiovasc Res* 27: 1542-1550, 1993.

18. **Mallet, R. T., and R. Bünger.** Energetic modulation of cardiac inotropism and sarcoplasmic reticular Ca^{2+} uptake. *Biochim Biophys Acta* 1224: 22-32, 1994.

19. **Mallet, R. T., and J. Sun.** Mitochondrial metabolism of pyruvate is required for its enhancement of cardiac function and energetics. *Cardiovasc Res* 42: 149-161, 1999.

20. **Marcus, M.** Difference in the regulation of coronary perfusion to the right and left ventricles. In: *The Coronary Circulation in Health and Disease*, edited by McGraw-Hill. New York:, 1983, pp. 93-122.

21. **Massie, B. M., S. Schaefer, J. Garcia, M. D. McKirnan, G. G. Schwartz, J. A. Wisneski, M. W. Weiner, and F. C. White.** Myocardial high-energy phosphate and substrate metabolism in swine with moderate left ventricular hypertrophy. *Circulation* 91: 1814-1823, 1995.

22. **Mertes, H., S. G. Sawada, T. Ryan, D. S. Segar, R. Kovacs, J. Foltz, and H. Feigenbaum.** Symptoms, adverse effects, and complications associated with dobutamine stress echocardiography. Experience in 1118 patients. *Circulation* 88: 15-19, 1993.

23. **Middleton, K. M., A. H. Henderson, and M. J. Lewis.** Catecholamine-induced necrosis and hypertrophy. Hemodynamic factors. *Adv Myocardiol* 6: 339-348, 1985.

24. **Mochizuki, S., and J. R. Neely.** Control of glyceraldehyde-3-phosphate dehydrogenase in cardiac muscle. *J Mol Cell Cardiol* 11: 221-236, 1979.

25. **Murakami, H., S. J. Kim, and H. F. Downey.** Persistent right coronary flow reserve at low perfusion pressure. *Am J Physiol* 256: H1176-184, 1989.

26. **Neely, J.R., and H.E. Morgan.** Relationship between carbohydrate and lipid metabolism and energy balance of heart muscle. *Annual Review of Physiology* 36: 413-459, 1974.

27. **Opie, L. H.** Substrate utilization and glycolysis in the heart. *Cardiology* 56: 2-21, 1971.

28. **Persoon-Rothert, M., E. J. van der Valk-Kokshoorn, J. M. Egas-Kenniphaas, I. Mauve, and A. van der Laarse.** Isoproterenol-induced cytotoxicity in neonatal rat heart cell cultures is mediated by free radical formation. *J Mol Cell Cardiol* 21: 1285-1291, 1989.

29. **Polgreen, K. E., G. J. Kemp, K. Clarke, and G. K. Radda.** Transsarcolemmal movement of inorganic phosphate in glucose-perfused rat heart: a ^{31}P nuclear magnetic resonance spectroscopic study. *J Mol Cell Cardiol* 26: 219-228, 1994.

30. **Rahimtoola, S. H.** The hibernating myocardium. *Am Heart J* 117: 211-221, 1989.

31. **Rovetto, M. J., W. F. Lamberton, and J. R. Neely.** Mechanisms of glycolytic inhibition in ischemic rat hearts. *Circ Res* 37: 742-751, 1975.

32. **Scholz, P. M., J. Kedem, B. V. Cheinberg, and H. R. Weiss.** The oxygen wasting effect of isoproterenol is altered by chemical denervation and cardiac hypertrophy. *Basic Res Cardiol* 91: 308-318, 1996.

33. **Schulz, R., B. D. Guth, K. Pieper, C. Martin, and G. Heusch.** Recruitment of an inotropic reserve in moderately ischemic myocardium at the expense of metabolic recovery. A model of short-term hibernation. *Circ Res* 70: 1282-1295, 1992.

34. **Schulz, R., S. Miyazaki, M. Miller, E. Thaulow, G. Heusch, J. Ross, Jr., and B. D. Guth**. Consequences of regional inotropic stimulation of ischemic myocardium on regional myocardial blood flow and function in anesthetized swine. *Circ Res* 64: 1116-1126, 1989.

35. **Schwartz, G. G., C. R. Greyson, J. A. Wisneski, J. Garcia, and S. Steinman**. Relation among regional O_2 consumption, high-energy phosphates, and substrate uptake in porcine right ventricle. *Am J Physiol* 266: H521-530, 1994.

36. **Singal, P. K., N. Kapur, K. S. Dhillon, R. E. Beamish, and N. S. Dhalla.** Role of free radicals in catecholamine-induced cardiomyopathy. *Can J Physiol Pharmacol* 60: 1390-1397, 1982.

37. **Singh, S., F. C. White, and C. M. Bloor.** Myocardial morphometric characteristics in swine. *Circ Res* 49: 434-441, 1981.

38. **Stanley, W. C., G. D. Lopaschuk, J. L. Hall, and J. G. McCormack.** Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions. Potential for pharmacological interventions. *Cardiovasc Res* 33: 243-257, 1997.

39. **Tune, J. D., R. T. Mallet, and H. F. Downey.** Insulin improves contractile function during moderate ischemia in canine left ventricle. *Am J Physiol* 274: H1574-1581, 1998.

40. **van der Vusse, G. J., J. F. Glatz, H. C. Stam, and R. S. Reneman.** Fatty acid homeostasis in the normoxic and ischemic heart. *Physiol Rev* 72: 881-940, 1992.

41. **Veech, R. L., J. W. Lawson, N. W. Cornell, and H. A. Krebs.** Cytosolic phosphorylation potential. *J Biol Chem* 254: 6538-6547, 1979.

42. **Yanagi, S., K. Takeuchi, T. Takeda, M. Ishikawa, and I. Miura.** Effects of inotropic stimulation on phosphate compounds in ischaemic canine hearts. *Cardiovasc Res* 27: 1435-1443, 1993.

43. **Yonekura, S., N. Watanabe, and H. F. Downey.** Transmural variation in autoregulation of right ventricular blood flow. *Circ Res* 62: 776-781, 1988.

44. **Zhang, J., G. Path, V. Chepuri, D. C. Homans, H. Merkle, K. Hendrich, K. Ugurbil, R. J. Bache, and A. H. From.** Effects of dobutamine on myocardial blood flow, contractile function, and bioenergetic responses distal to coronary stenosis: implications with regard to dobutamine stress testing. *Am Heart J* 129: 330-342, 1995.

45. **Zhang, J., C. Toher, M. Erhard, Y. Zhang, K. Ugurbil, R. J. Bache, T. Lange, and D. C. Homans.** Relationships between myocardial bioenergetic and left ventricular function in hearts with volume-overload hypertrophy. *Circulation* 96: 334-343, 1997.

46. **Zhou, Z., R. D. Lasley, J. O. Hegge, R. Bunger, and R. M. Mentzer, Jr.** Myocardial stunning: a therapeutic conundrum. *J Thorac Cardiovasc Surg* 110: 1391-1401, 1995.

Table **1**. *Right ventricular hemodynamics.*

RCP (mmHg)	AoP (mmHg)	HR (beats/min)	RCBF (ml/min/g)	RVP (mmHg)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)
			Baseline			
100 (U)	102 ± 2	125 ± 5	0.51 ± 0.02	27 ± 2	435 ± 25	-347 ± 42
100 (L)	104 ± 4	128 ± 9	0.50 ± 0.02	27 ± 3	442 ± 20	-345 ± 27
100 (H)	105 ± 5	129 ± 7	0.50 ± 0.02	27 ± 2	444 ± 41	-342 ± 20
			Hypoperfusion, pre-dobutamine			
40 ± 2 (U)	100 ± 4	125 ± 7	0.23 ± 0.03*	26 ± 2	380 ± 19*	-298 ± 39
41 ± 2 (L)	107 ± 3	125 ± 6	0.23 ± 0.02*	26 ± 3	390 ± 15*	-296 ± 19
38 ± 3 (H)	108 ± 3	137 ± 6	0.23 ± 0.01*	25 ± 2	386 ± 49*	-293 ± 22
			5 min dobutamine			
40 ± 2 (U)	100 ± 4	125 ± 8	0.23 ± 0.03*	26 ± 2	394 ± 24	-300 ± 40
41 ± 2 (L)	109 ± 3	137 ± 8	0.26 ± 0.02*†	28 ± 3	417 ± 27	-347 ± 17
38 ± 3 (H)	107 ± 5	162 ± 3§	0.29 ± 0.02*†	27 ± 2	475 ± 53†	-323 ± 32
			15 min dobutamine			
40 ± 2 (U)	101 ± 4	124 ± 8	0.22 ± 0.03*	26 ± 2	390 ± 15	-299 ± 38
41 ± 2 (L)	110 ± 3	135 ± 8	0.26 ± 0.02*†	28 ± 3	425 ± 27	-349 ± 17
38 ± 3 (H)	107 ± 5	166 ± 3§	0.29 ± 0.02*†	27 ± 2	472 ± 65†	-335 ± 37

Values are means ± SEM. RCP, right coronary perfusion pressure; AoP, mean aortic pressure; RCBF, right coronary blood flow; HR, heart rate; RVP, right ventricular peak systolic pressure; $+dP/dt_{max}$, maximum rate of pressure development; $-dP/dt_{min}$, maximum rate of relaxation. U, *Group 1* (untreated hypoperfusion, n = 9); L, *Group 2* (low-dose dobutamine, n = 13); H, *Group 3* (high-dose dobutamine, n = 6). * $p < 0.05$ vs. respective baseline value at 100 mmHg; † $p < 0.05$ vs. pre-treatment value, same group; ‡ $p < 0.05$ vs. pre-treatment value, same period. § $p < 0.05$ vs. all values.

Table **2**. *Right coronary venous blood gases and myocardial lactate and glucose uptake.*

RCP (mmHg)	Pvo_2 (mmHg)	$Pvco_2$ (mmHg)	Lactate Uptake (μmol/min/g)	Glucose Uptake (μmol/min/g)
		Baseline		
100 (U)	36.7 ± 1.7	42.0 ± 2.5	0.17 ± 0.02	0.12 ± 0.01
100 (L)	36.9 ± 2.0	42.1 ± 1.9	0.20 ± 0.07	0.12 ± 0.03
100 (H)	36.5 ± 2.7	42.8 ± 1.1	0.17 ± 0.02	0.12 ± 0.03
		Hypoperfusion, pre-dobutamine		
40 ± 2 (U)	29.7 ± 1.8*	48.9 ± 1.8*	0.04 ± 0.02*	0.18 ± 0.01*
41 ± 2 (L)	28.5 ± 1.5*	47.3 ± 2.1*	0.03 ± 0.02*	0.18 ± 0.03*
38 ± 3 (H)	28.8 ± 1.1*	48.7 ± 2.1*	0.03 ± 0.04*	0.18 ± 0.02*
		5 min dobutamine		
40 ± 2 (U)	29.3 ± 1.8*	49.6 ± 2.0*	0.04 ± 0.02*	0.17 ± 0.01*
41 ± 2 (L)	26.2 ± 1.2*†	49.3 ± 2.0*	-0.02 ± 0.04*†	0.25 ± 0.02*†‡
38 ± 3 (H)	25.2 ± 1.8*†	53.7 ± 3.7*†	-0.04 ± 0.07*†	0.25 ± 0.03*†‡
		15 min dobutamine		
40 ± 2 (U)	29.2 ± 1.6*	50.0 ± 2.1*	0.03 ± 0.02*	0.17 ± 0.01*
41 ± 2 (L)	26.3 ± 1.2*†	48.7 ± 2.0*	-0.02 ± 0.04*†	0.25 ± 0.02*†‡
38 ± 3 (H)	15.7 ± 1.3§	63.6 ± 5.0§	-0.28 ± 0.09§	0.29 ± 0.03*†‡

Values are means ± SEM. Pvo_2, $Pvco_2$, right coronary venous Po_2, Pco_2. U, *Group 1* (untreated hypoperfusion, n = 9); L, *Group 2* (low-dose dobutamine, n = 13); H, *Group 3* (high-dose dobutamine, n = 6). * $p < 0.05$ vs. respective baseline value at 100 mmHg; † $p < 0.05$ vs. pre-treatment value, same group; ‡ $p < 0.05$ vs. pre-treatment value, same period. § $p < 0.05$ vs. all values.

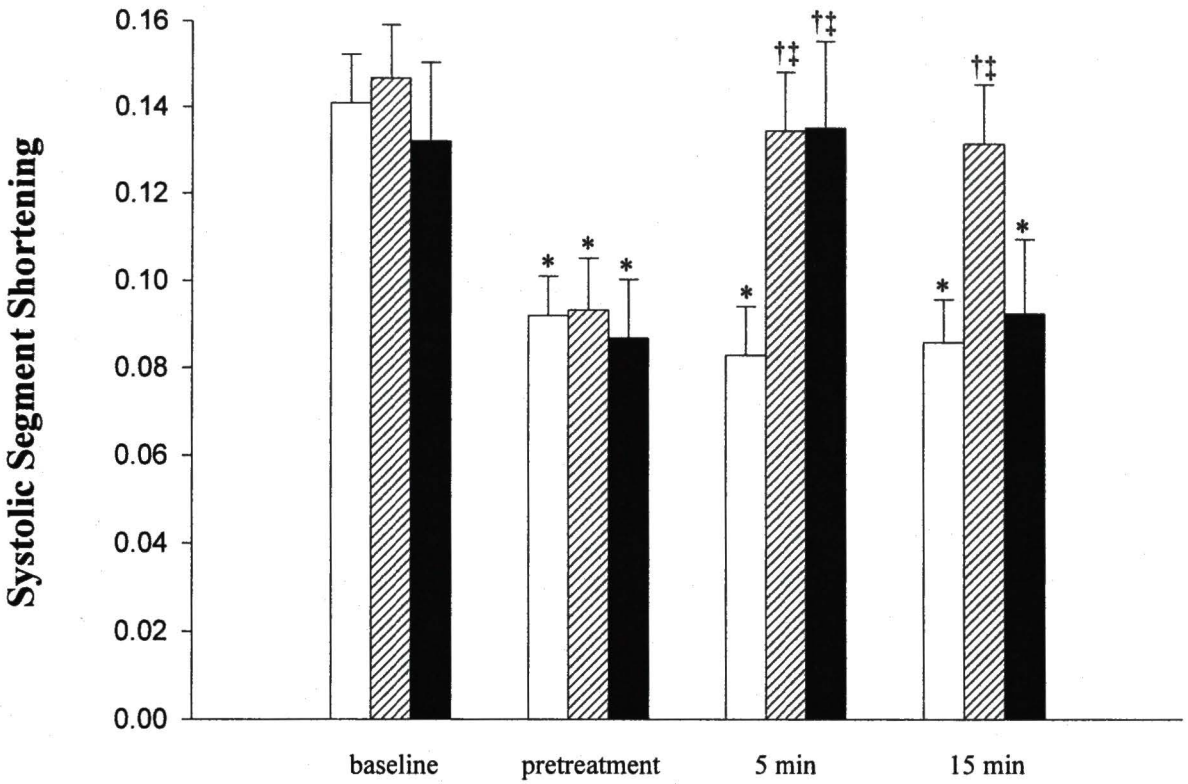


Figure 1. *Systolic segment shortening (SS) of right ventricular myocardium.* Baseline values (means ± SEM) were obtained at 100 mm Hg right coronary perfusion pressure (RCP); pretreament values were obtained 15 min after RCP was lowered to 40 mm Hg. Other values were obtained at 5 and 15 min dobutamine infusion in Groups 2 and 3, and at the same points in untreated Group 1. O pen bars: untreated hypoperfusion (Group 1; n = 9)hatched bars: low-dose dobutamine treatment (Group 2; n = 13); solid bars: high-dose dobutamine (Group 3; n = 6). * $p < 0.05$ vs. baseline; † $p < 0.05$ vs. pretreatment; ‡ $p < 0.05$ vs. Group 1.

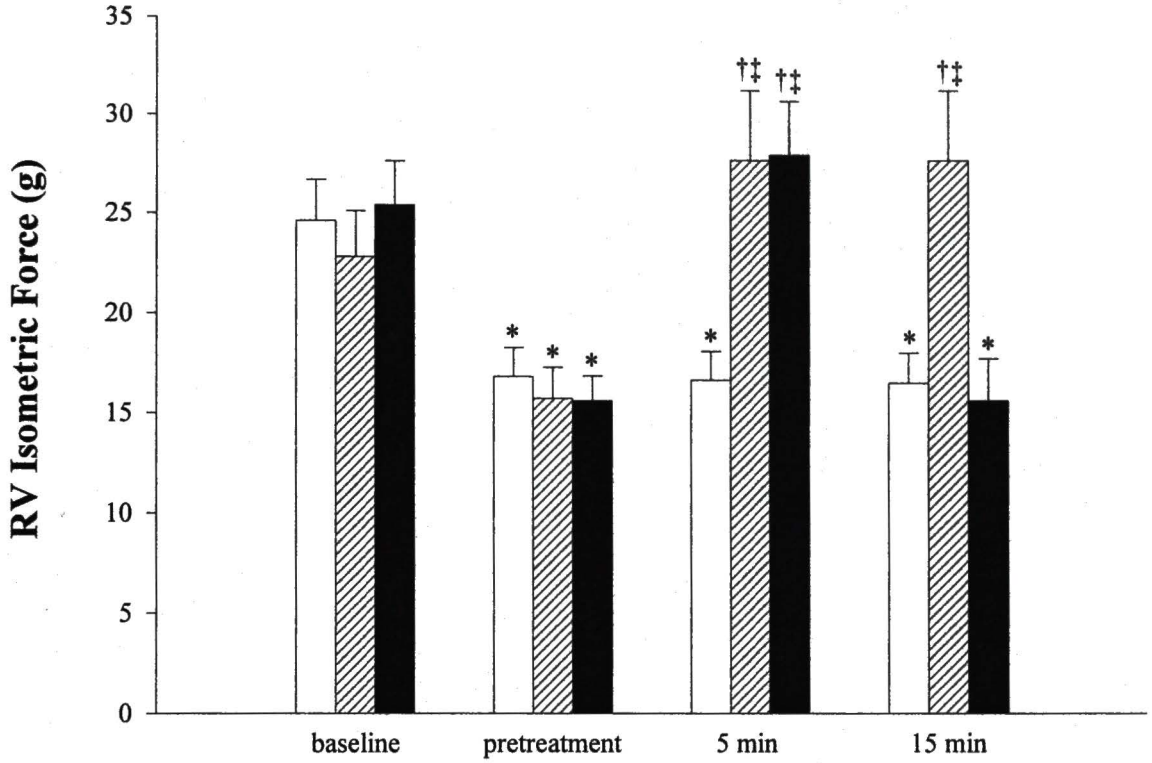


Figure 2. *Systolic isometric force of right ventricular myocardium.* Treatment groups, time points, and significance are the same as in Figure 1.

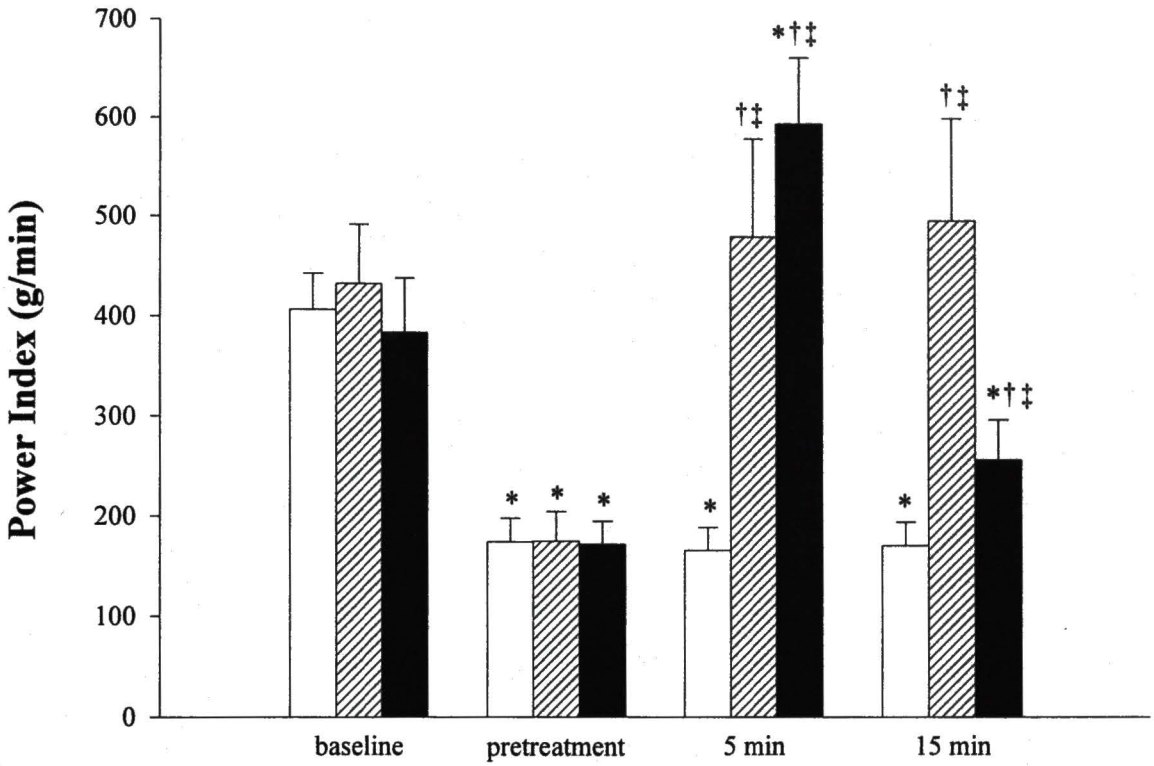


Figure 3. *Right ventricular regional power.* Treatment groups, time points, and significance symbols are the same as in Figure 1.

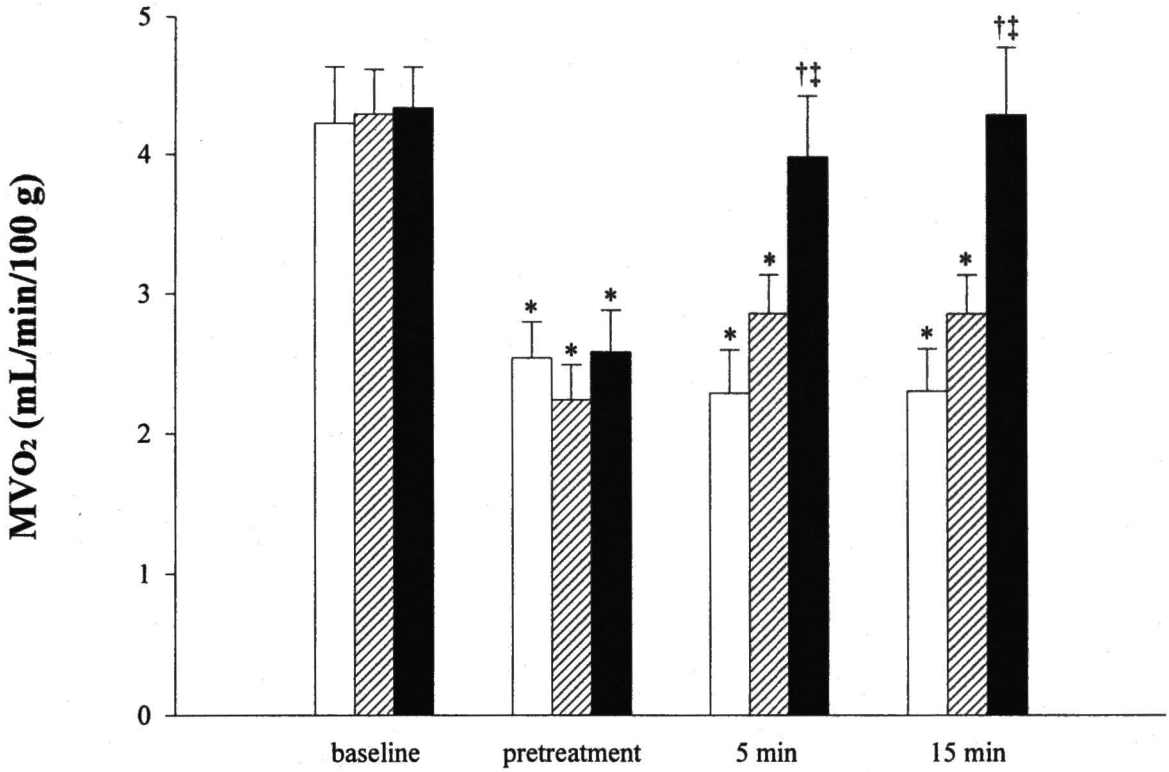


Figure 4. *Right ventricular myocardial oxygen consumption (MVO_2).* Treatment groups, time points, and significance symbols are the same as in Figure 1.

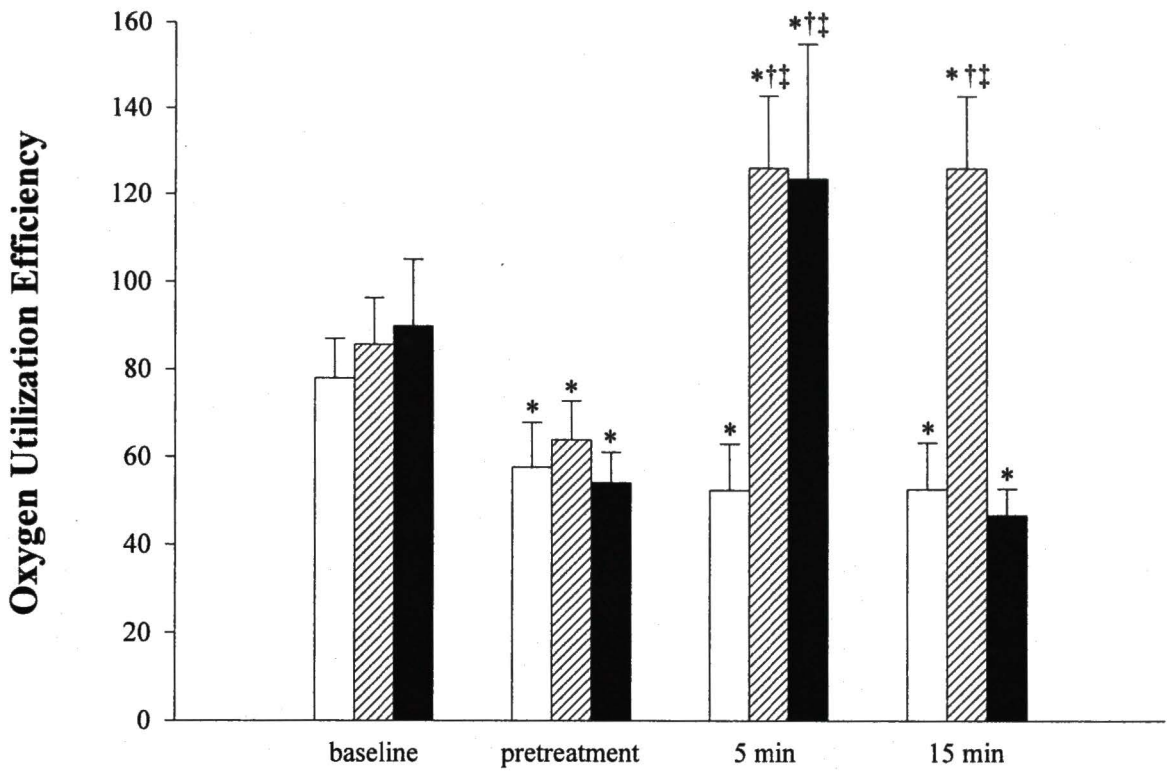


Figure 5. *Right ventricular myocardial oxygen utilization efficiency (O_2UE).* Treatment groups, time points, and significance symbols are the same as in Figure 1.

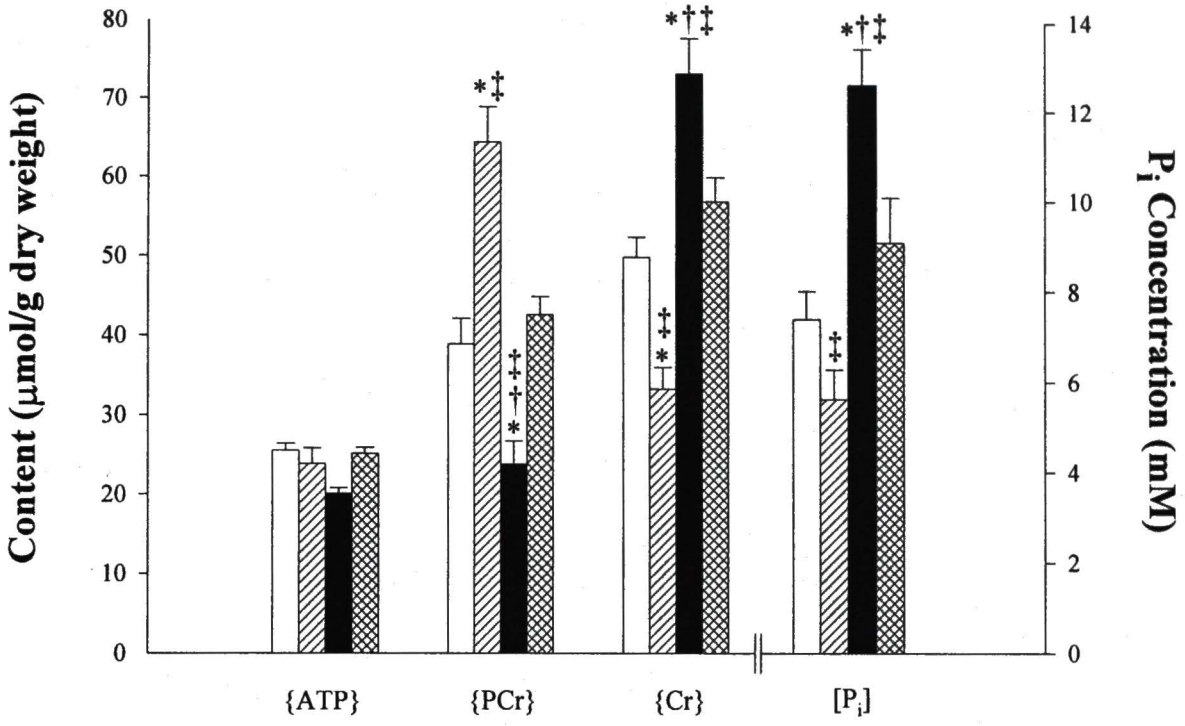


Figure 6. *Right ventricular myocardial energy metabolites.* ATP, phosphocreatine, (PCr), creatine (Cr), and inorganic phosphate (P_i) were measured in myocardium freezed-clamped at 30 min untreated hypoperfusion (Group 1; open bars), 15 min low-dose dobutamine (Group 2; hatched bars), 15 min high-dose dobutamine (Group 3; solid bars), or at 75 min nonischemic time control perfusion (Group 4; cross-hatched bars). { }: metabolite contents (μmol/g dry wt); [ ]: intracellular concentration (mM). * $p < 0.05$ vs. control Group 1; † $p < 0.05$ vs. Group 2; ‡ $p < 0.05$ vs. Group 4.

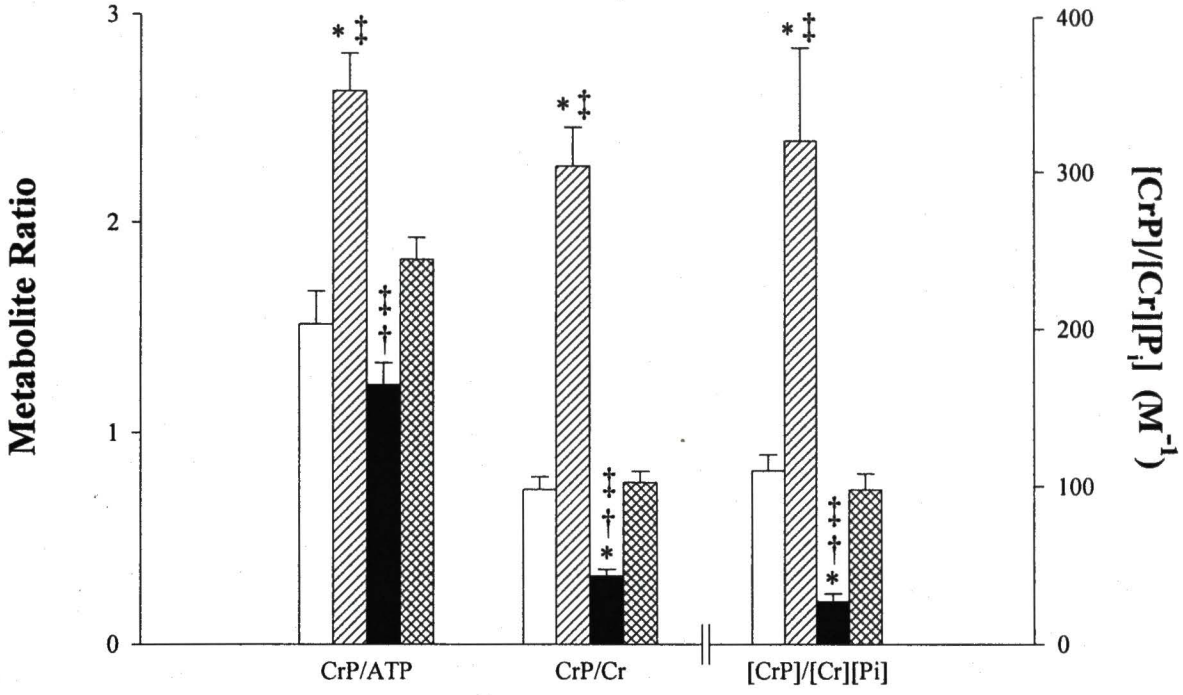


Figure 7. *Energy metabolite ratios and phosphocreatine phosphorylation potential in right ventricular myocardium.* Values are from the same experiments as in Figure 6. { }: metabolite contents (μmol/g dry wt); [ ]: intracellular concentration (mM). * $p < 0.05$ vs. control Group 1; † $p < 0.05$ vs. Group 2; ‡ $p < 0.05$ vs. Group 4.

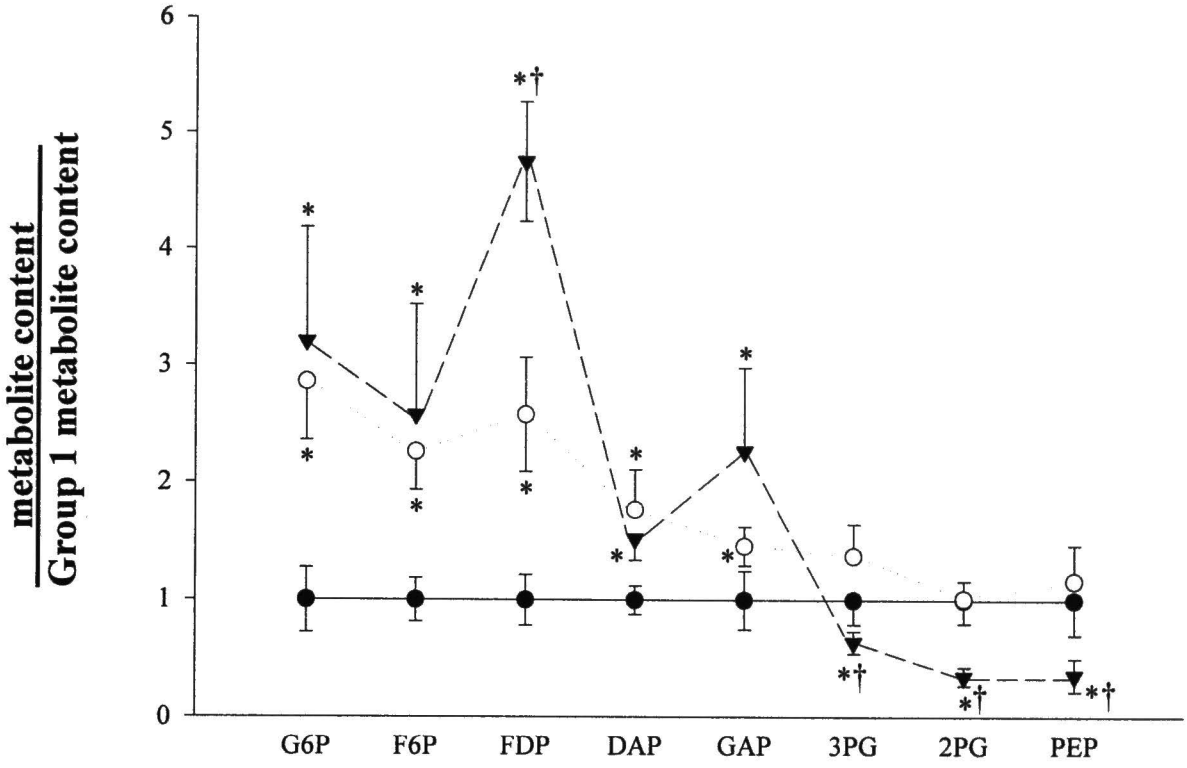


Figure 8. *Crossover plots of glycolytic intermediates in right ventricular myocardium.* Metabolite contents in untreated hypoperfusion (Group 1; closed circles) are assigned a value of untiy; metabolite contents in low-dose dobutamine treated myocardium (Group 2; open circles) and high-dose dobutamine treated myocardium (Group 3; triangles) are normalized to the respective Group 1 contents. G6P: glucose-6-phosphate; F6P: fructose-6-phosphate; FDP: fructose-1,6-*bis*-phosphate; DAP: dihydroxyacetone phosphate; GAP: glyceraldehyde-3-phosphate; 3PG: 3-phosphoglycerate; 2PG: 2-phosphoglycerate; PEP: phosphoenolpyruvate. Group 1 contents (μmol/ g dry wt): G6P (0.66 ± 0.18), F6P (0.19 ± 0.04), FDP (0.20 ± 0.04), DAP (0.15 ± 0.02), GAP (0.05 ± 0.01), 3PG (0.37 ± 0.11), 2PG (0.17 ± 0.03), PEP (0.07 ± 0.02).* $p < 0.05$ vs. control Group 1; † $p < 0.05$ vs. Group 2; ‡ $p < 0.05$ vs. Group 4.

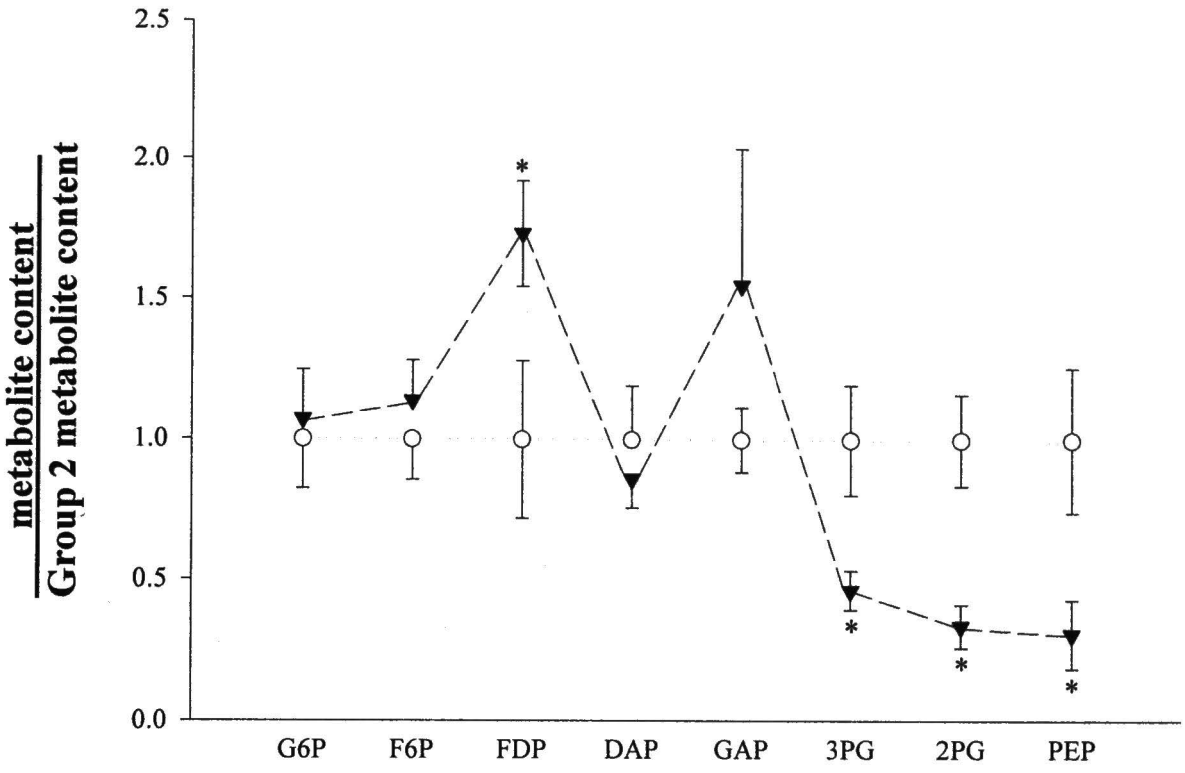


Figure 9. *Glycolytic crossover plot in dobutamine-treated myocardium.* Metabolite contents in low-dose dobutamine treated myocardium (Group 2; open circles) are assigned a value of unity, and metabolite contents in high-dose dobutamine treated myocardium (Group 3; triangles) are normalized to the respective Group 2 values. Abbreviations as in Figure 8. * $p < 0.05$ vs. Group 2.

CHAPTER III

CONCLUSION

This study investigated the effects of two doses of dobutamine on regional contractile function, oxygen demand, and cytosolic energy reserves of canine right ventricular myocardium during coronary hypoperfusion. Functional and metabolic responses of this hypoperfused myocardium to dobutamine depended heavily on the applied concentration of the β-adrenergic agent. Inotropic stimulation with low-dose dobutamine significantly increased RV regional contractile function without a concomitant increase in MVO_2. Remarkably, low-dose dobutamine stimulation did not deplete but instead sharply increased high energy phosphate reserves and cytosolic phosphorylation potential. On the other hand, a six-fold higher dose of dobutamine produced a biphasic contractile response: RV regional systolic function initially increased but later fell to pretreatment values despite continued dobutamine infusion. High-dose dobutamine also depleted high energy phosphates, indicating a renewed metabolic supply-demand imbalance. Both dobutamine doses stimulated glucose metabolism, but glycolysis became limited at the level of glyceraldehyde 3-phosphate dehydrogenase during high-dose dobutamine stimulation. From this we can conclude that:

1) chronotropic response to dobutamine may be the main determinant of myocardial energy supply:demand balance during coronary hypoperfusion.

2) glycolytic limitation may have contributed to the depletion of energy reserves during high-dose dobutamine treatment

3) carefully selected, low doses of dobutamine can produce favorable increases in both myocardial performance and energy reserves, but higher concentrations of dobutamine are energetically costly and cannot sustain improved performance.

APPENDIX

ADDITIONAL TABLES AND GRAPHS INCLUDING

INTERMEDIATE CORONARY PERFUSION

PRESSURES (60 AND 50 mm Hg)

Table 1. *Right ventricular hemodynamics.*

RCP (mmHg)	AoP (mmHg)	HR (beats/min)	RCBF (ml/min/g)	RVP (mmHg)	+dP/dt (mmHg/s)	-dP/dt (mmHg/s)
100 (U)	102 ± 2	125 ± 5	0.51 ± 0.02	27 ± 2	435 ± 25	-347 ± 42
100 (L)	104 ± 4	128 ± 9	0.50 ± 0.02	27 ± 3	442 ± 20	-345 ± 27
100 (H)	105 ± 5	129 ± 7	0.50 ± 0.02	27 ± 2	444 ± 41	-342 ± 20
60 (U)	102 ± 2	126 ± 5	0.35 ± 0.02*†	27 ± 2	460 ± 24†	-321 ± 38
60 (L)	106 ± 3	127 ± 7	0.33 ± 0.01*†	26 ± 3	462 ± 23†	-316 ± 23
60 (H)	104 ± 3	132 ± 7	0.35 ± 0.01*†	27 ± 2	462 ± 34†	-310 ± 16
50 (U)	102 ± 3	128 ± 6	0.29 ± 0.02*†	27 ± 2	466 ± 28†	-310 ± 40
50 (L)	106 ± 3	126 ± 7	0.27 ± 0.02*†	26 ± 3	454 ± 16†	-305 ± 23
50 (H)	106 ± 4	134 ± 7	0.27 ± 0.01*†	25 ± 2	458 ± 40†	-302 ± 21
40 ± 2 (U)	100 ± 4	125 ± 7	0.23 ± 0.03‡	26 ± 2	380 ± 19‡	-298 ± 39
41 ± 2 (L)	107 ± 3	125 ± 6	0.23 ± 0.02*	26 ± 3	390 ± 15*	-296 ± 19
38 ± 3 (H)	108 ± 3	137 ± 6	0.23 ± 0.01*	25 ± 2	386 ± 49*	-293 ± 22
40 ± 2 (U)	100 ± 4	125 ± 8	0.23 ± 0.03‡	26 ± 2	394 ± 24	-300 ± 40
41 ± 2 (L)	109 ± 3	137 ± 8	0.26 ± 0.02*†	28 ± 3	417 ± 27	-347 ± 17
38 ± 3 (H)	107 ± 5	162 ± 3§	0.29 ± 0.02*†	27 ± 2	475 ± 53†	-323 ± 32
40 ± 2 (U)	101 ± 4	124 ± 8	0.22 ± 0.03‡	26 ± 2	390 ± 15	-299 ± 38
41 ± 2 (L)	110 ± 3	135 ± 8	0.26 ± 0.02*†	28 ± 3	425 ± 27	-349 ± 17
38 ± 3 (H)	107 ± 5	166 ± 3§	0.29 ± 0.02*†	27 ± 2	472 ± 65†	-335 ± 37

Values are means ± SEM. RCP, right coronary perfusion pressure; AoP, mean aortic pressure; RCBF, right coronary blood flow; HR, heart rate; RVP, right ventricular peak systolic pressure; $+dP/dt_{max}$, maximum rate of pressure development; $-dP/dt_{min}$, maximum rate of relaxation. U, *Group 1* (untreated hypoperfusion, n = 9); L, *Group 2* (low-dose dobutamine, n = 13); H, *Group 3* (high-dose dobutamine, n = 6). * $p < 0.05$ vs. respective baseline value at 100 mm Hg; † $p < 0.05$ vs. pre-treatment value, same group; ‡ $p < 0.05$ vs. pre-treatment value, same period. § $p < 0.05$ vs. all values.

Table 2. *Right coronary venous blood gases and myocardial lactate and glucose uptake.*

RCP (mm Hg)	Pvo_2 (mm Hg)	$Pvco_2$ (mm Hg)	Lactate Uptake (μmol/min/g)	Glucose Uptake (μmol/min/g)
100 (U)	36.7 ± 1.7	42.0 ± 2.5	0.17 ± 0.02	0.12 ± 0.01
100 (L)	36.9 ± 2.0	42.1 ± 1.9	0.20 ± 0.07	0.12 ± 0.03
100 (H)	36.5 ± 2.7	42.8 ± 1.1	0.17 ± 0.02	0.12 ± 0.03
60 (U)	34.3 ± 1.6*†	44.0 ± 2.4†	0.14 ± 0.04†	0.14 ± 0.01
60 (L)	32.7 ± 1.6*†	43.0 ± 1.8†	0.17 ± 0.05†	0.15 ± 0.03
60 (H)	33.2 ± 1.3*†	43.3 ± 1.1†	0.15 ± 0.02†	0.15 ± 0.01
50 (U)	32.3 ± 1.6*†	44.6 ± 2.5†	0.13 ± 0.03†	0.16 ± 0.02
50 (L)	30.6 ± 1.1*†	44.3 ± 1.8†	0.13 ± 0.05†	0.16 ± 0.02
50 (H)	31.5 ± 1.3*†	43.3 ± 1.3†	0.13 ± 0.05†	0.16 ± 0.03
40 ± 2 (U)	29.7 ± 1.8*	48.9 ± 1.8*	0.04 ± 0.02*	0.18 ± 0.01*
41 ± 2 (L)	28.5 ± 1.5*	47.3 ± 2.1*	0.03 ± 0.02*	0.18 ± 0.03*
38 ± 3 (H)	28.8 ± 1.1*	48.7 ± 2.1*	0.03 ± 0.04*	0.18 ± 0.02*
40 ± 2 (U)	29.3 ± 1.8*	49.6 ± 2.0*	0.04 ± 0.02*	0.17 ± 0.01*
41 ± 2 (L)	26.2 ± 1.2*†	49.3 ± 2.0*	-0.02 ± 0.04*†	0.25 ± 0.02*†‡
38 ± 3 (H)	25.2 ± 1.8*†	53.7 ± 3.7*†	-0.04 ± 0.07*†	0.25 ± 0.03*†‡
40 ± 2 (U)	29.2 ± 1.6*	50.0 ± 2.1*	0.03 ± 0.02*	0.17 ± 0.01*
41 ± 2 (L)	26.3 ± 1.2*†	48.7 ± 2.0*	-0.02 ± 0.04*†	0.25 ± 0.02*†‡
38 ± 3 (H)	15.7 ± 1.3§	63.6 ± 5.0§	-0.28 ± 0.09§	0.29 ± 0.03*†‡

Values are means ± SEM. Pvo_2, $Pvco_2$, right coronary venous Po_2, Pco_2. U, *Group 1* (untreated hypoperfusion, n = 9); L, *Group 2* (low-dose dobutamine, n = 13); H, *Group 3* (high-dose dobutamine, n = 6). * $p < 0.05$ vs. respective baseline value at 100 mm Hg; † $p < 0.05$ vs. pre-treatment value, same group; ‡ $p < 0.05$ vs. pre-treatment value, same period. § $p < 0.05$ vs. all values.

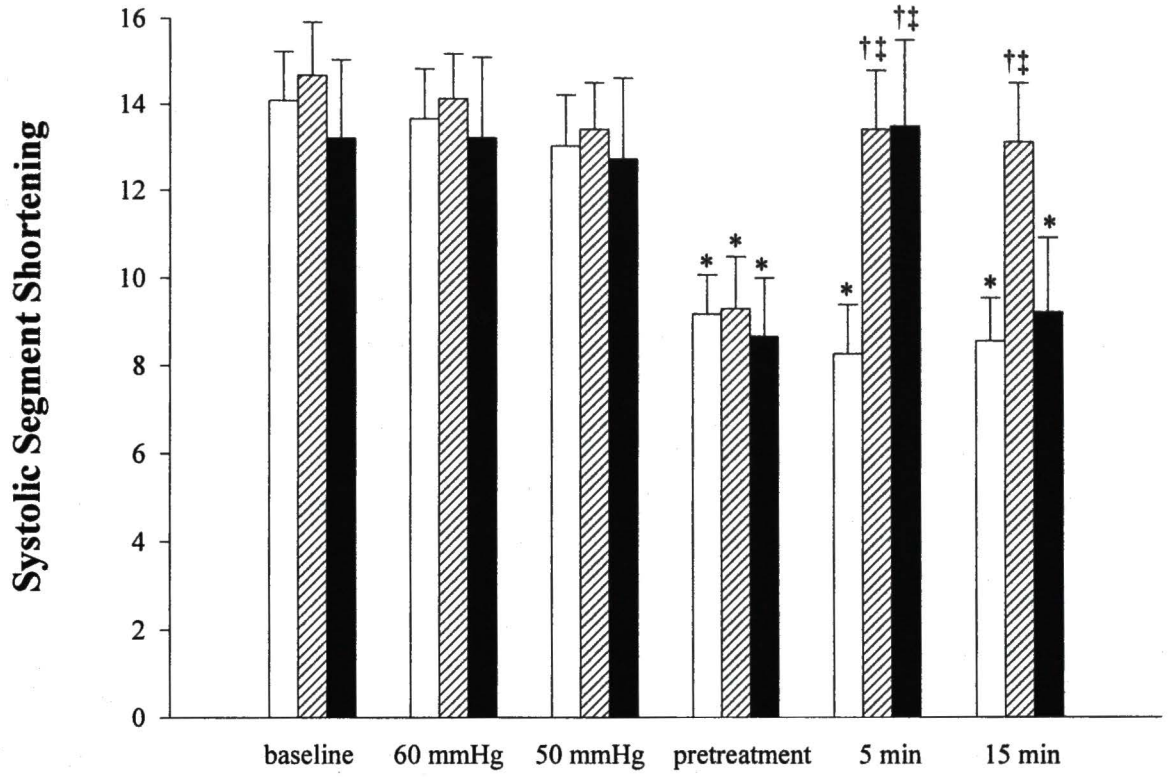


Figure 1. *Systolic segment shortening (%SS) of right ventricular myocardium.* Baseline values (means ± SEM) were obtained at 100, 60, and 50 mmHg right coronary perfusion pressure (RCP); pretreament values were obtained 15 min after RCP was lowered to 40 mmHg. Other values were obtained at 5 and 15 min dobutamine infusion in Groups 2 and 3, and at the same points in untreated Group 1. Open bars: untreated hypoperfusion (Group 1; n = 9); hatched bars: low-dose dobutamine treatment (Group 2; n = 13); solid bars: high-dose dobutmaine (Group 3; n = 6). * $p < 0.05$ vs. baseline, and 50 mmHg; † $p < 0.05$ vs. pretreatment; ‡ $p < 0.05$ vs. Group 1.

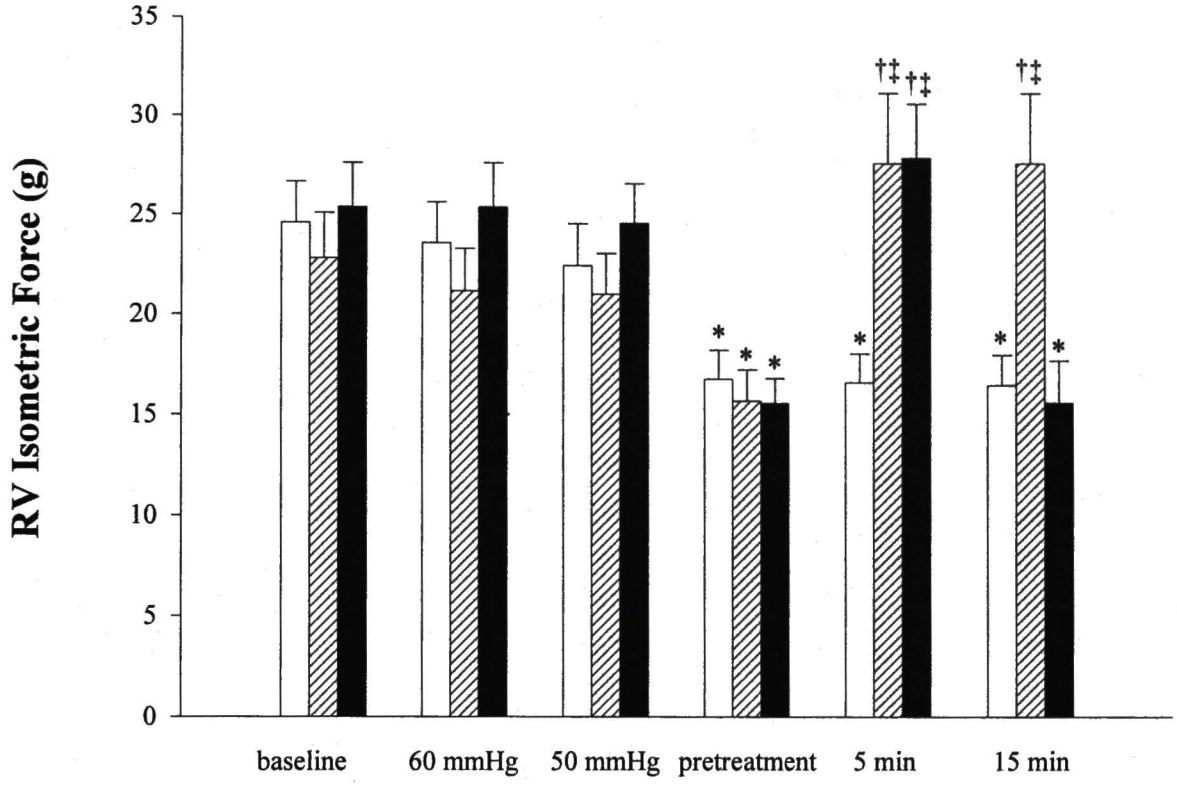


Figure 2. *Systolic isometric force of right ventricular myocardium.* Treatment groups, time points, and significance are the same as in Figure 1.

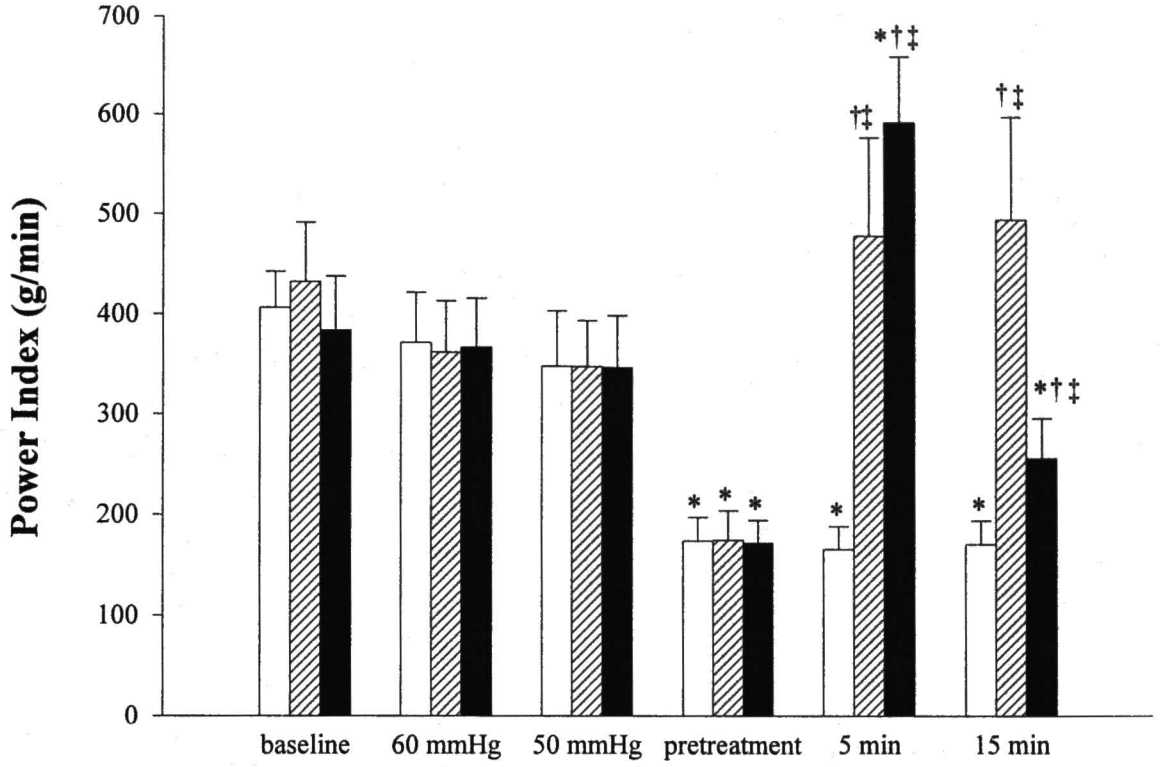


Figure 3. *Right ventricular regional power.* Treatment groups, time points, and significance symbols are the same as in Figure 1.

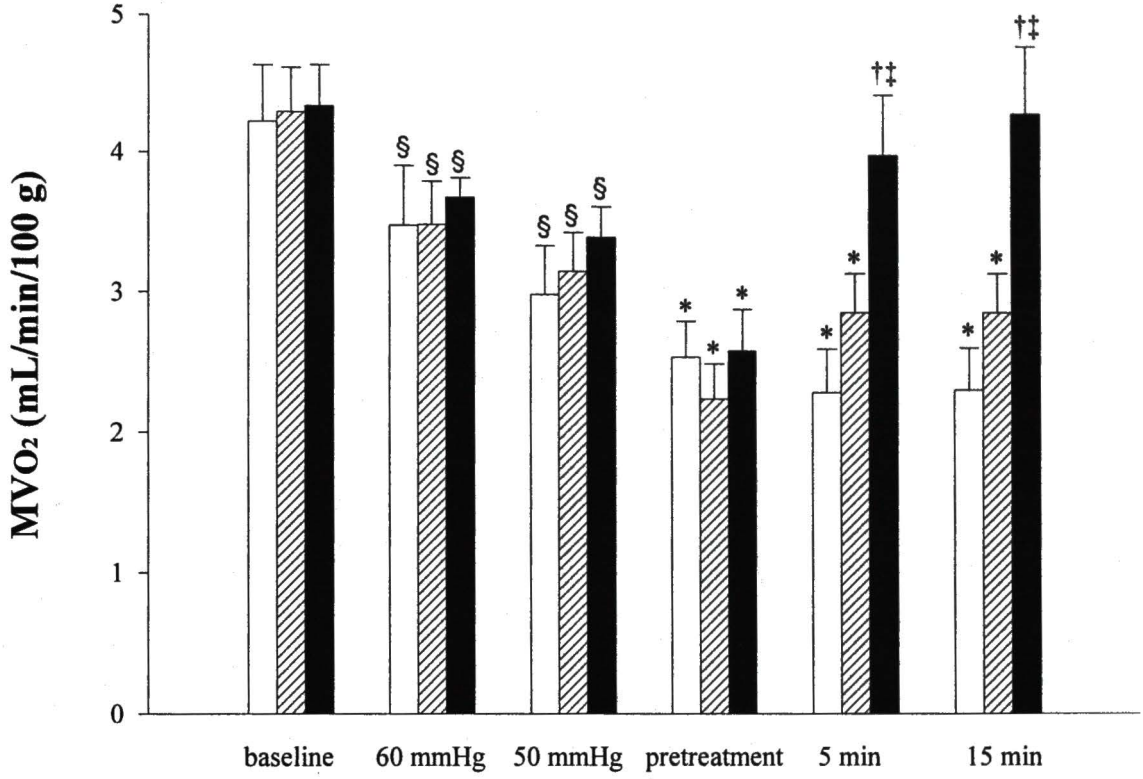


Figure 4. *Right ventricular myocardial oxygen consumption (MVO_2).* Treatment groups, time points, and significance symbols are the same as in Figure 1, except § $p < 0.05$ vs. baseline.

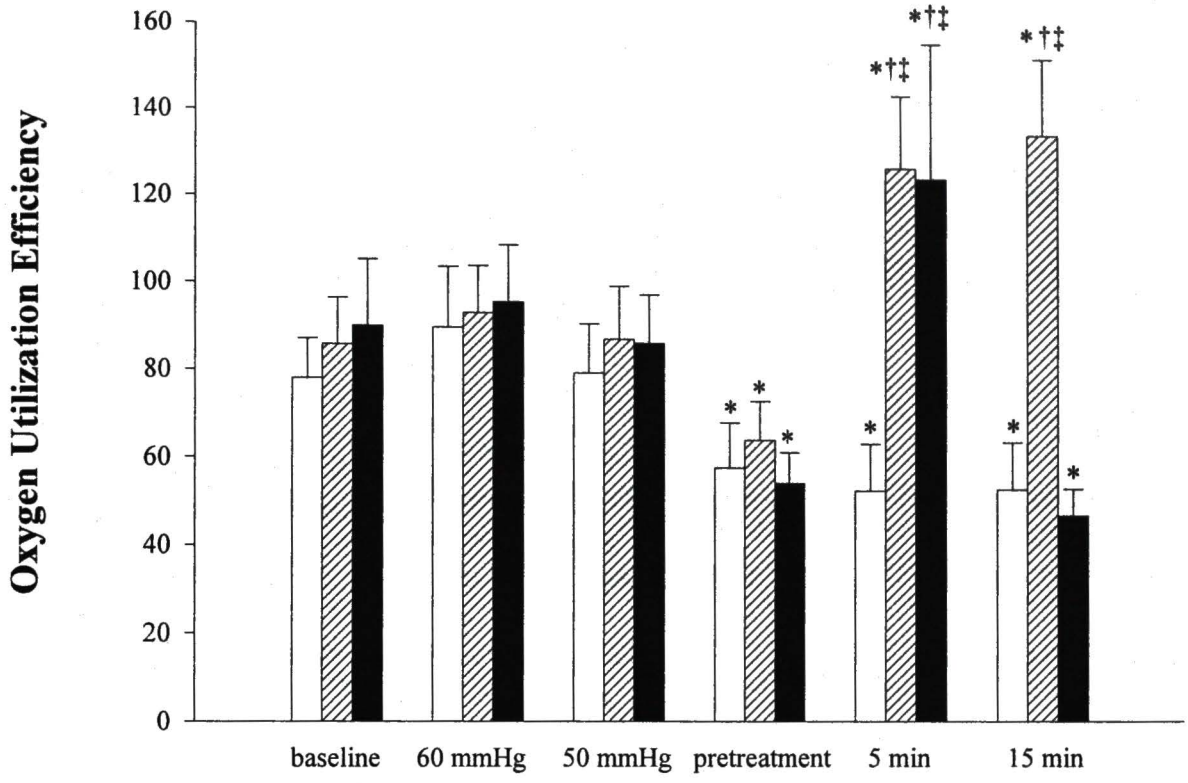


Figure 5. *Right ventricular myocardial oxygen utilization efficiency (O_2UE).* Treatment groups, time points, and significance symbols are the same as Figure 1.

REFERENCES

1. **Ahlquist, R. P.** Adrenergic receptors and others. Fourteenth Annual Becton, Dickinson and Company Oscar Schwidetzky Memorial Lecture. *Anesth Analg* 58: 510-515, 1979.

2. **Allen, D. G., C. G. Nichols, and G. L. Smith.** The effects of changes in muscle length during diastole on the calcium transient in ferret ventricular muscle. *J Physiol (Lond)* 406: 359-370, 1988.

3. **Arai, A. E., G. A. Pantely, C. G. Anselone, J. Bristow, and J. D. Bristow.** Active downregulation of myocardial energy requirements during prolonged moderate ischemia in swine. *Circ Res* 69: 1458-1469, 1991.

4. **Armiger, L. C., J. B. Gavin, and P. B. Herdson.** Mitochondrial changes in dog myocardium induced by neutral lactate in vitro. *Lab Invest* 31: 29-33, 1974.

5. **Baller, D., H. G. Wolpers, J. Zipfel, A. Hoeft, and G. Hellige.** Unfavorable effects of ventricular pacing on myocardial energetics. *Basic Res Cardiol* 76: 115-123, 1981.

6. **Bian, X., A. G. Williams, Jr., P. A. Gwirtz, and H. F. Downey.** Right coronary autoregulation in conscious, chronically instrumented dogs. *Am J Physiol* 275: H169-175, 1998.

7. **Braunwald, E.** On the difference between the heart's output and its contractile state. *Circulation* 43: 171-174, 1971.

8. **Brazy, P., and L. Mandel.** Does availability of inorganic phosphate regulate cellular oxidative metabolism? *News Physiol Sci* 1: 100-103, 1986.

9. **Chesnais, J. M., E. Coraboeuf, M. P. Sauviat, and J. M. Vassas.** Sensitivity to H, Li and Mg ions of the slow inward sodium current in frog atrial fibres. *J Mol Cell Cardiol* 7: 627-642, 1975.

10. **Cobbe, S. M., and P. A. Poole-Wilson.** The time of onset and severity of acidosis in myocardial ischaemia. *J Mol Cell Cardiol* 12: 745-760, 1980.

11. **Coetzee, W. A.** Channel-mediated calcium current in the heart. *Cardiovasc Drugs Ther* 1: 447-459, 1988.

12. **Cohen, D., P. Savard, R. D. Rifkin, E. Lepeschkin, and W. E. Strauss.** Magnetic measurement of S-T and T-Q segment shifts in humans. Part II: Exercise-induced S-T segment depression. *Circ Res* 53: 274-279, 1983.

13. **Dell'Italia, L. J., M. R. Starling, R. Blumhardt, J. C. Lasher, and R. A. O'Rourke.** Comparative effects of volume loading, dobutamine, and nitroprusside in patients with predominant right ventricular infarction. *Circulation* 72: 1327-1335, 1985.

14. **Francis, G. S.** Dobutamine in Chronic Congestive Heart Failure. In: *Dobutamine*, edited by K. Chatterjee. New York: NCM Publishers, Inc, 1989.

15. **Goodlett, M., K. Dowling, L. J. Eddy, and J. M. Downey.** Direct metabolic effects of isoproterenol and propranolol in ischemic myocardium of the dog. *Am J Physiol* 239: H469-H476, 1980.

16. **Greyson, C., J. Garcia, M. Mayr, and G. G. Schwartz.** Effects of inotropic stimulation on energy metabolism and systolic function of ischemic right ventricle. *Am J Physiol* 268: H1821-1828, 1995.

17. **Guth, B. D., J. F. Martin, G. Heusch, and J. Ross, Jr.** Regional myocardial blood flow, function and metabolism using phosphorus-31 nuclear magnetic resonance spectroscopy during ischemia and reperfusion in dogs. *J Am Coll Cardiol* 10: 673-681, 1987.

18. **Guth, B. D., J. A. Wisneski, R. A. Neese, F. C. White, G. Heusch, C. D. Mazer, and E. W. Gertz.** Myocardial lactate release during ischemia in swine. Relation to regional blood flow. *Circulation* 81: 1948-1958, 1990.

19. **He, M. X., S. Wang, and H. F. Downey.** Correlation between myocardial contractile force and cytosolic inorganic phosphate during early ischemia. *Am J Physiol* 272: H1333-341, 1997.

20. **Hearse, D. J.** Ischemia at the crossroads? *Cardiovasc Drugs Ther* 2: 9-15, 1988.

21. **Hearse, D. J.** Oxygen deprivation and early myocardial contractile failure: a reassessment of the possible role of adenosine triphosphate. *Am J Cardiol* 44: 1115-1121, 1979.

22. **Hearse, D. J., and D. M. Yellon.** Why are we still in doubt about infarct size limitation? The experimentalist's viewpoint. In: *Therapeutic approaches to myocardial infarct size limitation*, edited by D. J. Hearse, D.M. New York: Raven Press, 1984, p. 17-41.

23. **Hodgson, J. M., and G. B. Mancini.** Relation between graded, subcritical impairments of coronary flow reserve and regional myocardial dysfunction induced by atrial pacing in dogs. *J Am Coll Cardiol* 5: 1116-1124, 1985.

24. **Itoya, M., R. T. Mallet, Z. P. Gao, A. G. Williams, Jr., and H. F. Downey.** Stability of high-energy phosphates in right ventricle: myocardial energetics during right coronary hypotension. *Am J Physiol* 271: H320-328, 1996.

25. **Jacobus, W. E., I. H. Pores, S. K. Lucas, M. L. Weisfeldt, and J. T. Flaherty.** Intracellular acidosis and contractility in the normal and ischemic heart as examined by 31P NMR. *J Mol Cell Cardiol* 14 Suppl 3: 13-20, 1982.

26. **Kammermeier, H., P. Schmidt, and E. Jungling.** Free energy change of ATP-hydrolysis: a causal factor of early hypoxic failure of the myocardium? *J Mol Cell Cardiol* 14: 267-277, 1982.

27. **Karliner, J. S., and J. Ross, Jr.** Left ventricular performance after acute myocardial infarction. *Prog Cardiovasc Dis* 13: 374-391, 1971.

28. **Katz, A. M., and H. H. Hecht.** Editorial: the early "pump" failure of the ischemic heart. *Am J Med* 47: 497-502, 1969.

29. **Katzel, L. I., J. L. Fleg, M. J. Busby-Whitehead, J. D. Sorkin, L. C. Becker, E. G. Lakatta, and A. P. Goldberg.** Exercise-induced silent myocardial ischemia in master athletes. *Am J Cardiol* 81: 261-265, 1998.

30. **Kentish, J. C.** The effects of inorganic phosphate and creatine phosphate on force production in skinned muscles from rat ventricle. *J Physiol (Lond)* 370: 585-604, 1986.

31. **Kentish, J. C., and D. G. Allen.** Is force production in the myocardium directly dependent upon the free energy change of ATP hydrolysis? *J Mol Cell Cardiol* 18: 879-884, 1986.

32. **Kihara, Y., W. Grossman, and J. P. Morgan.** Direct measurement of changes in intracellular calcium transients during hypoxia, ischemia, and reperfusion of the intact mammalian heart. *Circ Res* 65: 1029-1044, 1989.

33. **Koretsune, Y., M. C. Corretti, H. Kusuoka, and E. Marban.** Mechanism of early ischemic contractile failure. Inexcitability, metabolite accumulation, or vascular collapse? *Circ Res* 68: 255-262, 1991.

34. **Kubler, W., and A. M. Katz.** Mechanism of early "pump" failure of the ischemic heart: possible role of adenosine triphosphate depletion and inorganic phosphate accumulation. *Am J Cardiol* 40: 467-471, 1977.

35. **Kusachi, S., O. Nishiyama, K. Yasuhara, D. Saito, S. Haraoka, and H. Nagashima.** Right and left ventricular oxygen metabolism in open-chest dogs. *Am J Physiol* 243: H761-H766, 1982.

36. **Kusuoka, H., M. L. Weisfeldt, J. L. Zweier, W. E. Jacobus, and E. Marban.** Mechanism of early contractile failure during hypoxia in intact ferret heart: evidence for modulation of maximal Ca^{2+}-activated force by inorganic phosphate. *Circ Res* 59: 270-282, 1986.

37. **Lawless, C. E., and H. S. Loeb.** Pharmacokinetics and pharmacodynamics of dobutamine. In: *Dobutamine: Ten year review.* New York: NCM Publishers, Inc., 1989, p. 33-47.

38. **Lee, J. A., and D. G. Allen.** Mechanisms of acute ischemic contractile failure of the heart. Role of intracellular calcium. *J Clin Invest* 88: 361-367, 1991.

39. **Lee, S. C., and H. F. Downey.** Downregulation of oxygen demand in isoprenaline stimulated canine myocardium. *Cardiovasc Res* 27: 1542-1550, 1993.

40. **Liedtke, A. J.** Alterations of carbohydrate and lipid metabolism in the acutely ischemic heart. *Prog Cardiovasc Dis* 23: 321-336, 1981.

41. **Liedtke, A. J., S. Nellis, and J. R. Neely.** Effects of excess free fatty acids on mechanical and metabolic function in normal and ischemic myocardium in swine. *Circ Res* 43: 652-661, 1978.

42. **Liedtke, A. J., S. H. Nellis, and O. D. Mjos.** Effects of reducing fatty acid metabolism on mechanical function in regionally ischemic hearts. *Am J Physiol* 247: H387-H394, 1984.

43. **Maekawa, K., M. Yokoyama, Y. Katada, Y. Ishikawa, K. Fujiwara, T. Mizutani, and H. Fukuzaki.** A study on myocardial ischemia in the left ventricular wall with special reference to electrographic and metabolic changes. *Jpn Heart J* 21: 215-224, 1980.

44. **Marcus, M.** Difference in the regulation of coronary perfusion to the right and left ventricles. In: *The Coronary Circulation in Health and Disease*, edited by McGraw-Hill. New York:, 1983, p. 93-122.

45. **Marshall, R. C., W. W. Nash, M. M. Bersohn, and G. A. Wong.** Myocardial energy production and consumption remain balanced during positive inotropic stimulation when coronary flow is restricted to basal rates in rabbit heart. *J Clin Invest* 80: 1165-1171, 1987.

46. **Maseri, A.** *Ischemic heart disease : a rational basis for clinical practise and clinical research*. New York: Churchill Livingstone, 1995.

47. **Masuyama, T., M. Uematsu, Y. Doi, K. Yamamoto, T. Mano, J. Naito, H. Kondo, R. Nagano, M. Hori, and T. Kamada.** Abnormal coronary flow dynamics at rest and during tachycardia associated with impaired left ventricular relaxation in humans: implication for tachycardia-induced myocardial ischemia. *J Am Coll Cardiol* 24: 1625-1632, 1994.

48. **Messerli, F. H., D. Aristizabal, and F. Soria.** Reduction of left ventricular hypertrophy: how beneficial? *Am Heart J* 125: 1520-1524, 1993.

49. **Miyamae, M., S. A. Camacho, W. D. Rooney, G. Modin, H. Z. Zhou, M. W. Weiner, and V. M. Figueredo.** Inorganic phosphate and coronary perfusion pressure mediate contractile dysfunction during mild ischemia. *Am J Physiol* 273: H566-H572, 1997.

50. **Neely JR, M. H.** Relationship between carbohydrate and lipid metabolism and energy balance of heart muscle. *Ann Rev Physiol* 36: 413-459, 1974.

51. **Opie, L. H.** *The heart : physiology, from cell to circulation.* Philadelphia: Lippincott-Raven, 1998.

52. **Opie, L. H., and O. L. Bricknell.** Role of glycolytic flux in effect of glucose in decreasing fatty-acid- induced release of lactate dehydrogenase from isolated coronary ligated rat heart. *Cardiovasc Res* 13: 693-702, 1979.

53. **Pantely, G. A., S. A. Malone, W. S. Rhen, C. G. Anselone, A. Arai, J. Bristow, and J. D. Bristow.** Regeneration of myocardial phosphocreatine in pigs despite continued moderate ischemia. *Circ Res* 67: 1481-1493, 1990.

54. **Rahimtoola, S. H.** The hibernating myocardium. *Am Heart J* 117: 211-221, 1989.

55. **Rauch, U., K. Schulze, B. Witzenbichler, and H. P. Schultheiss.** Alteration of the cytosolic-mitochondrial distribution of high-energy phosphates during global myocardial ischemia may contribute to early contractile failure. *Circ Res* 75: 760-769, 1994.

56. **Reimer, K. A., J. E. Lowe, M. M. Rasmussen, and R. B. Jennings.** The wavefront phenomenon of ischemic cell death. 1. Myocardial infarct size vs duration of coronary occlusion in dogs. *Circulation* 56: 786-794, 1977.

57. **Rooke, G. A., and E. O. Feigl.** Work as a correlate of canine left ventricular oxygen consumption, and the problem of catecholamine oxygen wasting. *Circ Res* 50: 273-286, 1982.

58. **Rovetto, M. J., W. F. Lamberton, and J. R. Neely.** Mechanisms of glycolytic inhibition in ischemic rat hearts. *Circ Res* 37: 742-751, 1975.

59. **Ruffolo, R. R., Jr.** The pharmacology of dobutamine. *Am J Med Sci* 294: 244-248, 1987.

60. **Ruffolo, R. R., Jr., T. A. Spradlin, G. D. Pollock, J. E. Waddell, and P. J. Murphy.** Alpha and beta adrenergic effects of the stereoisomers of dobutamine. *J Pharmacol Exp Ther* 219: 447-452, 1981.

61. **Saito, D., T. Shiraki, K. Inoue, A. Kajiyama, S. Takemoto, S. Hori, T. Takamura, and H. Kono.** Reduced vasodilator response of the right coronary artery to myocardial ischemia in the hypertrophied right ventricle. *Jpn Circ J* 60: 247-253, 1996.

62. **Saupe, K. W., F. R. Eberli, J. S. Ingwall, and C. S. Apstein.** Hypoperfusion-induced contractile failure does not require changes in cardiac energetics. *Am J Physiol* 276: H1715-H723, 1999.

63. **Schmidt-Ott, S. C., C. Bletz, C. Vahl, W. Saggau, S. Hagl, and J. C. Ruegg.** Inorganic phosphate inhibits contractility and ATPase activity in skinned fibers from human myocardium. *Basic Res Cardiol* 85: 358-366, 1990.

64. **Schulz, R., B. D. Guth, K. Pieper, C. Martin, and G. Heusch.** Recruitment of an inotropic reserve in moderately ischemic myocardium at the expense of metabolic recovery. A model of short-term hibernation. *Circ Res* 70: 1282-1295, 1992.

65. **Singh, S., F. C. White, and C. M. Bloor.** Myocardial morphometric characteristics in swine. *Circ Res* 49: 434-441, 1981.

66. **Sonnenblick, E. H., W. H. Frishman, and T. H. LeJemtel.** Dobutamine: a new synthetic cardioactive sympathetic amine. *N Engl J Med* 300: 17-22, 1979.

67. **Stanley, W. C., J. L. Hall, C. K. Stone, and T. A. Hacker.** Acute myocardial ischemia causes a transmural gradient in glucose extraction but not glucose uptake. *Am J Physiol* 262: H91-H96, 1992.

68. **Tennant, R.** Factors concerned in the arrest of contraction in an ischemic myocardial area. *Am J Physiol* 113: 677-682, 1935.

69. **Watanabe, I., T. A. Johnson, J. Buchanan, C. L. Engle, and L. S. Gettes.** Effect of graded coronary flow reduction on ionic, electrical, and mechanical indexes of ischemia in the pig. *Circulation* 76: 1127-1134, 1987.

70. **Watanabe, T., Y. Akutsu, H. Yamanaka, T. Michihata, O. Okazaki, T. Katagiri, and K. Harumi.** Exercise-induced ST-segment depression: imbalance between myocardial oxygen demand and myocardial blood flow. *Acta Cardiol* 55: 25-31, 2000.

71. **Wissner, S. B.** The effect of excess lactate upon the excitability of the sheep Purkinje fiber. *J Electrocardiol* 7: 17-26, 1974.

72. **Xiang, J. Z., and J. C. Kentish.** Effects of inorganic phosphate and ADP on calcium handling by the sarcoplasmic reticulum in rat skinned cardiac muscles. *Cardiovasc Res* 29: 391-400, 1995.

73. **Yanagi, S., K. Takeuchi, T. Takeda, M. Ishikawa, and I. Miura.** Effects of inotropic stimulation on phosphate compounds in ischaemic canine hearts. *Cardiovasc Res* 27: 1435-1443, 1993.

74. **Yonekura, S., N. Watanabe, and H. F. Downey.** Transmural variation in autoregulation of right ventricular blood flow. *Circ Res* 62: 776-781, 1988.