# Myocardial Energy Production and Consumption Remain Balanced during Positive Inotropic Stimulation When Coronary Flow Is Restricted to Basal Rates in Rabbit Heart

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## Abstract

The effect on myocardial energy balance of increasing oxygen demand without altering basal myocardial perfusion rate was assessed in isolated, isovolumic, retrograde blood perfused rabbit hearts. Myocardial energy requirements were increased with paired stimulation. The capacity of rapid paired stimulation to increase mechanical energy consumption was demonstrated in the presence of increased perfusion with the rate  $\times$  pressure product and oxygen consumption increasing 86 and 148%, respectively, compared with control values. In contrast, rapid paired stimulation under constant, basal flow conditions did not alter the rate  $\times$  pressure product, while oxygen extraction and consumption increased only 40% relative to control. Myocardial ATP, creatine-phosphate, and lactate content were identical under control and constant flow-paired stimulation conditions. The results of this study indicate that no detectable energy imbalance was produced by rapid paired stimulation with flow held constant at basal rates. These results suggest that the myocardium does not increase mechanical energy expenditure in response to inotropic or rate stimulation in the presence of restricted flow reserve and are inconsistent with the concept of "demand-induced" or "relative" myocardial ischemia.

## Introduction

Stimuli that increase oxygen demand have been shown to have a detrimental effect on ischemic myocardium as evidenced by a decline in ischemic and postischemic contractile performance, accelerated lactate production and depletion of myocardial high energy phosphate stores (1–7). However, all of these studies were performed under conditions in which variable degrees of flow reduction were produced concomitantly with increased oxygen demand. In the presence of critically impaired oxygen delivery and cellular washout, increasing oxygen requirements might have a more adverse effect on the balance between energy production and consumption than in the absence of diminished myocardial blood flow. At present, little information is available on the ability of increased oxygen demand to alter myocardial function and energy metabolism in the absence of reduced perfusion.

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This study assessed the effect of inotropic stimulation on the balance between oxidative energy production and mechanical energy consumption under constant, basal flow conditions. Paired stimulation was used to increase myocardial inotropy (8, 9). The experimental preparation was an isolated, isovolumically contracting retrograde blood perfused rabbit heart. Myocardial high energy phosphate content, oxygen consumption, lactate metabolism, developed pressure and the rate × pressure product were measured to evaluate steady-state myocardial energy balance during basal flow-paired stimulation (CF-PS).<sup>1</sup> The data obtained were compared to that observed during paired stimulation with increased perfusion (IF-PS) and during single and paired stimulation with reduced myocardial blood flow. The results of this study indicate that no detectable myocardial energy imbalance<sup>2</sup> was produced with inotropic stimulation of myocardial oxygen demand in the absence of diminished perfusion.

## **Methods**

Perfusate composition. Hearts were perfused with a modified Tyrode's solution containing 20% oxygenated bovine red cells and 15 g/liter bovine serum albumin (free fatty acid free, Sigma Chemical Co., St. Louis, MO). The specific electrolyte concentrations were (in mM): NaCl, 110.0; CaCl<sub>2</sub>, 2.5; KCl, 6.0; MgCl<sub>2</sub>, 1.0; NaH<sub>2</sub>PO<sub>4</sub>, 0.435; NaHCO<sub>3</sub>, 32.0. Bovine serum albumin was prepared by overnight dialysis at 4°C against a large volume of buffer and filtration through a 0.8- $\mu$ m Millipore filter. Dextrose, 11 mM, in the presence of 2.5 mU/ml insulin was provided as substrate. Red cell glycolysis produced an average arterial lactate concentration of 0.31 mM (range, 0.11-0.59 mM).

Bovine erythrocytes were used because of the relative independence of bovine oxyhemoglobin dissociation from 2,3-diphosphoglycerate concentration (10, 11). Fresh cow blood was collected at a local slaughterhouse in polyethylene bottles with sufficient sodium heparin for anticoagulation. The blood was transported on ice and immediately centrifuged at 4°C for 20 min at 2,600 g. After aspirating the plasma and buffy coat, the cells were washed three times and stored in calcium-free buffer. All cells were washed daily and used within 5 d of harvesting. Red cells were oxygenated by washing five times in ice-cold perfusate that had been equilibrated with 100% oxygen. Final perfusate pH was adjusted to between 7.30 and 7.40 by equilibrating the buffer in which the red cells were suspended for myocardial perfusate was placed on ice and a mixture of 100% O<sub>2</sub> and 98%/2% O<sub>2</sub>/CO<sub>2</sub> was blown over the surface of the suspended red cells in sufficient quantity

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<sup>1.</sup> Abbreviations used in this paper: CF-PS, constant flow-paired stimulation; IF-PS, increased flow-paired stimulation; PCA, perchloric acid; RFI, reduced flow ischemia.

<sup>2.</sup> As employed in this study, the term "energy imbalance" refers to the potential dissociation of mechanical energy consumption from oxidative energy production and consequent high energy phosphate depletion during constant flow-paired stimulation.

to maintain oxygen content and pH constant over the 60-min experimental times employed in this study.

Before entry into the heart, the red blood cell containing perfusate was heated to 37°C using a water jacketed heating coil and bubble trap. An in-line Swank transfusion filter (13  $\mu$ m exclusion; model IL 200, Pioneer Viggo, Inc., Beaverton, OR) was used to filter red cell aggregates. This blood perfusion technique resulted in an average myocardial oxygen delivery under nonischemic, control conditions of 7.74±0.22  $\mu$ mol/g wet wt min<sup>-1</sup>.

*Experimental preparation.* After administration of 10 mg of sodium heparin and 200 mg sodium pentobarbital via an ear vein, hearts from nonfasted, male New Zealand white rabbits (2.0–2.5 kg) were excised through a medium sternotomy and arrested in ice-cold saline. The aorta was rapidly cannulated to allow retrograde perfusion with red blood cell containing perfusate. Flow was held constant at  $\sim$  2.0 ml/g wet wt min<sup>-1</sup> using a peristaltic pump (Gilson Minipuls 2; Gilson Co., Inc., Worthington, OH). The red blood cell containing perfusate was not recirculated.

Preparation of isovolumically beating hearts was similar to that previously reported by other investigators (10, 12-14). Briefly, an apical drain was inserted through the left atrium into the left ventricle to allow drainage of fluid from the Thebesian circulation. After the atrioventricular node was crushed to allow controlled stimulation, a fluidfilled latex balloon connected to a pressure transducer (Gould-Statham P23ID, Gould Inc., Oxnard, CA) was inserted into the left ventricle via the left atrium and mitral valve. A coronary venous sampling catheter and needle thermistor (Bailey Instruments Co., Inc., Saddle Brook, NJ) were inserted into the right ventricle via the right atrium. The venae cavae and pulmonary artery were then ligated so that all coronary venous drainage flowed out the sampling catheter without exposure to the atmosphere. Stimulating electrodes from a stimulator (Grass SD-44, Grass Instrument Co., Quincy, MA) were placed against the left and right ventricles and 4 V, 4 ms stimuli were delivered at a rate of 180/min.

The volume of the left ventricular balloon was adjusted until enddiastolic pressure was between 2 and 5 mm/Hg (mean EDP = 4 mmHg, mean vol =  $2.7\pm0.1$  ml). End-diastolic pressure remained constant throughout each experiment except in some hearts with increased pressure development where it was sometimes necessary to add 0.1-0.2 ml to the balloon volume to keep EDP from declining. Since total balloon volume did not change by > 10% and EDP remained constant, loading conditions for individual hearts remained essentially constant throughout each experiment.

After the preparation was complete, the isolated heart was placed in a water jacketed container to help maintain coronary venous temperature at 37°C as measured by the needle thermistor in the right ventricle. An equilibration period of 15 min preceded any experimental intervention. A preparation was accepted as suitable for study if it developed at least 80 mm of Hg pressure (peak systolic pressure minus end-diastolic pressure) at an end-diastolic pressure  $\leq 5$  mmHg, and if end-diastolic and systolic pressure were stable over the equilibration period.

Control conditions were  $181\pm1.0$  single stimuli/min and a flow of  $2.11\pm0.1$  ml/g wet wt min<sup>-1</sup>. Paired stimulation of myocardial contractility (8, 9) was chosen over pharmacologic methods because of the absence of known direct effects on metabolism, vasoactivity or myocardial toxicity (15–17). The paired stimulus coupling interval was held constant at between 100 and 120 ms such that a single functional contraction resulted from each pair of stimuli. The constant coupling interval also produced a relatively uniform level of inotropic stimulation (9) during both increased flow and constant flow-paired stimulation experiments. The maximal paired stimulus frequency evaluated (~ 240 paired stimuli/min) represents near maximal stimulation since faster paired stimulus rates frequently produced ventricular fibrillation.

Data collection and analytical procedures. All reported measurements were taken at least 15 min after each experimental intervention to allow time for an apparent steady state to be reached. In experiments evaluating the effect of progressive changes in paired stimulus and/or perfusion rates on mechanical function and oxidative metabolism, each heart served as its own control with serial measurements taken at the stimulus and flow rates indicated. In hearts used for analysis of myocardial high energy phosphate and lactate content, the paired stimulus rate was maintained constant during CF-PS at 240/min. Similarly, flow was reduced and held constant at ~ 0.4 ml/g wet wt min<sup>-1</sup> during reduced flow ischemia.

Isovolumic developed pressure recorded continuously from the fluid-filled ventricular balloon on a recorder (HP-7404A, Hewlett Packard Co., Palo Alto, CA) was used to assess myocardial function. An estimate of mechanical energy expenditure was obtained from the stimulus rate  $\times$  peak developed pressure product. Arterial and venous lactate and pyruvate concentrations were determined spectrophotometrically using the lactate dehydrogense procedure (18) while the corresponding oxygen concentrations were measured directly with an oxygen analyzer (Lex O<sub>2</sub> Con; Lexington Instruments, Waltham, MA). All data used to estimate metabolite consumption were acquired in duplicate.

Metabolic rates for oxygen and lactate were computed from measured arterial and venous concentration differences and the predetermined flow rate while oxygen extraction fraction was directly analyzed from arterial and venous concentrations. Arterial samples were taken from a side-arm placed just above the aortic cannula. Arterial and venous samples were placed on ice until the end of the experiment and then centrifuged to separate "plasma" from red cells. After addition of 0.5 ml of 6% perchloric acid (PCA) to 1.0 ml of perfusate, lactate and pyruvate "plasma" concentrations were determined on the same experimental day they were acquired.

Hearts used for analysis of tissue metabolites were smash-frozen with Wollenberger clamps cooled to the temperature of liquid nitrogen. Hearts were extracted with 6% PCA and neutralized with 5 M  $K_2CO_3$ . Myocardial ATP and creatine phosphate content were measured using the glucose-6-PO<sub>4</sub> dehydrogenase enzyme assay and tissue lactate was estimated with the lactate dehydrogenase procedure (18).

Data are expressed as the mean $\pm$ SE. Statistical analysis of functional and metabolic rate data acquired sequentially in individual hearts was performed using an analysis of variance with repeated measures, while comparisons between experimental groups were performed using an analysis of variance. Data expressed as percent change were analyzed using the Wilcoxon signed-rank test. The significance of measured changes in tissue ATP, creatine phosphate, and lactate content was assessed with a multiple comparisons analysis. A *P* value < 0.05 was considered significant.

# Results

The data illustrated in Fig. 1 are from experiments evaluating the functional and metabolic stability of the retrograde blood perfused rabbit heart during 60 min of control stimulation and perfusion. Oxygen consumption and extraction (a), lactate consumption and the lactate/pyruvate ratio (b), and developed pressure and the rate times pressure product (c) were all within the range previously reported by other investigators employing isolated blood perfused hearts (10, 12–14, 19). Stability of the current preparation was indicated by statistical analysis of this data that did not reveal any significant time-related simple trends in any of the physiologic parameters assessed.

Fig. 2 illustrates data from hearts in which oxygen demand was progressively raised by graded increases in the paired stimulus rate while holding flow constant at  $2.06\pm0.09$  ml/g wet wt min<sup>-1</sup> (CF-PS). Oxygen consumption and extraction fraction increased to ~ 40% greater than control at a paired stimulus frequency of 164/min and then exhibited a plateau as the paired stimulus rate was increased further to 242 paired stimuli/min (*a*, *P* < 0.05 vs. control). Lactate production and the venous lactate/pyruvate ratio tended to increase minimally at



Figure 1. Stability of blood perfused Langendorff. Evaluation of oxygen consumption and extraction fraction (a), lactate consumption and venous lactate/pyruvate ratio (b) and developed pressure and the rate  $\times$  pressure product (c) during control conditions. Measurements were taken every 15 min for a total of 60 min. In this and subsequent illustrations, lactate extraction is denoted with a + and lactate production with a -. For discussion, see text. Data are mean±SE for six hearts.

faster paired stimulus rates (b, P < 0.05 vs. control), indicating only a small increase in anaerobic glycolysis during CF-PS. Developed pressure initially increased above control values at the slowest paired stimulus rate evaluated and then declined at faster paired stimulus frequencies (c, P < 0.05 vs. control). The virtually linear decline in developed pressure with increasing paired stimulus frequencies reflected stability in the rate  $\times$  pressure product (P > 0.05 vs. control) despite variation in the rate of paired stimulation from 124 to 242/min.

One potential explanation for the inability to alter the rate  $\times$  pressure product with increasing paired stimulus frequencies at constant flow is that rapid electrical stimulation might have a deleterious effect on ventricular function independent of limitations in oxygen supply. To evaluate this possibility, developed pressure, the rate times pressure product and oxygen and lactate consumption were assessed as the paired stimulus rate was raised from 160 to 240/min in the presence of parallel increases in myocardial perfusion (IF-PS, Table I).



Figure 2. Oxidative metabolism and ventricular function during CF-PS. Effect on oxygen consumption and extraction (a), lactate metabolism (b) and developed pressure and the rate  $\times$  pressure product (c) of progressive increase in paired stimulus rate holding flow constant. Time-sequence of data acquisition is similar to Fig. 1 except that a preliminary control measurement was taken at the end of the equilibration period. Statistical analysis of preischemic control values did not reveal any significant differences between the data presented here vs. Fig. 1. Data are mean±SE for seven hearts. For discussion, see text. \*P < 0.05 vs. control.

At faster paired stimulus rates, isovolumic pressure development declined slightly despite the increase in perfusion. However, at 240 paired stimuli/min, developed pressure, the rate  $\times$  pressure product and oxygen consumption were 41, 86, and 148% greater than corresponding control values, respectively. Lactate consumption also increased by 55% while oxygen extraction decreased minimally. Comparing these results to those obtained at 240 paired stimuli/min in the absence of increased perfusion (Fig. 2), pressure development, the rate × pressure product and oxygen consumption were all significantly greater during IF-PS (P < 0.01 for all comparisons). In addition, the increases in the rate  $\times$  pressure product and oxygen consumption during IF-PS are comparable to elevations in hemodynamic indices of global oxygen consumption and nonischemic myocardial blood flow reported in previous studies evaluating the effect of exercise, pacing-induced tachycardia and catecholamine infusion on regionally ischemic myocardial function (1, 2, 6, 7, 20).

Table I. Increased Paired Stimulation Frequency with Increased Myocardial Perfusion

	Control (180 SS/min)	160 PS/min	200 PS/min	240 PS/min
Oxygen consumption,				
µmol/g wet wt min <sup>-1</sup>	3.31±0.10	5.59±0.30	6.97±0.31	8.23±0.51*
Oxygen extraction fraction	0.45±0.02	0.42±0.02	0.42±0.03	0.41±0.03*
Lactate metabolism,				
$\mu$ mol/g wet wt min <sup>-1</sup>	+0.11±0.03	+0.12±0.03	+0.16±0.05	+0.17±0.08*
Developed pressure, mmHg	103±2.0	164±6.1	154±3.2	145±4.0*
Rate $\times$ pressure product,				
mmHg $ imes$ beats/min $ imes$ 10 <sup>-3</sup>	18.8±0.3	26.7±1.0	30.8±0.8	35.0±0.9*
Flow rates, $ml/g$ wet wt $min^{-1}$	1.9±0.15	3.3±0.20	4.1±0.21	5.1±0.33*

Data evaluating the effect of rapid electrical stimulation on myocardial performance and lactate and oxygen metabolism in the presence of increased myocardial blood flow. Time-sequence of data acquisition was similar to that employed in Fig. 2. Data are mean $\pm$ SE for five hearts. \* P < 0.01 vs. CF-PS (Fig. 2).

To evaluate the independent contribution of increased perfusion (21) to the improved mechanical performance observed during IF-PS, developed pressure, the rate  $\times$  pressure product, oxygen consumption and oxygen extraction were assessed during graded increases in myocardial blood flow under control stimulation conditions (180 single stimuli/min) (Fig. 3). At the fastest perfusion rate evaluated (5.0±0.35 ml/g wet wt min<sup>-1</sup>), oxygen consumption (a), developed pressure (c) and the rate  $\times$  pressure product (d) increased 37, 21, and 21% compared with basal flow values, respectively. The extraction fraction for oxygen declined 50% (b). Comparing these results



Figure 3. Comparison of the effect of increasing flow during paired stimulation vs. single stimulation. Data are expressed as percent changed from pre-experimental control values that were statistically identical. Relative changes in oxygen consumption are illustrated in a, oxygen extraction fraction in b, developed pressure in c and the rate  $\times$  pressure product in d. Data obtained during IF-PS same as Table I. Data are mean±SEM for five hearts. \*P < 0.05.

to those obtained during IF-PS, the percent increases in developed pressure, the rate  $\times$  pressure product and oxygen consumption were considerably greater with paired stimulation, while the percent decline in oxygen extraction fraction was significantly attenuated. These observations indicate that the increase in the rate  $\times$  pressure product observed during IF-PS was primarily a consequence of rapid paired stimulation and not accelerated blood flow. These results also indicate that rapid electrical pacing alone was not responsible for the inability to alter the rate  $\times$  pressure product during CF-PS.

To allow evaluation of the response of the current preparation to myocardial ischemia, isolated blood perfused rabbit hearts were subjected to progressive reductions in myocardial blood flow (Fig. 4). Oxygen consumption (a), developed pressure and the rate  $\times$  pressure product (c) declined as the severity of flow restriction was increased despite an increase in oxygen extraction fraction. Lactate production became pronounced and the venous lactate/pyruvate ratio increased (b). These results are consistent with previously published data obtained in several different preparations during reduced oxygen delivery (5, 6, 13, 22, 23) and indicate the comparability of the current isolated blood perfused rabbit heart to previously employed experimental preparations.

Fig. 5 illustrates myocardial ATP, creatine-phosphate and lactate content after 15 and 60 min under constant flow-paired stimulation, control, increased flow-paired stimulation and reduced flow ischemic conditions. During control and IF-PS, whole tissue ATP, creatine-phosphate and lactate content were statistically identical at 15 and 60 min and comparable to previously reported values observed in isolated buffer perfused and in situ blood perfused hearts (3, 5). During CF-PS at both time periods, no significant depletion of myocardial ATP and creatine-phosphate content or lactate accumulation was observed compared with either control or IF-PS. In contrast, in the presence of an 80% flow restriction, significant myocardial high energy phosphate depletion and lactate accumulation were observed.

Prior investigations demonstrating a detrimental effect of increased oxygen demand on myocardial high energy phosphate stores during critically impaired myocardial perfusion did not employ paired stimuli to increase myocardial energy requirements. Therefore, the ability of paired stimulation to alter myocardial ATP, creatine-phosphate and lactate content was assessed during reduced flow ischemia (Table II). In con-



Figure 4. Oxidative metabolism and ventricular function during reduced flow ischemia. The effect on oxygen consumption and extraction fraction (a), lactate metabolism (b) and developed pressure and the rate  $\times$  pressure product (c) of progressive reductions in myocardial perfusion during control stimulation. Format and time-sequence of data acquisition are identical to Fig. 2. Data are mean±SEM for six hearts. \*P < 0.05 vs. control.

trast to the results obtained during unaltered basal perfusion, there was an accelerated depletion of myocardial ATP and creatine-phosphate content and an insignificant tendency for lactate to accumulate during inotropic stimulation of oxygen demand in the presence of reduced myocardial blood flow.

### Discussion

The inability to alter the rate  $\times$  pressure product, the maintained oxygen consumption and normal myocardial high energy phosphate stores indicate that no myocardial energy imbalance was detected during constant flow-rapid paired stimulation. The apparent balance between energy production and consumption during CF-PS was observed despite evidence that rapid paired stimulation was capable of increasing energy utilization during IF-PS to levels previously observed in studies evaluating the effect of altered oxygen demand on ischemic



Figure 5. Myocardial high energy phosphate and lactate content. Comparison of myocardial ATP (a), creatine phosphate (b) and lactate (c) content under control, IF-PS, CF-PS, and reduced flow ischemic conditions. Paired stimulus rate during IF-PS and CF-PS was 240/min. Myocardial metabolite content was determined after 15 and 60 min of experimental elapsed time. Failure of substantial increase in myocardial lactate content at 15 min during reduced flow ischemia to achieve statistical significance was due to variability between preparations at this time period. Data are mean±SE for four to seven hearts. Cr-P, creatine phosphate; C,

control; RFI, reduced flow ischemia. \*P < 0.005, x, P < 0.01.

myocardial function and metabolism (1, 2, 6, 7, 20). These observations imply that the myocardium does not increase mechanical energy expenditure in response to inotropic or rate stimulation in the presence of inadequate flow reserve. These results also suggest that production of myocardial ischemia by increasing oxygen demand might depend primarily on the concomitant maldistribution of transmural myocardial blood flow previously observed in other studies evaluating "demand-induced" or "relative" myocardial ischemia (1, 20).

Table II. Effect of Increased Oxygen Demand on Myocardial High Energy Phosphate and Lactate Content during Reduced Myocardial Perfusion

				T
		ATP	CrP	Lactate
15 min	RFI	16.4±0.8	29.8±2.9	24.5±4.6
	RFI + PS	11.4±0.7*	16.2±2.9*	41.7±4.2
45 min	RFI	14.0±0.9	26.0±3.5	42.5±5.7
	RFI + PS	10.1±0.8*	14.6±0.8*	57.3±3.0

Effect of paired stimulation on myocardial ATP, creatinine-phosphate and lactate content during reduced flow ischemia. Myocardial blood flow averaged  $0.41\pm0.07$  and  $0.42\pm0.12$  ml/g wet wt min<sup>-1</sup> during reduced flow ischemia and reduced flow ischemia plus paired stimulation, respectively. Evaluation of the effect of paired stimulation on myocardial high energy phosphate and lactate content during reduced blood flow was performed at a paired stimulus frequency of 180/min, with a 145–150-ms delay for a maximum of 45 min because of electrical instability of ischemic hearts during rapid stimulation. Data are mean±SE of four to five hearts. CrP, creatine phosphate; RFI, reduced flow ischemia; RFI + PS, reduced flow ischemia + paired stimulation. \* P < 0.01.

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During CF-PS, the rate  $\times$  pressure product remained constant as the paired stimulus rate was increased because of an absolute fall in isovolumic developed pressure. This decline in mechanical performance was observed despite the presence of normal myocardial high energy phosphate stores, maintained myocardial perfusion, absent lactate accumulation and continued oxidative energy production. These results are quite different from those observed after sudden cessation of myocardial perfusion in which contractile performance declines in the presence of high energy phosphate depletion, diminished wash-out of metabolic end-products and lactate accumulation as well as severe reduction in oxidative energy production. Since these latter metabolic abnormalities have been thought to contribute to contractile dysfunction during myocardial ischemia, their absence suggests the need to consider some other mechanism to explain the decrease in isovolumic developed pressure during CF-PS. The most obvious explanation for these observations is that rapid paired stimulation under basal flow conditions might have depleted a strategically placed ATP microcompartment regulating contractile protein function and/or calcium metabolism (4, 24). However, the current data do not exclude the existence of other regulatory mechanism(s) and additional information is needed to characterize the nature of the physiologic signal relating flow-restricted mechanical energy consumption and energy production.

The current results are consistent with a previously published report by Gallagher and co-workers evaluating the relationship between regional ventricular function and blood flow at rest and during exercise (20). According to their results, increasing oxygen demand with exercise did not appear to affect ischemic ventricular performance independent of associated reductions in myocardial blood flow: the negative effect of exercise on ischemic ventricular function could be explained by a secondary reduction in myocardial perfusion during stress-provoked tachycardia and possibly altered diastolic function. Based on the similarity of the function-flow relationship at rest and during exercise, these investigators postulated that the myocardium down-regulates energy expenditure under oxygen-restricted conditions and that relative or demand-induced ischemia might not exist except during the initial seconds of altered myocardial energy requirements. In the current report, the inability to detect a significant energy imbalance during CF-PS supports and extends this contention and, in addition, demonstrates that the explanation for the observed down regulation of mechanical energy consumption does not involve whole tissue high energy phosphate depletion or lactate accumulation.

In contrast to CF-PS, increasing oxygen demand in the presence of reduced myocardial blood flow accelerated the decline in myocardial ATP and creatine phosphate content. During diminished myocardial perfusion, the buildup of toxic metabolic end-products, the increased relative percentage of non-contractile energy consumption and/or altered calcium homeostasis might adversely affect the myocardium's response to stimuli that increase energy requirements.

The current myocardial oxygen extraction fraction of  $\sim 0.4$  under control conditions is lower than the 0.65–0.75 extraction fraction reported in basal humans (25). It is unlikely that this low oxygen extraction could have accounted for the maintenance of a normal myocardial energy balance during CF-PS. The 40% increase in oxygen consumption due to al-

tered extraction during CF-PS was much smaller than the 148% increase in oxygen consumption due to accelerated delivery during IF-PS. Furthermore, the current values for oxygen extraction fraction are comparable to those reported for other isolated blood perfused heart preparations in which myocardial oxygen extraction ranged from 0.20 to 0.45 (10, 12-14, 19).

In this study, the peak systolic pressure  $\times$  stimulus rate product was used to estimate the isovolumic pressure-related cardiac workload. Although a well-defined hemodynamic index of mechanical energy consumption (26), potential problems may arise when comparing rate × pressure products during altered inotropic states or with different contraction durations. The observation that the rate  $\times$  pressure product increased during IF-PS but remained unaltered during CF-PS is presumably unaffected by this potential inaccuracy since myocardial inotropy and contraction duration were changed in an identical fashion. However, potential differences in mechanical workload during control single stimulation and CF-PS might be underestimated by the rate  $\times$  pressure product. The substantially greater rise in oxygen consumption than rate × pressure product when comparing values obtained during paired stimulation to control probably reflects this underestimation.

In contrast to the results of the current investigation, several previous studies have demonstrated a decline in myocardial ATP and creatine phosphate content during increased cardiac work in the presence of unrestricted myocardial perfusion (27, 28). In part, these disparate results might be explained by the use of different experimental preparations: previous investigations evaluating work-related changes in myocardial high energy phosphate stores employed the buffer perfused working rat heart. However, inadequate substrate delivery also appeared to play an important role in the previously observed depletion of myocardial high energy phosphate stores since the more marked changes in ATP and creatine phosphate content occurred when glucose was provided as the sole substrate. In the present study, myocardial substrate delivery might have been improved by inclusion of insulin in the perfusate.

During CF-PS, the modest lactate production observed in the presence of unaltered tissue lactate content presumably reflects accelerated lactate synthesis and/or membrane transport under conditions of adequate cellular washout. The acceleration of lactate production during CF-PS in the blood perfused Langendorff preparation was much less marked than previously reported by us in the isolated arterially perfused rabbit septum (22). This disparity might be accounted for by the considerable differences in basal aerobic and anaerobic metabolic rates in these two experimental models: control oxygen consumption and glucose oxidation (unpublished data) are increased approximately fourfold and lactate production is markedly reduced in the blood perfused Langendorff preparation compared with that observed in the septum.

The inability to dissociate energy consumption and production during CF-PS suggests that stress-provoked myocardial ischemia in chronic coronary artery disease patients with normal basal blood flow might reflect primarily reduced diastolic myocardial blood flow and not demand-induced oxygen supply demand imbalance. The previously observed maldistribution of myocardial blood flow that occurs during stressrelated tachycardia in experimental animals with partial coronary artery obstruction provides additional support for this possibility (1, 20).

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