Increased Oxygen Cost of Contractility in Stunned Myocardium of Dog

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Recent studies have shown that myocardial oxygen consumption does not proportionally decrease with the deterioration of contractile function in stunned myocardium. To investigate this disproportion, we studied the end-systolic pressure–volume relation and the relation between oxygen consumption per beat (\( \dot{V}O_2 \)) and systolic pressure–volume area (PVA, a measure of total mechanical energy) in stunned hearts. In the \( \dot{V}O_2 \)-PVA relation, \( \dot{V}O_2 \) can be divided into PVA-dependent and PVA-independent fractions. In excised cross-circulated dog left ventricles, a 15-minute normothermic global ischemia followed by 60–120 minutes of reperfusion significantly decreased the ventricular contractility index (E\(_{\text{max}}\)) by \(~40\%\), but the PVA-independent \( \dot{V}O_2 \) did not significantly decrease. Oxygen cost of PVA, defined as the slope of the \( \dot{V}O_2 \)-PVA relation, was slightly decreased in stunned hearts. Restoration of the depressed E\(_{\text{max}}\) to the preischemic control level by calcium infusion increased the PVA-independent \( \dot{V}O_2 \) to 137±27% of control level (\( p<0.01 \)). Oxygen cost of contractility, defined as the slope of the relation between PVA-independent \( \dot{V}O_2 \) and E\(_{\text{max}}\), increased from 0.0011±0.0003 to 0.0023±0.0005 ml O\(_2\)·ml·mm Hg\(^{-1}\)·beat\(^{-1}\) per 100 g myocardium in control and stunned hearts, respectively (\( p<0.01 \)). From these new findings, we conclude that the unchanged \( \dot{V}O_2 \), despite the depressed contractility in stunned myocardium, is mainly due to the increased oxygen cost of contractility. (Circulation Research 1991;69:975–988)

A brief period of myocardial ischemia followed by reperfusion results in prolonged dysfunction without necrosis, which is known as myocardial stunning.\(^1\) Previous investigators have reported normal\(^2\) or even increased\(^3,4\) myocardial oxygen consumption (\( \dot{V}O_2 \)) in stunned myocardium despite contractile dysfunction, although some studies\(^5,6\) have shown reductions in both \( \dot{V}O_2 \) and mechanical performance. In these studies,\(^1,5,6\) myocardial contractile function was evaluated by segment shortening or systolic developed pressure, both of which are load dependent. Therefore, myocardial contractility per se of stunned myocardium has not been assessed in a load-independent manner. In addition, \( \dot{V}O_2 \) was not analyzed by differentiating its mechanical and nonmechanical fractions. Thus, the relation between myocardial contractile dysfunction and \( \dot{V}O_2 \) in stunned myocardium has not been fully elucidated.

We considered that this problem can be appropriately investigated by using both systolic pressure–volume area (PVA, a measure of total mechanical energy) and a contractility index (E\(_{\text{max}}\)) as two primary correlates of \( \dot{V}O_2 \).\(^7\) (See Table 1 for a glossary of terms.) PVA is the area in the pressure–volume (P–V) diagram circumscribed by the end-systolic P–V line, end-diastolic P–V curve, and the systolic P–V trajectory (Figure 1A).

The physiological significance of the PVA and E\(_{\text{max}}\) follows. E\(_{\text{max}}\), the slope of the end-systolic P–V relation, sensitively reflects ventricular contractility largely independent of ventricular loading conditions (Figure 1A).\(^7\) PVA correlates linearly with \( \dot{V}O_2 \) in a load-independent manner in a stable contractile state (Figure 1B). The slope of the \( \dot{V}O_2 \)-PVA relation in a given E\(_{\text{max}}\) means the “oxygen cost of PVA.”\(^7\) \( \dot{V}O_2 \) can be divided at the \( \dot{V}O_2 \) intercept of the \( \dot{V}O_2 \)-PVA relation into PVA-dependent and PVA-independent components (Figure 1B). The PVA-independent \( \dot{V}O_2 \) is considered to be equivalent to the energy utilization for nonmechanical activities consisting of excitation–contraction (E–C) coupling and basal metabolism.\(^7,8\) The \( \dot{V}O_2 \)-PVA relation is elevated in a parallel manner with an enhancement of E\(_{\text{max}}\) (Figure 1B).
The PVA-independent $\dot{V}O_2$ increases with increases in $E_{\text{max}}$ (Figure 1D).7,9,10 The slope of the relation between the PVA-independent $\dot{V}O_2$ and $E_{\text{max}}$ means the "oxygen cost of contractility" (Figure 1D).11 The $\dot{V}O_2$--PVA--$E_{\text{max}}$ relation has been thoroughly reviewed by Suga.7

The present purpose was to investigate, by means of the $\dot{V}O_2$--PVA--$E_{\text{max}}$ relation, the disproportionate increase in $\dot{V}O_2$ relative to the depressed contractility in stunned myocardium.4--7 This dissociation could result from an increased oxygen cost of PVA, an increased oxygen cost of contractility, an increased basal metabolism, or their combinations. To this end, we simultaneously assessed $E_{\text{max}}$ and the $\dot{V}O_2$--PVA relation in control and stunned myocardium after a 15-minute normothermic global ischemia in the excised cross-circulated dog heart preparation.

**Figure 1.** Panel A: Schematic illustration of end-systolic pressure (P)–volume (V) relation line (ESPVR), the slope of which is defined as Emax, and systolic pressure–volume area (PVA), defined as the sum of external work (EW) and potential energy (PE). Vo is the volume at which peak systolic pressure is zero. Panel B: Schematic illustration of left ventricular oxygen consumption ($\dot{V}O_2$) versus PVA relation and two components of $\dot{V}O_2$: PVA-independent $\dot{V}O_2$ (b) and PVA-dependent $\dot{V}O_2$ ($\dot{V}O_2$–b) at a stable Emax. Horizontal dashed line passing through b divides $\dot{V}O_2$ into PVA-dependent and PVA-independent $\dot{V}O_2$. Slope a of the $\dot{V}O_2$–PVA relation is designated as oxygen cost of PVA. Panel C: Parallel upward shifts of the $\dot{V}O_2$–PVA relation line caused by increases in PVA-independent $\dot{V}O_2$ (b) with enhancement of Emax. Slope a remains unchanged. Panel D: Relation between Emax and PVA-independent $\dot{V}O_2$ (b). Slope c of this relation is designated as oxygen cost of Emax (contractility). Extrapolated y intercept (d) of this relation means PVA-independent $\dot{V}O_2$ per beat extrapolated to zero Emax.

**Materials and Methods**

**Surgical Preparation**

Experiments were performed on the excised cross-circulated dog heart preparation as previously described in detail.7 Briefly, two mongrel dogs (12–19 kg) were anesthetized with sodium pentobarbital (30 mg/kg i.v.) after premedication with ketamine hydrochloride (7 mg/kg i.m.). Blood was heparinized (1,000 units/kg body wt) in both dogs. The common carotid arteries and external jugular vein were cannulated in the larger dog (support dog) for cross circulation of the heart preparation.

The chest of the smaller dog (heart donor dog) was opened midsternally under artificial ventilation. The arterial and venous cross-circulation tubes from the support dog were cannulated into the left subclavian artery and the right ventricle (RV) via the right atrial appendage, respectively. The heart–lung section was isolated from the systemic and pulmonary circulation by ligating the azygos vein, descending aorta, brachiocephalic artery, inferior and superior venae ca-
væ, and bilateral pulmonary hili. The supported beating heart was excised from the chest after cross circulation was started. There was no interruption of coronary circulation during surgery.

The arterial cross-circulation tube served as the coronary arterial perfusion tube. The venous cross-circulation tube was disconnected in the middle: the heart-side tube hydrostatically drained coronary venous blood return into a beaker 30 cm below the heart, and the support dog-side tube was connected to a funnel 5–10 cm above the support dog. A roller pump continuously pumped coronary venous blood from the beaker into the funnel.

The left atrium was opened and the chordae tendineae were cut. A thin latex balloon (unstressed volume, ~50 ml) mounted on a rigid connector (14 mm i.d.) was placed in the left ventricle (LV) and secured at the mitral annulus. A miniature pressure gauge (model P-7, Konigsberg Instruments, Inc., Pasadena, Calif.) was placed inside the apical end of the balloon to measure LV pressure. The balloon was connected to a volume servo pump, which accurately controlled and measured LV volume.7 The LV epicardial electrocardiogram was recorded to trigger the volume command signal of the servo pump. Heart rate was held constant at 150 beats/min by left atrial pacing.

Coronary blood flow was measured with an electromagnetic flowmeter (model MVF-2100, Nihon Kohden, Japan) by placing an in-line probe (model FF-050T, Nihon Kohden) in the coronary venous drainage tube from the right heart. We neglected LV thebesian venous blood flow because of its small fraction (<3%) in the total coronary flow.7 Coronary arteriovenous O2 content difference (AVO2D) was measured continuously with a custom-made dual-channel oximeter (Erma PWA-200S),12 which functions in the same principle as the Avox system (Avox Systems, Inc., San Antonio, Tex.).13 This analyzer was calibrated against an IL-282-CO oximeter in each experiment. The bypassed venous blood was returned to the coronary venous tube upstream of the flowmeter.

The temperature of the heart was monitored and maintained constant near 36°C throughout the experiment with heaters placed on the coronary arterial perfusion tube and under the box containing the heart. The systemic arterial blood pressure of the support dog served as the coronary perfusion pressure of the excised heart. We gave indomethacin (0.5–1.0 mg/kg i.v.) to minimize systemic hypotension under cross circulation; our experience in cross-circulated dog hearts (unpublished) and a study in cross-circulated rabbit hearts14 have shown that this dose of indomethacin maintains the support animal’s arterial pressure without noticeably affecting coronary blood flow, ventricular mechanics, and energetics. When the mean perfusion pressure decreased to <80 mm Hg, we restored it by slowly transfusing 50–100 ml whole blood that had been collected from the heart donor dog or infusing 50–100 ml 10% dextran 40 solution as needed. Arterial pH, Po2, and PCO2 of the support dog were maintained within their physiological ranges by using supplemental oxygen and intravenous sodium bicarbonate if necessary.

**Experimental Protocol**

We performed two series of experiments. In the first series, we primarily compared Emax and the VO2–PVA relation between preischemic control and postischemic contractions. In the second series, we primarily compared the oxygen cost of contractility between control and postischemic contractions. Figure 2 shows the time courses of the protocols in the two series.

**Series 1: Control loading run.** In a group of 10 hearts, LV end-diastolic volume was widely varied with the servo pump to cover a wide range of PVA in the preischemic control contractile state. Stroke volume was arbitrarily set, usually within 3–10 ml, because we have shown that changes in stroke volume do not affect the VO2–PVA relation.7 Electrocardiogram, LV pressure, LV volume, coronary blood flow, and AVO2D were measured in steady-state contractions under each LV loading condition. These measurements were repeated at five or six different LV volumes including a positive finite V0 in each heart. V0 is the LV volume at which peak isovolumic pressure was zero. PVA is zero at V0.

**Series 1: Ischemia.** LV was kept mechanically unloaded at V0 during ischemia. We arbitrarily chose V0 to standardize mechanical loading conditions during ischemia among hearts. To avoid any air ejection into the decompressed ascending aorta and hence prevent occurrence of any air emboli in the coronary circulation, we stopped LV pressure development by inducing ventricular fibrillation with 60-Hz electric sine waves of 3–4 V immediately before the coronary occlusion. The heart was then subjected to a 15-minute normothermic global ischemia by occluding the coronary arterial perfusion tube. Ventricular fibrillation disappeared when ischemic arrest occurred within a few minutes.
Series 1: Reperfusion. After the 15-minute global ischemia, the heart was reperfused by gradually releasing the occlusion over 1 minute. All ischemically arrested hearts soon started to fibrillate spontaneously. The heart was electrically defibrillated (5–10 J), and atrial pacing was restarted at the same rate as the preischemic rate (150 beats/min). Our previous study has shown that fibrillation and defibrillation do not significantly depress postfibrillatory $E_{max}$ except within the initial 5 minutes after defibrillation.15

Series 1: Postischemic loading runs. Loading runs similar to the preischemic control loading run were performed at 20, 60, and 120 minutes after the end of ischemia. Between these runs, the LV was kept mechanically unloaded at $V_0$. We arbitrarily chose $V_0$ again to standardize mechanical loading conditions among hearts. We considered such a load standardization important, because loading conditions affected $V_0$ and might thereby affect the severity of and recovery from stunning.

Lactate concentration was measured in coronary arterial and venous blood with a lactate analyzer (model 23L, Yellow Springs Instrument Co., Yellow Springs, Ohio) at the maximal PVA in each of the control and 20-minute, 60-minute, and 120-minute postischemic loading runs.

Series 1: Calcium run. In eight of the 10 hearts after the 120-minute postischemic loading run, both end-diastolic and stroke volumes were set to intermediate values so that ejection pressure was 50–70 mm Hg before $E_{max}$ enhancement. Calcium (1% CaCl$_2$) was administered into the coronary arterial tube at a gradually increased infusion rate (0.02–0.1 meq/min) until $E_{max}$ was raised to the control level. Measurements were made in steady state (usually 3 minutes) under the final infusion rate. We performed this run to quantify the PVA-independent $V_O_2$ by the method explained later in “Data Analysis.”

We used calcium rather than catecholamines as a positive inotropic agent, because calcium increases contractility without involving complex phosphorylation processes that augment the calcium uptake rate of sarcoplasmic reticulum and decrease the calcium sensitivity of contractile proteins.16

Series 1: Sham. To assess the influence of the lapse of time per se in series one, a second group of five hearts was subjected to sham experiments without ischemia. Three loading runs were performed at identical times with the control loading run and 60- and 120-minute postischemic loading runs in series one. We tacitly assumed that there would be no change in LV mechanics and energetics at 20 minutes in the sham group.

Series 2: Control loading and calcium runs. In a third group of eight hearts, the same control loading run as in series one was first performed. Then, the same calcium run as in series one was performed to quantify preischemic control oxygen cost of contractility. $E_{max}$ was enhanced by calcium infusion until it was nearly doubled from the control level. Then, the calcium infusion was stopped, and a 10–15-minute rest period was allowed for the return of contractility to the preenhanced control level.

Series 2: Ischemia and reperfusion. The heart was subjected to the same 15-minute ischemia as in series one, followed by a 60-minute reperfusion.

Series 2: Postischemic loading and calcium runs. The postischemic loading and second calcium runs were performed at 60 minutes of reperfusion in a similar manner to the preischemic calcium runs.

Series 2: KCl arrest. The heart was finally arrested at $V_0$ by injecting KCl (3–4 ml of 0.75 eq/l) into the coronary arterial tube over 0.5 minutes, followed by continuous KCl infusion (1–3 meq/min) in the same way as before.7,8 When both coronary flow and AVO$_2$D tracings were stabilized (usually 15 minutes of arrest), VO$_2$ was measured as basal metabolic VO$_2$ under KCl arrest.

Series 2: Sham. A fourth group of six hearts was subjected to sham experiments for series two without ischemia. Two sets of the loading and calcium runs were repeated with the same interval as that between the control and postischemic runs in series two. KCl arrest was also performed.

After these experimental protocols, the LV including the septum and the RV free wall of all hearts were weighed. The LV and RV weights were $77\pm15$ and $24\pm5$ g in series one and $78\pm12$ and $28\pm3$ g in series two, respectively. These LV and RV weights were used to normalize $E_{max}$ PVA, and $V_O_2$ for 100 g LV.

Then, the LV was cut into five short-axis slices from the apex to the base, each of which was incubated in triphenyltetrazolium chloride (at 37°C for 20 minutes) for identification of tissue necrosis. Their cross sections were photographed in color.17

Data Analysis

Cardiac analog signals were sampled at 2-msec intervals, and the digital data were analyzed with a signal processor (model 7T-18, NEC San-ei, Japan).

Contractility index. LV contractile state was assessed by $E_{max}$ of individual contractions in the same way as before (Figures 1A and 3A).7 $E_{max}$ was computed as the maximal value of instantaneous ratio of P(t)/[V(t)–$V_0$], where P(t) and V(t) are instantaneous ventricular pressure and volume, respectively, in each contraction. $E_{max}$ in a loading run was obtained as a mean of differently loaded contractions in each stable contractile state. $E_{max}$ (in mm Hg/ml) was normalized for 100 g LV and expressed as follows: mm Hg · ml$^{-1}$ · 100 g $^{-1}$ = mm Hg/(ml/100 g)$^{-1}$.7,9,13,14 Note that 100 g, not (100 g)$^{-1}$, appears in the unit.

Pressure–volume area. PVA is a specific area in the P–V diagram that is circumscribed by the end-systolic P–V relation line (or $E_{max}$ line), the end-diastolic P–V relation curve, and the systolic P–V trajectory (Figures 1A and 3A).7 PVA represents the total mechanical energy generated by each contraction of LV.7 We calculated PVA of each beat from the digitized P(t) and V(t) data in the same way as before.7 PVA was
The PVA dependence and PVA independence of these terms have been validated experimentally.\textsuperscript{7,18}

In both Equations 1 and 2, coefficient a or the slope of the Vo\textsubscript{2}–PVA line obtained at a given E\textsubscript{max} has been designated as “oxygen cost of PVA” or “oxygen cost of mechanical energy” (Figure 1B).\textsuperscript{7}

This cost means Vo\textsubscript{2} per unit PVA or mechanical energy. Therefore, the reciprocal of a represents “contractile efficiency” or the efficiency of energy conversion from PVA-dependent Vo\textsubscript{2} to PVA (Figure 1B).\textsuperscript{7}

Vo\textsubscript{2} has dimensions as follows: ml O\textsubscript{2} \cdot beat\textsuperscript{-1}, and PVA has dimensions as follows: mm Hg ml \cdot beat\textsuperscript{-1}. Therefore, the unit of the oxygen cost of PVA is as follows: ml O\textsubscript{2}/(mm Hg \cdot ml), that is, ml O\textsubscript{2} \cdot mm Hg\textsuperscript{-1} \cdot ml\textsuperscript{-1}. However, 1 ml O\textsubscript{2} is approximately equivalent to 20 J and 1 mm Hg \cdot ml is equivalent to 1.33 \times 10\textsuperscript{-4} J. Therefore, after appropriate conversions of both Vo\textsubscript{2} and PVA, the unit of the oxygen cost of PVA can be dimensionless, and the unit of the contractile efficiency is also dimensionless. The units of both the oxygen cost of PVA and the contractile efficiency do not include LV weight and therefore are not affected whether or not both Vo\textsubscript{2} and PVA are normalized for LV weight.

The oxygen cost of PVA and the contractile efficiency can be considered to represent the overall efficiency of the energy conversion from oxidative phosphorylation through crossbridge cycling.\textsuperscript{7} A greater coefficient a means a greater oxygen cost of PVA and a smaller contractile efficiency, suggesting an increased inefficiency of both or either of oxidative phosphorylation and crossbridge cycling.\textsuperscript{7,14}

Constant b represents the Vo\textsubscript{2} intercept of the Vo\textsubscript{2}–PVA line, as well as actually measured Vo\textsubscript{2} of unloaded contraction at V\textsubscript{0} (Figure 1B). Constant b means the PVA-independent Vo\textsubscript{2}, and it sensitively changes with E\textsubscript{max}.\textsuperscript{7,9} It consists of Vo\textsubscript{2} for both E–C coupling and basal metabolism.\textsuperscript{7}

Equation 1 and its coefficient a and constant b can be obtained by applying linear regression analysis to Vo\textsubscript{2}–PVA data points obtained in a stable contractile state. They were determined in a preischemic control loading run and in 20-, 60-, and 120-minute postischemic runs in series one and in preischemic control loading and 60-minute postischemic runs in series two.

Coefficient c in Equation 2 represents the slope of the PVA-independent Vo\textsubscript{2} (b) versus E\textsubscript{max} relation (Figure 1D). Constant d represents PVA-independent Vo\textsubscript{2} extrapolated to zero E\textsubscript{max}. Zero E\textsubscript{max} means no E–C coupling but electrical excitation at a given heart rate. Coefficient c and constant d can be determined by plotting increasing b against gradually enhanced E\textsubscript{max}.\textsuperscript{9,10} We have recently developed a convenient method to determine them.\textsuperscript{11}

The method is schematically explained in Figure 3. First, a reference Vo\textsubscript{2}–PVA relation is obtained at control E\textsubscript{max} level, and a and b are determined (Figure 3B). Second, E\textsubscript{max} is gradually increased in several steps, whereas steady-state PVA and Vo\textsubscript{2} are normalized for 100 g LV. Its dimensions are as follows: mm Hg \cdot ml \cdot beat\textsuperscript{-1} \cdot 100 g\textsuperscript{-1}.

Oxygen consumption. Vo\textsubscript{2} was determined as the product of coronary flow and AVo\textsubscript{2}D. Vo\textsubscript{2} per beat (ml O\textsubscript{2}/beat) was obtained by dividing Vo\textsubscript{2} per minute by heart rate in a steady state. All raw Vo\textsubscript{2} data (ml O\textsubscript{2}/beat) were plotted against the raw PVA in preischemic control and 20-minute, 60-minute, and 120-minute postischemic loading runs, as shown in Figures 4E–4H.

Vo\textsubscript{2}–PVA relation, oxygen cost of PVA, contractile efficiency, and oxygen cost of contractility. Previous studies by us\textsuperscript{7,9,11} and others\textsuperscript{10} have shown that Vo\textsubscript{2} can be empirically formulated as

\[ \dot{\text{Vo}}_2 = aPVA + b \]  
(1)

\[ \dot{\text{Vo}}_2 = aPVA + cE_{\text{max}} + d \]  
(2)

where aPVA means the PVA-dependent Vo\textsubscript{2} and b or cE\textsubscript{max} + d means the PVA-independent Vo\textsubscript{2} (Figure 1B).\textsuperscript{7}
determined in contractions with both end-diastolic and stroke volumes fixed (Figure 3A). Third, obtained Vo2-PVA data points are plotted (Figure 3B). From the individual Vo2-PVA data point at each enhanced E\textsubscript{max} an increase in PVA-independent Vo2 is determined by Vo2-(aPVA+b), where a is assumed to remain unchanged during E\textsubscript{max} enhancement. This plus b, which is equal to Vo2-aPVA, is the PVA-independent Vo2 at this enhanced E\textsubscript{max}. Plotting all resultant Vo2-aPVA against corresponding E\textsubscript{max} yields the relation between PVA-independent Vo2 and E\textsubscript{max} such as shown in Figure 1D. The slope of this relation gives c as "oxygen cost of contractility," and the y intercept gives d as "zero-E\textsubscript{max} PVA-independent Vo2." We assumed constancy of a during each calcium run based on the already established parallelism of two Vo2-PVA relations obtained at control and a nearly doubled E\textsubscript{max}.7,9

Coefficient c and constant d were determined by this method in all calcium runs in series one and two. The oxygen cost of contractility, c, has dimensions as follows: (ml O\textsubscript{2}/beat)/(mm Hg/ml), or ml O\textsubscript{2}· min Hg\textsuperscript{-1}· ml· beat\textsuperscript{-1}. When PVA-independent Vo2 is normalized for 100 g LV, it has dimensions as follows: ml O\textsubscript{2}· min· beat\textsuperscript{-1}· 100 g\textsuperscript{-1}. E\textsubscript{max} after normalization has dimensions as follows: mm Hg· ml\textsuperscript{-1}· 100 g. Therefore, c has dimensions as follows: ml O\textsubscript{2}· min· mm Hg\textsuperscript{-1}· ml· beat\textsuperscript{-1}· 100 g\textsuperscript{-2} (in the same manner as before).11 (Note: 100 g\textsuperscript{-2} appears because Vo2 normalized for 100 g LV has 100 g in the denominator, but the dimensions of E\textsubscript{max} normalized for 100 g LV have 100 g in the numerator.)

In these calculations, PVA-independent Vo2 was always corrected for LV by the following method.11 We assumed that Vo2 consisted of Vo2 of both the LV and RV. LV Vo2 consisted of Vo2 for PVA, E\textsubscript{max} and basal metabolism. RV Vo2 consisted of Vo2 for E\textsubscript{max} and basal metabolism, because PVA was zero in the unloaded RV. We tacitly assumed that Vo2 for both E-C coupling and basal metabolism per unit myocardium mass was comparable between the LV and RV, although the LV was variably loaded and the RV was always unloaded. To obtain the PVA-independent Vo2 of the LV alone, we divided the biventricular PVA-independent Vo2 by LV weight/(LV and RV weight). This PVA-independent Vo2 of LV was normalized for 100 g LV and correlated with E\textsubscript{max}.

Statistics

Analysis of variance (ANOVA)19 was applied to compare each of E\textsubscript{max} PVA-independent Vo2, coronary flow, AVO\textsubscript{2}D, and the slope of the Vo2-PVA relations among preischemic control and postischemic loading runs in series one (Figure 5). When ANOVA showed statistical significance by F test, the mean values were compared by the least significant difference method.19 ANOVA was also applied to compare E\textsubscript{max} and PVA-independent Vo2 among control, 120-minute postischemic loading, and calcium runs in series one (Figure 6). Analysis of covariance (ANCOVA)19 was applied to compare the two regression lines of PVA-independent Vo2 on E\textsubscript{max} during control and postischemic calcium runs in each heart (Figure 7C). Statistical

**FIGURE 4.** Pressure–volume loop trajectories in control (panel A), 20-minute (panel B), 60-minute (panel C), and 120-minute (panel D) loading runs in series one and regression lines in individual runs (panels E–H) corresponding to the upper panels. VO2, oxygen consumption per beat; PVA, pressure–volume area. The solid diagonal lines indicate the linear regression lines. The inner and outer pairs of the dashed lines around the linear regression lines are the 95% confidence limits of the regression lines and data points, respectively. All data of these eight panels were obtained from the same heart.
significance of the differences in the slopes of the regression lines in individual heart was tested by F test. Differences of the mean values between two groups were tested by paired t test. Values of p<0.05 were considered statistically significant. Data are presented as mean±SD.

Results

Series One

Figure 4 shows the pressure-volume and VO2-PVA relations obtained in the preischemic control and 20-minute, 60-minute, and 120-minute postischemic loading runs in one representative experiment. The P–V trajectories became taller with increases in volume load from V0 in every loading run (Figures 4A–4D). Emax values of individual contractions in a given loading run were close to each other with a small coefficient of variation (11.7±3.9% in different loading runs). Emax (mean of variably loaded contractions) was 9.9 mm Hg·ml⁻¹·100 g in the control loading run, decreased to 31% of control at 20 minutes, and recovered to >50% of control at 60 and 120 minutes of reperfusion. VO2 always increased linearly with increases in PVA in each loading run (Figures 4E–4H). The slope of the VO2-PVA relation was 1.70×10⁻³ ml O₂·mm Hg⁻¹·ml⁻¹ in the control loading run and slightly decreased during the reperfusion period, especially at 20 minutes. PVA-independent VO2, which was equal to the directly measured VO2 of unloaded contraction at V0, was 0.024 ml O₂·beat⁻¹·100 g⁻¹ in the control loading run and did not change except for a slight decrease at 20 minutes of reperfusion.

Table 2 summarizes the changes in Emax, coronary flow, AVO₂D, PVA-independent VO₂, the slope of the VO₂-PVA relation, and contractile efficiency in 10 stunned hearts in series one. These coronary flow and AVO₂D values are those obtained at V0, which was arbitrarily chosen as a common loading condition to preischemic control and postischemic hearts.

Figure 5 shows their percent changes from the control values, in comparison to the data of the sham group. Emax markedly decreased at 20 minutes of reperfusion (41±16% of control) and slightly recovered at 60 minutes of reperfusion (64±15% of control) (Figure 5A). No more recovery of Emax was observed at 120 minutes of reperfusion (63±23% of control). Emax of the sham group decreased to some extent at 60 and 120 minutes of observation, but their decreases were not significant. Coronary flow was higher (Figure 5B) and AVO₂D was lower during reperfusion compared with control.

Despite the marked decreases in Emax, PVA-independent VO2 did not significantly decrease except at 20 minutes of reperfusion (82±12% of control) (Figure 5C). It did not change with time in the sham group.

The slope of the VO₂-PVA relation, or the oxygen cost of PVA, decreased significantly at 20 minutes of reperfusion (69±16% of control), returned toward preischemic level at 60 minutes of reperfusion, and again decreased significantly at 120 minutes of reperfusion.

![Figure 6](image_url)
fusion (81±14%) (Figure 5D). The smaller slope after 60 minutes of ischemia (10% smaller than control) was not statistically significant (compare with series two below). The slope gradually decreased even in the sham group, and its decrease at 120 minutes of observation (79±14%) was significant (p<0.05). Therefore, although the slope was significantly lower than control at 120 minutes after ischemia, it did not differ from the sham group (p>0.2 by unpaired t test). Contractile efficiency changed reciprocally with the changes in the slope of the Vo2-PVA relation.

End-diastolic pressure at the same end-diastolic volume (21.7±0.2 ml) did not significantly increase at any time after the onset of reperfusion (6.2±2.9, 7.5±4.0, 7.4±4.1, and 6.6±4.2 mm Hg in control, 20-minute, 60-minute, and 120-minute loading runs, respectively).

The duration of QRS complex of the LV epicardial electrocardiogram measured at Vo2 only slightly increased from the control value of 74±8 msec to 81±12, 80±10, and 79±9 msec in 20-minute, 60-minute, and 120-minute loading runs, respectively (all p<0.05 versus control).

Figure 6 shows relative magnitudes of Emax and PVA-independent Vo2 in the 120-minute loading run and the postischemic calcium run. The calcium run performed after 120 minutes of reperfusion considerably increased Emax to 7.2±3.2 mm Hg · ml⁻¹ · 100 g⁻¹, which was similar to the preischemic control Emax (Table 2). However, PVA-independent Vo2 increased considerably to 0.034±0.008 ml O2 · ml⁻¹ · beat⁻¹ · 100 g⁻¹ (Table 2), which was 37±27% greater than the preischemic control value. Oxygen cost of contractility determined as incremental ratio of PVA-independent Vo2 to Emax was 0.00474±0.00289 ml O2 · ml⁻¹ · mm Hg⁻¹ · beat⁻¹ · 100 g⁻².

Coronary venous blood lactate concentration was always lower than coronary arterial level in all hearts. The differences were 1.7±1.1, 0.9±1.8, 1.0±0.5, and 1.2±0.9 mmol/l in control, 20-minute, 60-minute, and 120-minute loading runs, respectively. Although there was a tendency to decrease, no postischemic value was significantly smaller than the preischemic control value (p>0.05).

**Series Two**

Changes in the LV mechanics and energetics variables due to 15-minute ischemia and 60-minute reperfusion were similar to those observed in series one. Table 3 summarizes the mecanhanoenergetic data. Stunning significantly decreased Emax by 43±16%, PVA-independent Vo2 by 12±9%, and slope of the Vo2-PVA relation (i.e., the oxygen cost of PVA) by 27±9%. Contractile efficiency changed reciprocally with the slope of the Vo2-PVA relation.

Unlike series one, the decrease in the slope at 60 minutes of reperfusion was significant (p<0.01). If the preischemic control and 60-minute postischemic data of the Vo2-PVA slope were pooled from series one and two, the preischemic slope was (1.95±0.38)×10⁻⁵, and the postischemic slope was (1.59±0.31)×10⁻⁵ ml O2 · mm Hg⁻¹ · ml⁻¹, the latter significantly smaller than the former (p<0.05). In the sham group, neither Emax PVA-independent Vo2, the slope, nor contractile efficiency changed significantly between the first and second loading runs.

Figure 7 shows the Vo2-PVA data points during the preischemic calcium (Figure 7A) and postischemic calcium (Figure 7B) runs in a representative heart. With increases in Emax, the Vo2-PVA data point moved linearly in a right-upward direction from the preenhanced point on or near the reference Vo2-PVA relation in the respective run. After calculating PVA-independent Vo2 values from these moving Vo2-PVA data, Figure 7C shows the regression lines of the PVA-independent Vo2 on Emax during these two calcium runs. The relation was higher and steeper in stunned myocardium than in myocardium subjected to sham experiments. ANCOVA showed a significant difference (p<0.05) in the slope between the two regression lines in this heart. All the other hearts showed similar results.
**Table 2. Changes in Cardiac Mechanics and Energetics in Series 1**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>20 min</th>
<th>60 min</th>
<th>Postischemic</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax (mm Hg·ml⁻¹·100 g)</td>
<td>7.8±2.6</td>
<td>3.0±0.9*</td>
<td>4.9±1.5*</td>
<td>4.8±2.1*</td>
<td></td>
</tr>
<tr>
<td>Coronary flow (ml·min⁻¹·100 g⁻¹)</td>
<td>62±31</td>
<td>96±37*</td>
<td>76±24†</td>
<td>95±37*</td>
<td></td>
</tr>
<tr>
<td>AVo₂D (vol%)</td>
<td>7.3±3.8</td>
<td>3.4±1.3*</td>
<td>5.1±2.0*</td>
<td>4.1±1.7*</td>
<td></td>
</tr>
<tr>
<td>PVA-independent Vo₂ (ml O₂·beat⁻¹·100 g⁻¹)</td>
<td>0.024±0.006</td>
<td>0.019±0.003*</td>
<td>0.022±0.004†</td>
<td>0.023±0.004†</td>
<td></td>
</tr>
<tr>
<td>Slope (10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹)</td>
<td>1.84±0.38</td>
<td>1.25±0.33*</td>
<td>1.64±0.34†</td>
<td>1.50±0.43*</td>
<td></td>
</tr>
<tr>
<td>Contractile efficiency (%)</td>
<td>37.9±8.6</td>
<td>57.2±15.3*</td>
<td>43.2±7.3†</td>
<td>47.8±10.6*</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD for control and postischemic 20-minute, 60-minute, and 120-minute loading runs. Emax, slope of end-systolic pressure–volume relation line; AVo₂D, coronary arteriovenous O₂ content difference; PVA, pressure–volume area; Vo₂, oxygen consumption per beat; PVA-independent Vo₂, directly measured Vo₂ of unloaded contraction at ventricular volume at which peak isovolumic pressure is zero (Vo₂); Slope, slope of the Vo₂–PVA relation in loading run; Contractile efficiency, reciprocal of slope after converting the slope value in 10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹ to a percent value using 1 ml O₂=20 J and 1 mm Hg·ml=1.33×10⁻⁴ J. Coronary flow and AVo₂D were measured at Vo₂.

*P<0.01 compared with control; †P>0.05 compared with control (p=NS).

Figure 8A compares the oxygen cost of contractility between the preischemic control and 60-minute postischemic calcium runs in all eight hearts. The oxygen cost of contractility increased by 123±90% from preischemic control in stunned hearts. The oxygen cost of contractility remained constant between the first and second calcium runs in the sham group, as shown in Figure 8B. The oxygen cost of contractility in the 120-minute postischemic calcium run in series one (0.00474 ml O₂·ml·mm Hg⁻¹·beat⁻¹·100 g⁻²) was significantly greater than the value in the 60-minute postischemic run in series two (p<0.05 by unpaired t test).

Zero-E_{\text{max}} PVA-independent Vo₂ (d) (Figure 1D) was 0.0166±0.0027 ml O₂·beat⁻¹·100 g⁻¹ or 2.48±0.42 ml O₂·min⁻¹·100 g⁻¹ in preischemic control and 0.0115±0.0032 ml O₂·beat⁻¹·100 g⁻¹ or 1.74±0.54 ml O₂·min⁻¹·100 g⁻¹ in stunned hearts. Both per-beat and per-minute values in stunned hearts were smaller by 30±17% than preischemic control values (p<0.05).

KCl-arrest basal metabolic Vo₂ was 1.03±0.30 ml O₂·min⁻¹·100 g⁻¹ in 60-minute postischemic hearts. This value did not significantly differ from that in the sham group (1.21±0.15 ml O₂·min⁻¹·100 g⁻³, p>0.1 by unpaired t test). Thus, Vo₂ for KCl-arrest basal metabolism was not significantly elevated in stunned hearts.

The KCl-arrest basal metabolic Vo₂ was significantly smaller than the zero-E_{\text{max}} PVA-independent Vo₂. This difference may partly be due to the gradual decrease in KCl-arrest basal metabolism with time during arrest and partly due to the fact that zero-E_{\text{max}} PVA-independent Vo₂ contains energy utilization for electrical excitation of cell membrane by the Na⁺,K⁺-ATPase pump.

**Myocardial Necrosis**

No area of necrosis was observed on visual inspection of triphenyltetrazolium chloride–stained LV slices from any of the stunned hearts in series one and two.

**Discussion**

The major findings of the present study are as follows: 1) Despite the considerably depressed myocardial contractility, the PVA-independent Vo₂ decreased only little in stunned myocardium. 2) The oxygen cost of PVA was smaller and the contractile efficiency was greater in stunned hearts. 3) A similarly decreased oxygen cost of PVA was obtained in

**Table 3. Changes in Cardiac Mechanics and Energetics in Series 2**

<table>
<thead>
<tr>
<th></th>
<th>Stunned group</th>
<th>60 min</th>
<th>Postischemic</th>
<th>Sham group</th>
<th>First run</th>
<th>Second run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emax (mm Hg·ml⁻¹·100 g)</td>
<td>7.4±1.5</td>
<td>4.2±1.3*</td>
<td>4.8±0.9</td>
<td>5.6±0.8†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary flow (ml·min⁻¹·100 g⁻¹)</td>
<td>74±36</td>
<td>94±40†</td>
<td>57±37</td>
<td>47±11†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVo₂D (vol%)</td>
<td>5.6±2.6</td>
<td>3.7±1.7†</td>
<td>6.1±2.2</td>
<td>6.2±1.4†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PVA-independent Vo₂ (ml O₂·beat⁻¹·100 g⁻¹)</td>
<td>0.023±0.004</td>
<td>0.020±0.003‡</td>
<td>0.020±0.003</td>
<td>0.020±0.004‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope (10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹)</td>
<td>2.09±0.30</td>
<td>1.52±0.24*</td>
<td>1.74±0.16</td>
<td>1.74±0.29†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contractile efficiency (%)</td>
<td>32.6±4.0</td>
<td>44.6±8.0*</td>
<td>38.6±3.3</td>
<td>39.2±6.0†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD for control and postischemic 60-minute loading runs. Emax, slope of end-systolic pressure–volume relation line; AVo₂D, coronary arteriovenous O₂ content difference; PVA, pressure–volume area; Vo₂, oxygen consumption per beat; PVA-independent Vo₂, directly measured Vo₂ of unloaded contraction at ventricular volume at which peak isovolumic pressure is zero (Vo₂); Slope, slope of the Vo₂–PVA relation in loading run; Contractile efficiency, reciprocal of slope after converting the slope value in 10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹ to a percent value using 1 ml O₂=20 J and 1 mm Hg·ml=1.33×10⁻⁴ J. Coronary flow and AVo₂D were measured at Vo₂.

*P<0.05 compared with control or sham first run; †P>0.05 compared with control or sham first run (p=NS); ‡P<0.01 compared with control or sham first run.
sham-operated hearts at comparable times of observation. 4) When the depressed contractility of stunned myocardium was restored to preischemic control level, the PVA-independent VO\textsubscript{2} markedly exceeded the control level. 5) Most important, the oxygen cost of contractility was significantly higher in stunned myocardium than in control.

Because either KCl-arrest basal metabolism or zero-E\textsubscript{max} PVA-independent VO\textsubscript{2} was not increased in stunned myocardium, the first and fourth phenomena were clearly accounted for by the last findings. Therefore, the previously reported relatively high VO\textsubscript{2} despite decreased contractile performance\textsuperscript{1-4} seems to be attributable to the increased oxygen cost of contractility in stunned myocardium rather than to any increases in oxygen cost of PVA, zero-E\textsubscript{max} PVA-independent VO\textsubscript{2}, and KCl-arrest basal metabolic VO\textsubscript{2}. The present study is the first, to the best of our knowledge, that indicates explicitly a markedly increased cost of contractility (E\textsubscript{max}) in stunned myocardium.

Contractility Reserve

The depressed E\textsubscript{max} and its rise to the control level with calcium (Figure 6) qualify our postischemic hearts as stunned.\textsuperscript{1} This contractile reserve suggests that the depressed contractility was not due to the deficiency of energy supply.\textsuperscript{5,20,21} Because it is not the purpose of the present study to elucidate the mechanism of the depressed contractility in stunned myocardium, we will not further discuss the pathogenesis of the depressed contractility.

Oxygen Cost of Contractility

The increased PVA-independent VO\textsubscript{2} at the contractility restored to the preischemic control level in series one (Figure 6) indicates an increased VO\textsubscript{2} component for myocardial nonmechanical activities, independent of PVA. The PVA-independent VO\textsubscript{2} consists of energy utilization for basal metabolism and E-C coupling.\textsuperscript{7}

Because neither KCl-arrest basal metabolic VO\textsubscript{2} nor zero-E\textsubscript{max} PVA-independent VO\textsubscript{2} was increased by stunning, an increase in VO\textsubscript{2} for E-C coupling seems to be the factor most responsible for the increased PVA-independent VO\textsubscript{2} at the E\textsubscript{max} restored with calcium. This view is consistent with the increased oxygen cost of contractility in stunned myocardium proved in this study.

Although the PVA-independent VO\textsubscript{2} corresponds to VO\textsubscript{2} of mechanically unloaded contraction with zero PVA (Figures 1 and 3), there remains residual myocardial contraction for ventricular shape changes.\textsuperscript{18,22} We can assume that such a residual mechanical energy at zero PVA is negligibly small based on the following two observations: 1) VO\textsubscript{2} did not significantly decrease even when ventricular volume was decreased from V\textsubscript{0} to complete collapse with a negative pressure in the ventricle.\textsuperscript{22} 2) VO\textsubscript{2} of variably preloaded but rapidly ejecting contraction with zero PVA was not significantly greater than VO\textsubscript{2} of unloaded contraction at V\textsubscript{0}.\textsuperscript{16} The former condition minimized ventricular shape changes, and the latter condition considerably increased ventricular shape and size changes. Both these observations indicate that ventricular shape changes that are produced by residual crossbridge cycling at zero PVA require no significant VO\textsubscript{2}.

We used the oxygen cost of PVA (a) obtained in the control loading run to determine oxygen cost of contractility (c) in each calcium run in either preischemic or stunned period. This assumption of constancy of a is based on the parallelism of two VO\textsubscript{2} - PVA relation lines at control E\textsubscript{max} and a stably enhanced E\textsubscript{max} with calcium as well as many other inotropic agents.\textsuperscript{7,9,10} In this study, we tacitly assumed that this parallelism held even in the calcium run in stunned myocardium, although a was slightly smaller in stunned hearts (Tables 2 and 3). Unfortunately, we could not confirm the parallelism in stunned hearts, because it was difficult to maintain a stable level of calcium-enhanced E\textsubscript{max} at the end of a calcium run in stunned hearts.

Instead, we could estimate a possible maximum error of the oxygen cost of contractility by the aid of Figure 9. If the decreased a (1.59x10\textsuperscript{-5} ml O\textsubscript{2} \cdot mm Hg \textsuperscript{-1} \cdot ml\textsuperscript{-1}, the 60-minute postischemic mean of series one and two) were restored to the preischemic control (1.95x10\textsuperscript{-5} ml O\textsubscript{2} \cdot mm Hg \textsuperscript{-1} \cdot ml\textsuperscript{-1}, the mean of series one and two) with enhancement of E\textsubscript{max} by calcium, the difference of a (between lines 1 and 2 in Figure 9) would be 0.36x10\textsuperscript{-5} ml O\textsubscript{2} \cdot mm Hg \textsuperscript{-1} \cdot ml\textsuperscript{-1}. This difference multiplied by a representative maximum increment in PVA (600 mm Hg \cdot ml \cdot beat\textsuperscript{-1} \cdot 100 g\textsuperscript{-1} in the calcium run in stunned hearts yields 0.0022 ml O\textsubscript{2} \cdot beat\textsuperscript{-1} \cdot 100 g\textsuperscript{-1}. This difference in PVA-independent VO\textsubscript{2} (b1 - b2 in Figure 9) means only a 17%
overestimation of the initially calculated increase in PVA-independent VO₂ (b₁–b in Figure 9). This overestimation of PVA-independent VO₂ corresponds to an overestimation of the oxygen cost of contractility by only 0.0007 ml O₂ · ml · mm Hg⁻¹ · beat⁻¹ · 100 g⁻² when E_max is increased by 70% from 4.5 mm Hg · ml⁻¹ · 100 g (Tables 2 and 3). If the already determined oxygen cost of contractility (0.0023 ml O₂ · ml · mm Hg⁻¹ · beat⁻¹ · 100 g⁻²; Figure 8A) is corrected for this relatively small overestimation, the true cost would become 26% smaller than the value given in Figure 8A. However, we found that this correction did not affect the statistical significance of the increased oxygen cost of contractility in stunned hearts (Figure 8A).

Alternatively, we could recognize that the initially calculated increase in PVA-independent VO₂ (b₁–b in Figure 9) consists of two fractions related to the oxygen cost of contractility (b₂–b) and a change in oxygen cost of PVA (b₁–b₂) due to a change, if any, in a. Although the relative magnitudes of these two fractions remain yet unknown, we can consider the sum of them (i.e., the initially calculated oxygen cost of contractility) to be the overall measure of the oxygen cost of contractility. Then, the result in Figure 8A still indicates a significantly greater oxygen cost of contractility in stunned hearts.

The energy utilization of E–C coupling occurs at both the Na⁺,K⁺-ATPase pump and the Ca²⁺-ATPase pump.⁷,23 The Na⁺,K⁺-ATPase pump on the sarcolemma normally pumps 3 mol sodium, consuming 1 mol ATP to repolarize after depolarization of cell membrane in each cardiac cycle.⁷,23 The Ca²⁺-ATPase pump that exists mainly on the sarcoplasmic reticulum normally pumps 2 mol calcium, consuming 1 mol ATP to decrease cytosolic free calcium concentration and to relax the myocyte after contraction in each cardiac cycle.⁷,23 In addition to these constant coupling ratios, the amount of calcium involved in the E–C coupling is proportional to myocardial contractile force and contractility unless the responsiveness of the contractile machinery to calcium is changed.⁷,23 The ratio of contractility to calcium would decrease when the calcium responsiveness of the contractile machinery decreases. These relations seem to be the basis of the oxygen cost of contractility.⁷,11 Any decrease in the calcium/ATP coupling ratio or in the contractility/calcium relation or in both would increase the oxygen (or ATP) cost of contractility.

Although the elucidation of the mechanism of the increased oxygen cost of contractility is beyond the goal of the present study, we could discuss it as follows by taking a full advantage of the VO₂-PVA relation (Figure 10). The efficiency from VO₂ to E–C coupling is considered to be the product of the VO₂-to-ATP efficiency (the efficiency of oxidative phosphorylation to synthesize ATP [E₁]) and the efficiency from ATP to E–C coupling (E₂).⁷ On the other hand, the efficiency from VO₂ to PVA is considered to be the product of the same VO₂-to-ATP efficiency (E₁) and the ATP-to-PVA efficiency (the efficiency of the contractile machinery to generate total mechanical energy by hydrolyzing ATP [E₃]).⁷,24 Therefore, a change in the VO₂-to-ATP efficiency (E₁) will equally affect both nonmechanical...
(E1 × E2) and mechanical (E1 × E3) efficiencies if E2 and E3 remain unchanged.

We first discuss the possibility of a decrease in the \( \dot{V}O_2 \)-to-ATP efficiency \((E_1)\) as the mechanism of the increased oxygen cost of contractility. Since mitochondrial respiration has been reported intact in stunned myocardium,25,26 a decreased efficiency \((E_1)\) of oxidative phosphorylation is unlikely.27

Then, a decreased efficiency \((E_2)\) from ATP to E-C coupling seems more likely. One of the factors decreasing E2 would be a decreased coupling ratio of calcium to ATP in the Ca\(^{2+}\)-ATPase pump due to an increased calcium permeability of the sarcoplasmic reticulum membrane.28,29 Although a significant sarcoplasmic reticulum dysfunction involving a decreased calcium/ATP ratio progresses in ischemic hearts,28,30 the dysfunction partially recovers in stunned hearts.29,31 If the sarcoplasmic reticulum dysfunction involving the increased calcium permeability continued substantially in stunned hearts, the PVA-independent \( \dot{V}O_2 \) would be increased at any \( E_{\text{max}} \) and could account for the decreased E2. However, at zero \( E_{\text{max}} \), calcium involved in E-C coupling is minimal, and the zero-\( E_{\text{max}} \) PVA-independent \( \dot{V}O_2 \) may not need to be increased. This is consistent with our present result (Figure 7C).

Another factor decreasing E2 would be a decreased responsiveness of the contractile machinery to free calcium.32,33 The decreased responsiveness has two mechanisms: one is a shift of the sensitivity of the contractile machinery to a higher free calcium concentration and the other is a decrease in the maximally activated contractile force.32 Whichever mechanism is more involved, the decreased responsiveness means that more free calcium is needed to activate the contractile machinery to the same force level. For the sarcoplasmic reticulum to sequester more calcium, more ATP is consumed by the Ca\(^{2+}\)-ATPase pump,7,23,29,33 leading to a disproportionately increased PVA-independent \( \dot{V}O_2 \) at any positive \( E_{\text{max}} \). However, the zero-\( E_{\text{max}} \) PVA-independent \( \dot{V}O_2 \) does not need to be increased, because zero \( E_{\text{max}} \) means a minimal amount of calcium involved in E-C coupling. This is consistent with our present result (Figure 7C). Therefore, we cannot discriminate between these two factors as the mechanism of the decreased E2 in our study.

The QRS complex increased by 6–8 msec in stunned hearts. This suggests some decrease in the synchronicity of ventricular contraction depressing global contractility without decreasing \( \dot{V}O_2 \) for E-C coupling.34 However, this increase in the QRS duration could have decreased \( E_{\text{max}} \) by only a few percent,34 which is too small to account for the decrease in \( E_{\text{max}} \) in stunned heart.

Slope of \( \dot{V}O_2 \)-PVA Relation

Because the reciprocal of the slope of the \( \dot{V}O_2 \)-PVA relation has been considered to reflect the contractile efficiency from the PVA-dependent \( \dot{V}O_2 \) to PVA,7,9 the decreased slope of the \( \dot{V}O_2 \)-PVA relation that we found in stunned myocardium means an increased contractile efficiency. The contractile efficiency is equal to the product of E1 and E3. One possible explanation for the increased contractile efficiency is an increased E1 due to a partial shift of metabolism from aerobic to anaerobic (i.e., glycolytic). However, the coronary arteriovenous lactate difference was not significantly decreased in stunned myocardium in this study. A shift of metabolic substrate from free fatty acid to glucose or lactate could improve E1, because P/O ratios of lactate (3.0) and glucose (3.2) are 7–14% higher than that of fatty acid (2.8).35 A recent study6 has shown that preferential utilization of carbohydrates does not occur during early reperfusion. Further, an increased E1, if otherwise unchanged, would also result in a decreased oxygen cost of contractility, opposite in direction to the present result. Therefore, it is unlikely that the smaller slope of the \( \dot{V}O_2 \)-PVA relation and the slightly increased contractile efficiency have resulted from changes of metabolic substrates. However, a more direct metabolic analysis is mandatory to negate any metabolic changes in stunned hearts.

Another possible explanation for the increased contractile efficiency is an increased ATP-to-PVA efficiency \((E_3)\). Changes in myosin ATPase activity, crossbridge cycling rate, and its on/off time ratio are known to affect the economy of contraction,36 which might in turn affect the contractile efficiency,14 although the relation between the economy and the efficiency remains to be elucidated.7 The only condition in which a reduced contractile efficiency was reported in association with a reduced economy is hyperthyroidism in the rabbit.14

With respect to this aspect, our previous studies7,9,37 have shown that the slope of the \( \dot{V}O_2 \)-PVA relation was not significantly affected by various inotropic interventions, even by catecholamines, which are known to decrease economy of contraction.38 Even myocardial cooling did not affect the \( \dot{V}O_2 \)-PVA slope despite a presumably slowed crossbridge cycling rate.7

We observed, only under a severely decreased coronary perfusion pressure,37 a similar tilting down of the slope of the \( \dot{V}O_2 \)-PVA relation to that seen in stunned myocardium. We explained the decreased slope by gradual decreases in \( E_{\text{max}} \) with increases in end-diastolic volume and PVA under a very low coronary perfusion pressure. This explanation may not satisfactorily explain the decreased slope in stunned myocardium in the present study, because coronary flow was not restricted (Figure 5B) and \( E_{\text{max}} \) did not decrease even at high PVA levels (Figures 4C and 4D).

However, under a stably depressed contractility, \( E_{\text{max}} \) would gradually increase with increases in end-diastolic volume that were due to the downward convexity of the nonlinear end-systolic P–V relation.39 If so, the unchanged \( E_{\text{max}} \) that we observed despite increases in end-diastolic volume in the present stunned hearts might indicate a gradually de-
pressed contractility and thereby a gradually decreased VO₂ for E–C coupling with increases in PVA. The decreased slope of the VO₂–PVA relation observed in stunned hearts could then be interpreted to have resulted from a gradually decreased contractility despite unchanged Eₘₐₓ with increases in PVA.

Even the sham group of series one showed decreases in the slope of the VO₂–PVA relation in the second and third loading runs at 60 and 120 minutes of observation (Figure 5D). This suggests a possibility that the decreased slope of the VO₂–PVA relation observed in stunned hearts occurred by the lapse of time after surgical preparation rather than by stunning per se. Nevertheless, mechanisms of the decreased slope of the VO₂–PVA relation and the apparently increased contractile efficiency in stunned myocardium remain to be elucidated. An increase in contractile efficiency might be an adaptive mechanism of insulted myocardium to compensate for the increased oxygen cost of contractility and minimize the increase in total myocardial oxygen consumption. Nevertheless, the increased contractile efficiency was too small to cancel out the increased oxygen consumption due to the increased oxygen cost of contractility in stunned hearts.

We used indomethacin to minimize occurrence of the systemic arterial hypotension and to stabilize the arterial pressure level of the support dog. However, a recent study showed that prostaglandin (endogenous vasodilator prostaglandin) attenuated postischemic contractile dysfunction of stunned myocardium. This result suggests that indomethacin could worsen stunned myocardium and that the mechanism of isovolumic diastolic proportion we observed might have been exaggerated. Therefore, the results in our stunned heart model must be carefully extrapolated to clinical cases.

In conclusion, we studied the relation between myocardial contractility and oxygen consumption of globally stunned myocardium in the excised cross-circulated dog heart. We demonstrated no significant decrease in PVA-independent VO₂ despite a depressed myocardial contractility in stunned myocardium at 60–120 minutes of reperfusion after a 15-minute global ischemia. The oxygen cost of contractility (or Eₘₐₓ) quantified by the slope of the relation between PVA-independent VO₂ and Eₘₐₓ was two times greater in stunned myocardium compared with preischemic myocardium. Although the oxygen cost of PVA was slightly decreased, the increased oxygen cost of Eₘₐₓ can primarily account for the disproportionately high VO₂ for Eₘₐₓ in stunned myocardium.

Acknowledgments

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References


**KEY WORDS** ischemia reperfusion ventricular contractility total mechanical energy excitation-contraction coupling
Increased oxygen cost of contractility in stunned myocardium of dog.
Y Ohgoshi, Y Goto, S Futaki, H Taku, O Kawaguchi and H Suga

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