Effect of Coronary Hyperemia on $E_{\text{max}}$ and Oxygen Consumption in Blood-Perfused Rabbit Hearts

Energetic Consequences of Gregg’s Phenomenon

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To assess the relation between increases in contractile function and oxygen consumption ($\text{Vo}_2$) during increased coronary flow (Gregg’s phenomenon), we measured the end-systolic pressure-volume relation and the relation between $\text{Vo}_2$ and left ventricular systolic pressure-volume area (PVA, a measure of total mechanical energy output) in blood-perfused, isovolumically contracting rabbit hearts during control and intracoronary adenosine infusion. During adenosine infusion at a constant perfusion pressure (93±11 mm Hg), coronary flow increased by 99±76\% ($p<0.01$), and the slope of the end-systolic pressure-volume relation, $E_{\text{max}}$ (ventricular contractility index), increased by 18±15\% ($p<0.01$). When compared at the same left ventricular volume, PVA increased by 20±14\% ($p<0.01$) and $\text{Vo}_2$ by 19±15\% ($p<0.01$) with adenosine. The $\text{Vo}_2$–PVA relation was linear under each condition (both median $r=0.98$). With increased coronary flow, the $\text{Vo}_2$-intercept of the $\text{Vo}_2$–PVA relation (unloaded $\text{Vo}_2$) increased by 22±18\% ($p<0.01$) without a change in the slope; that is, a parallel upward shift was observed, indicating that the contractile efficiency (energy conversion efficiency of the contractile machinery) remained constant. These increases in $E_{\text{max}}$ and unloaded $\text{Vo}_2$ were not eliminated by $\beta$-adrenergic blockade with propranolol. We conclude that increased coronary flow with adenosine at a constant perfusion pressure augments both $E_{\text{max}}$ and the nonmechanical energetic cost for excitation–contraction coupling and basal metabolism via nonadrenergic mechanisms, without changing contractile efficiency. (Circulation Research 1991;68:482–492)

Increased coronary perfusion pressure and flow enhances left ventricular contractile function and oxygen consumption ($\text{Vo}_2$) in the isolated heart$^{1-3}$ or in the in situ heart.$^{4-8}$ This is known as Gregg’s phenomenon.$^{13}$ However, in most of the previous studies, the effects of increased perfusion pressure and increased coronary flow were not separated.$^{1-4,6-8}$ More importantly, the relation between increases in contractile function and $\text{Vo}_2$ during Gregg’s phenomenon has not been assessed; that is, the energetic cost for the increase in mechanical function is unclear.

Recently, left ventricular pressure-volume area (PVA) has been proposed as a measure of total mechanical energy generated by the ventricle.$^{14,15}$ PVA is the area circumscribed by the end-systolic and end-diastolic pressure–volume relation and the systolic segment of the pressure–volume trajectory. The relation between $\text{Vo}_2$ and PVA is linear and independent of ventricular loading conditions. The reciprocal of the slope of the linear $\text{Vo}_2$–PVA relation has been considered to reflect the chemomechanical energy transduction efficiency of the contractile machinery, or contractile efficiency,$^{15,16}$ while its $\text{Vo}_2$-intercept reflects the oxygen cost of excitation–contraction coupling and basal metabolism.$^{14,15}$ Using the $\text{Vo}_2$–PVA relation, one can analyte the relation between a mechanical phenomenon and its energetic cost.

Thus, the purpose of the present study was to assess the relation between the increases in mechanical function and $\text{Vo}_2$ resulting from Gregg’s phenomenon by using the $\text{Vo}_2$–PVA relation. To this

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end, we used the VO₂–PVA relation as a tool to relate ventricular mechanics to energetics. In addition, to separate the effect of increased coronary flow from that of increased perfusion pressure, we induced coronary hyperemia with adenosine while keeping perfusion pressure constant.

Materials and Methods

Heart Preparation

Experiments were performed on the isolated, cross-circulated (blood-perfused) rabbit heart supported by anesthetized intact rabbits. The details of the surgical procedure have been described elsewhere. In brief, four rabbits (one heart donor, one blood donor, and two supporters) were premedicated with fentanyl (0.044 mg/kg i.m.) and droperidol (2.2 mg/kg i.m.), anesthetized with ketamine (20 mg/kg i.m.) and xylazine (1 mg/kg i.m.), tracheotomized, and ventilated with respirators (Harvard Apparatus, South Natick, Mass.). The anesthesia thereafter was maintained with supplemental intramuscular ketamine and xylazine. The chest of the heart donor rabbit (weight, 3.4±0.6 kg [mean±SD]) was then opened, and the innominate artery was cannulated and connected to the perfusion circuit. Heparinized (1,500 units/kg) arterial blood from the carotid arteries of the two support rabbits flowed to an arterial reservoir, from which the donor heart was perfused by a pump. Coronary venous blood draining from the right ventricle of the donor heart was returned to the external jugular veins of the support rabbits. The supported beating heart was excised from the chest cavity after cross-circulation was started, so that there was no interruption of the coronary circulation during the surgery. Mean coronary perfusion pressure was monitored and kept at a constant level between 80 and 110 mm Hg throughout each experiment.

To measure left ventricular pressure and volume, a thin latex balloon (unstretched volume, 4 ml) mounted on a Y-connector was placed in the left ventricle and secured with a purse-string suture around the mitral valve ring. Coronary blood flow was measured at the coronary venous drainage tube with a graduated cylinder by timed collection. The coronary arteriovenous oxygen content difference was measured continuously with an AVOX analyzer (A-VOX systems, San Antonio, Tex.) that was calibrated with a Lex O₂-Con oxygen content analyzer (Lexington, Waltham, Mass.).

The temperature of the excised heart was maintained at 36–37°C. To maintain arterial pH, PO₂, and PCO₂ of the support rabbits within physiological ranges, supplemental oxygen was given or the respiratory rate was changed if necessary. In addition, indomethacin (1 mg/kg i.v.) was given to maintain mean arterial pressure of the support rabbits above 60 mm Hg.

Experimental Protocol

Experiments were performed on nine isolated hearts. Under baseline contractile conditions, coronary perfusion pressure, electrocardiogram, left ventricular pressure, coronary blood flow, and coronary arteriovenous oxygen content difference were measured during steady-state isovolumic contractions. Left ventricular volume was then varied within a range between V₀ (at which peak isovolumic pressure was zero) and an arbitrary maximal volume at which peak systolic pressure exceeded coronary perfusion pressure or diastolic pressure reached approximately 13 mm Hg. After approximately 3 minutes were allowed to elapse, measurements were repeated when ventricular pressure and arteriovenous oxygen content difference stabilized at each new ventricular volume. This set of measurements was termed the control run.

Adenosine was then infused into the coronary perfusion tubing with an infusion pump (Harvard) at a constant rate between 20 and 200 μg/min (mean, 79±73 μg/min). Coronary flow rate was increased with the pump to maintain perfusion pressure constant. Measurements were repeated in the same manner as the control run. This set of measurements was termed the adenosine run.

In four additional hearts, measurements before and during adenosine were made under β-blockade with propranolol to determine if increased delivery of catecholamines to the myocardium was responsible for the increased mechanical function and VO₂ observed with adenosine. Propranolol in a bolus dose of 40 μg was administered into the coronary perfusion tubing before the control measurements, followed by continuous infusion at a rate of 4 μg/min throughout the experiment. The rates of adenosine infusion in this series of experiments were 25 μg/min in two experiments and 50 μg/min in the other two experiments.

Heart rate was held constant by left atrial pacing throughout the experiment.

Data Analysis

End-systolic pressure–volume relation. Data were recorded on a pen recorder and stored on computer disk at a sampling interval of 5 ms for off-line data analysis with a PDP 11/73 computer (Digital Equipment Corp., Marlboro, Mass.). Left ventricular diastolic and end-systolic pressures were determined as the minimal and peak pressures of the isovolumic contractions, respectively. Left ventricular volume was determined as the sum of the volume of saline within the left ventricular balloon and the volume of the balloon walls and connector within the left ventricle.

Linear regression analysis was performed on the end-systolic pressure–volume relation to obtain the slope, E_{max} and the volume axis intercept, V₀. Both E_{max} and V₀ were normalized for left ventricular weight.

Left ventricular oxygen consumption. Left ventricular VO₂ was calculated as follows. First, the total VO₂ per minute (ml O₂·min⁻¹) was calculated as the product of coronary blood flow (ml·min⁻¹) and coronary arteriovenous oxygen content difference (vol %) divided by heart rate to yield total VO₂ per beat.
(ml O₂ · beat⁻¹). Left ventricular thebesian flow was neglected because it is only 1–4% of the total coronary blood flow in this preparation.⁷ Left ventricular Vo₂ was then obtained by subtracting right ventricular Vo₂ from the total Vo₂. Because the right ventricle was kept mechanically unloaded and collapsed by continuous hydrostatic drainage, right ventricular Vo₂ was considered to be minimal, constant, and independent of left ventricular loading conditions. This right ventricular unloaded Vo₂ was calculated as biventricular unloaded Vo₂ times right ventricular weight divided by biventricular weight. Because biventricular unloaded Vo₂ could vary with changes in ventricular contractile state, we determined the unloaded right ventricular Vo₂ in each run, that is, during the control and adenosine runs. Left ventricular Vo₂ was normalized for 100 g left ventricular weight (in ml O₂ · beat⁻¹ · 100 g⁻¹).

**Pressure–volume area.** Left ventricular systolic PVA is the area that is surrounded by the end-systolic and diastolic pressure–volume relations and the systolic pressure–volume trajectory in the pressure–volume diagram. It consists of both external mechanical work and elastic potential energy in ejecting contractions, or potential energy only in isovolumic contractions in which external work is zero.⁵ We calculated PVA as the sum of a triangular area formed by the three straight lines connecting the end-systolic, minimal diastolic, and V₀ points and a narrow crescent area between the straight line connecting V₀ and the minimal diastolic point and the curvilinear diastolic pressure–volume relation.¹⁷ The triangular area was calculated as (Pₑ–Pₜ) × (V–V₀)/2, where Pₑ and Pₜ are end-systolic and diastolic pressures and V is ventricular volume. The narrow crescent area was calculated as Pₑ × (V–V₀)/4, because the diastolic pressure–volume relation is reasonably approximated by a third power of V–V₀.¹⁴ PVA (in mm Hg · ml·beat⁻¹·100 g⁻¹) was normalized for left ventricular weight.

The relation between left ventricular Vo₂ and PVA was obtained in each run. Linear regression analysis was used to determine the slope (in ml O₂ · mm Hg⁻¹ · ml⁻¹) and the Vo₂-intercept (in ml O₂ · beat⁻¹ · 100 g⁻¹) of each Vo₂–PVA relation.

**Contractile efficiency.** PVA is theoretically an expression of total mechanical energy and has the dimensions of energy because 1 mm Hg·ml=1.33×10⁻⁶ J. Also, under normal aerobic conditions, 1 ml O₂ consumed by myocardium is approximately equivalent to 20 J.¹⁵ Therefore, both Vo₂ (ml O₂·beat⁻¹·100 g⁻¹) and PVA (mm Hg·ml·beat⁻¹·100 g⁻¹) can be converted into the same unit of energy (J·beat⁻¹·100 g⁻¹). Thus, the dimensionless ratio of PVA to excess Vo₂ above the unloaded Vo₂ is the fraction of total mechanical energy output to energy input that is used exclusively for mechanical contraction, which reflects the chemomechanical energy transduction efficiency of the contractile machinery, or contractile efficiency.¹⁵ This contractile efficiency was estimated as the reciprocal of the slope of the linear Vo₂–PVA relation.¹⁷,¹⁸

**Oxygen cost of contractility.** Enhancement of myocardial contractility increases the oxygen cost of excitation–contraction coupling, while Vo₂ for basal metabolism remains constant.¹⁴ Further, changes in E_max during a positive inotropic intervention linearly correlate with changes in the Vo₂-intercept of the Vo₂–PVA relation.²¹ Therefore, in the absence of a change in the slope of the Vo₂–PVA relation, the ratio of an increase in the Vo₂-intercept to an increase in E_max can be considered a measure of the oxygen cost of increased contractility.²² The effect of increased coronary flow with adenosine on the oxygen cost of increased E_max was assessed as Δ%Vo₂-intercept/Δ%E_max and compared with reported values with other inotropic interventions in the literature.

**Statistics**

Comparisons of paired variables before and during adenosine infusion were performed by paired t test. The slopes and intercepts of the end-systolic pressure–volume relation and Vo₂–PVA relation before and during adenosine infusion were also compared by paired t test on the assumption that the slope and intercept values of individual regression lines reasonably represented their true values because the correlation coefficients were close to unity in every heart. In addition, analysis of covariance²³ was performed to assess slope and elevation differences between the two Vo₂–PVA relations before and during adenosine. A value of p<0.05 was considered statistically significant. Data are presented as mean±SD unless otherwise indicated.

**Results**

**Hearts Without β-Blockade**

In the nine experiments without β-blockade, left and right ventricular weight averaged 4.4±0.6 and 1.4±0.3 g, respectively. Heart rate was kept constant at 174±25 beats·min⁻¹ and mean coronary perfusion pressure was kept constant at 93±11 mm Hg in the control run and 93±10 mm Hg in the adenosine run. Coronary blood flow varied slightly according to changes in ventricular loading conditions at a constant perfusion pressure. Mean coronary flow averaged 4.6±1.5 ml·min⁻¹ or 0.79±0.21 ml·min⁻¹·g⁻¹ during the control run, and 8.7±2.5 ml·min⁻¹ or 1.52±0.50 ml·min⁻¹·g⁻¹ during the adenosine run. The increase in mean coronary blood flow during the adenosine run averaged 99.3±78.5% (p<0.01).

Figure 1 shows recordings of perfusion pressure, electrocardiogram, left ventricular pressure, and coronary arteriovenous O₂ difference during the control and adenosine run in one heart without β-blockade. During adenosine infusion at a constant perfusion pressure, coronary blood flow increased from 4.5 to 9.2 ml·min⁻¹, and left ventricular end-systolic pressure increased from 75 to 100 mm Hg despite the constant ventricular volume. Coronary arteriovenous O₂ difference decreased. Other hearts showed similar results.
Comparisons of variables obtained during the control and adenosine runs were made at the same maximal measured left ventricular volume of 1.1±0.4 ml (Figure 2). During adenosine infusion, coronary blood flow at this ventricular volume increased by 93.8±75.2% from 5.1±1.7 to 9.2±2.6 ml/min (p<0.01). Left ventricular end-systolic (i.e., peak isovolumic) pressure increased by 18.8±11.5% from 90±13 to 107±13 mm Hg (p<0.01). Left ventricular diastolic pressure increased by 1.0±0.9 mm Hg from 6.0±3.6 to 6.9±3.5 mm Hg (p<0.05). Left ventricular PVA increased by 19.5±14.4% from 563±232 mm Hg·ml·beat⁻¹·100 g⁻¹ (or 0.075±0.031 J·beat⁻¹·100 g⁻¹) to 661±250 mm Hg·ml·beat⁻¹·100 g⁻¹ (or

**Figure 1.** Representative recordings of coronary perfusion pressure, electrocardiogram (ECG), left ventricular (LV) pressure, and arteriovenous (A-V) O₂ difference before and during adenosine infusion. Increased coronary blood flow (CBF) at a constant perfusion pressure resulted in an increase in peak isovolumic LV pressure and a decrease in A-V O₂ difference at the same LV volume (LVV).

**Figure 2.** Comparisons of variables obtained at the maximal measured left ventricular volume before and during adenosine (Adeno). Panel A: Coronary blood flow. Panel B: Left ventricular end-systolic pressure. Panel C: Left ventricular end-diastolic pressure. Panel D: Left ventricular systolic pressure-volume area (PVA). Panel E: Coronary arteriovenous (A-V) oxygen content difference. Panel F: Left ventricular oxygen consumption (VO₂). *p<0.05, **p<0.01 by paired t test. Mean±SEM is indicated.
0.088 ± 0.033 J·beat⁻¹·100 g⁻¹ (p<0.01). Coronary arteriovenous O₂ difference decreased by 33.0 ± 14.5% from 8.3 ± 2.2 to 5.5 ± 1.7 vol% (p<0.01). As a result, VO₂ at the maximal measured left ventricular volume increased by 19.2 ± 15.1% from 0.043 ± 0.008 ml O₂·beat⁻¹·100 g⁻¹ (or 0.86 ± 0.15 J·beat⁻¹·100 g⁻¹) to 0.051 ± 0.008 ml O₂·beat⁻¹·100 g⁻¹ (or 1.01 ± 0.16 J·beat⁻¹·100 g⁻¹ (p<0.01).

Figure 3 shows the end-systolic pressure–volume relations (panel A) and VO₂–PVA relations (panel B) obtained during the control and adenosine runs in one heart. With adenosine, the slope of the end-systolic pressure–volume relation, Eₘₐₓ, increased with a near constant Vₒ value, indicating that the ventricular contractile state is enhanced with increased coronary blood flow. The relations between VO₂ and PVA were highly linear during both the control and adenosine runs. With increased coronary blood flow, the relation shifted upward in a parallel manner.

All nine hearts showed highly linear end-systolic pressure–volume relations as indicated by the median correlation coefficient of 0.983 during the control run and 0.989 during the adenosine run. On the average, normalized Eₘₐₓ increased by 18.4 ± 15.4% from 7.9 ± 4.5 to 9.2 ± 5.0 mm Hg·ml⁻¹·100 g (Figure 4A, p<0.01), whereas normalized VO₂ did not change significantly (Figure 4B, 9.8 ± 2.8 versus 10.1 ± 2.7 ml·100 g⁻¹).

The VO₂–PVA relation was also highly linear in each heart: the median correlation coefficient of the nine hearts was 0.978 during the control run and 0.980 during the adenosine run. The slope of the VO₂–PVA relation did not change significantly with adenosine (Figure 4C, 2.18 ± 10⁻⁵ ± 0.61 ± 10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹ or 3.28 ± 0.91 [dimensionless] versus 2.19 ± 10⁻⁵ ± 0.60 ± 10⁻⁵ ml O₂·mm Hg⁻¹·ml⁻¹ or 3.30 ± 0.89 [dimensionless]), whereas the VO₂-intercept did increase by 22.1 ± 17.6% from 0.030 ± 0.004 ml O₂·beat⁻¹·100 g⁻¹ (or 0.60 ± 0.09 J·beat⁻¹·100 g⁻¹) to 0.036 ± 0.005 ml O₂·beat⁻¹·100 g⁻¹ (or 0.73 ± 0.11 J·beat⁻¹·100 g⁻¹) (Figure 4D, p<0.01). Directly measured unloaded VO₂ also increased significantly by the same amount from 0.030 ± 0.005 ml O₂·beat⁻¹·100 g⁻¹ (or 0.60 ± 0.09 J·beat⁻¹·100 g⁻¹) to 0.036 ± 0.005 ml O₂·beat⁻¹·100 g⁻¹ (or 0.73 ± 0.10 J·beat⁻¹·100 g⁻¹) (p<0.01). Analysis of covariance indicated no slope difference between the two linear regression lines during the control and adenosine runs in any of nine hearts, whereas it indicated a significant upward shift of the regression line during the adenosine run in all nine hearts (all p<0.01). Average contractile efficiency calculated from the slope of the VO₂–PVA relation did not differ between the two runs (32.1 ± 6.5% versus 32.1 ± 7.6%). Thus, the contractile efficiency remained constant during the adenosine run despite the doubled coronary blood flow, while mechanically unloaded left ventricular VO₂ increased significantly.

Oxygen cost of increased Eₘₐₓ, calculated as Δ%VO₂-intercept/Δ%Eₘₐₓ during adenosine, averaged 1.26 ± 0.80 (dimensionless). By unpaired t test, this was not significantly different (both p>0.2) from previously reported values for calcium (1.29 ± 1.41) and epinephrine (0.87 ± 0.38).14

Figure 5 shows the correlation between an increase in coronary blood flow and an increase in Eₘₐₓ (Figure 5A) or VO₂-intercept (Figure 5B) in the nine hearts without β-blockade. Increases in Eₘₐₓ and VO₂-intercept were correlated linearly with an increase in coronary blood flow, indicating that both increases in Eₘₐₓ and VO₂-intercept during adenosine infusion are attrib-
utable to the increased coronary blood flow, resulting from coronary vasodilation by adenosine.

Hearts With β-Blockade

In the four hearts studied under β-blockade, heart rate was held constant at 159±10 beats/min, and mean coronary perfusion pressure was kept constant at 87±5 mm Hg during the control run and 85±8 mm Hg during the adenosine run. With adenosine infusion, average coronary blood flow increased significantly by 75.5±48.8% (Figure 6A, p<0.05). Left ventricular end-systolic pressure at the maximal measured ventricular volume of 1.0±0.1 ml increased by 12.3±7.4% (Figure 6B, p<0.05). Simultaneously,

FIGURE 5. Correlations between increase in coronary blood flow (CBF) and increase in Emax (panel A) or increase in VO2-intercept of the VO2-pressure-volume area relation (panel B) during adenosine in nine experiments. Values are expressed as percent change from the respective control values.
Our results indicate that increased coronary blood flow produced by adenosine augments both the ventricular contractility index $E_{\text{max}}$ and nonmechanical energetic costs (excitation–contraction coupling plus basal metabolism) via non-$\beta$-adrenergic mechanisms, without changing the contractile efficiency.
This conclusion is based on the following major findings: 1) increased coronary blood flow with adenosine at a constant perfusion pressure increases $E_{\text{max}}$; 2) increased coronary blood flow increases the unloaded $V_0$ without changing the slope of the $V_0$-PVA relation, and 3) $\beta$-adrenergic blockade does not eliminate these increases in $E_{\text{max}}$ and unloaded $V_0$.

Since Gregg et al. observed that elevation of coronary perfusion pressure increases $V_0$, there have been many reports of increased cardiac contractile and metabolic function during increased coronary perfusion pressure and flow. However, in most of these studies, both perfusion pressure and coronary flow were increased simultaneously, and therefore, the effect of increased perfusion pressure could not be separated from that of increased flow. In addition, previously observed enhancement of contractile and metabolic function with elevated perfusion pressure and flow may have been due to an improvement in underlying ischemic conditions, especially in the subendocardial layer, because hearts were perfused with crystalloid solution 2.4-6 or the perfusion pressure was low (40-60 mm Hg) during control measurements. 1.4.5.9.10.12 In contrast, our cross-circulated heart preparation is perfused with arterial blood from intact support animals at a constant, normal perfusion pressure, eliminating the possibility of ischemia during the control measurements.

Arnold et al. claimed that perfusion pressure, rather than flow, is the primary determinant of Gregg’s phenomenon, because increased coronary flow during hypoxia at a constant perfusion pressure did not increase either left ventricular systolic pressure or $V_0$, whereas increased perfusion pressure during dextran administration at a constant flow did increase both left ventricular systolic pressure and $V_0$. However, a negative inotropic effect of hypoxia itself might have masked increases in both contractile function and $V_0$ during increased coronary flow, whereas an increase in intracellular calcium induced by the hypertonic dextran perfusate itself might have enhanced systolic pressure and $V_0$. In contrast, Abel and Reis considered coronary blood flow the independent determinant of contractile function and $V_0$ because increased blood flow produced by nitroglycerin at a constant perfusion pressure increased both $V_{\text{max}}$ and $V_0$, whereas decreased perfusion pressure with nitroglycerin at a constant blood flow did not change $V_{\text{max}}$ or $V_0$. Although they did not assess ventricular contractility using $E_{\text{max}}$, their results are in accordance with ours.

The relation between increased contractile function and $V_0$ during increased coronary flow has not been systematically analyzed previously. Although Miller et al. observed that decreasing left ventricular volume to keep systolic pressure constant during increased coronary blood flow eliminated the increase in $V_0$, they could not logically explain the mechanism. On the basis of our results, the observation of Miller et al. may be explained by the offsetting effects of a decrease in mechanical $V_0$ caused by decreased PVA (decreased ventricular volume) and an increase in unloaded $V_0$ (or nonmechanical $V_0$) resulting from increased coronary flow.

The present study demonstrates a constant contractile efficiency and a significant increase in unloaded $V_0$ during increased coronary blood flow. The finding that contractile efficiency remained constant despite a significant increase in $E_{\text{max}}$ indicates that the chemomechanical energy transduction efficiency (excess $V_0$ to PVA efficiency) of the contractile machinery did not change during increased coronary blood flow. This is in accordance with previous findings in which $E_{\text{max}}$ was enhanced with calcium and epinephrine. Thus, following all acute positive or negative inotropic interventions tested to date, the contractile efficiency has not changed. In contrast, our previous study has demonstrated a decreased contractile efficiency in hyperthyroid rabbit hearts in which the myosin isoform component is chronically altered.

The increase in unloaded $V_0$ with an increase in $E_{\text{max}}$ is also in accordance with previous reports. Intriguingly, the oxygen cost of enhanced $E_{\text{max}}$ during increased coronary flow was similar to that reported with calcium and epinephrine. The oxygen cost of $E_{\text{max}}$ would be affected by an uncoupling of mitochondrial oxidative phosphorylation, a change in the calcium:ATP coupling ratio in the sarcoplasmic reticulum, or an altered calcium sensitivity of the myofilaments. Therefore, the similar oxygen cost of enhanced $E_{\text{max}}$ between adenosine and calcium or epinephrine suggests that the above changes did not occur or were negligibly small during increased coronary flow with adenosine.

A potential source of error in the present study is our assumption that thebesian flow does not change during adenosine infusion. Although we did not accurately measure thebesian flow in the present study, we knew it was small in magnitude (0.1-0.4 ml/min under control conditions). Also, we did not note a remarkable change in the rate of blood dripping from the left ventricular vent during adenosine infusion. In addition, because we excluded left ventricular thebesian flow from the $V_0$ calculation, $V_0$ would have been underestimated if thebesian flow had increased during adenosine. However, the result we observed was opposite in direction; that is, $V_0$ increased during adenosine infusion. Therefore, the error caused by changes in thebesian flow, if any, cannot explain the increase in $V_0$ during adenosine infusion.

There are several possible mechanisms for increased $E_{\text{max}}$ and $V_0$ with increased coronary blood flow. First, the possibility of an increase in the inotropic state of individual myocytes caused by a direct effect of adenosine should be considered. However, most prior studies have shown that adenosine itself does not have a direct positive inotropic effect on mammalian ventricular myocytes or muscle strips. It does exert an indirect negative
inotropic effect by attenuating the positive inotropic action of isoproterenol. Unlike these studies, Bruckner et al. and Wilson and Broadley observed a direct positive inotropic effect of a high dose of adenosine (100–1,000 μM) in guinea pig ventricular muscles. However, the magnitude of the increase in tension was very small (less than 3%) at a concentration equivalent to that used in the present study (approximately 30 μM). Therefore, it is unlikely that any direct effect of adenosine can explain the increase in E\text{max} observed in our study.

Second, in the present study, pretreatment with propranolol did not suppress the increase in peak isovolumic pressure, E\text{max} or VO\text{2} during increased coronary flow with adenosine. Thus, the possibility that increased delivery of circulating catecholamines from the support animal to the isolated heart, caused by increased coronary flow, results in an enhanced E\text{max} is also remote.

Third, an increase in basal metabolism caused by increased flow is a possible mechanism for the increase in VO\text{2} during increased coronary flow. Gibbs and Kotsans reported a linear dependence of VO\text{2} for basal metabolism on coronary blood flow. However, the magnitude of increased VO\text{2} caused by this mechanism (0.4–0.7 ml O\text{2}·min^{-1}·100 g^{-1} for a 1 ml·min^{-1}·g^{-1} increase in flow) does not appear to be large enough to account for the total increase in VO\text{2} during increased coronary flow in the present study (1.34 ml O\text{2}·min^{-1}·100 g^{-1} for a 0.73 ml·min^{-1}·g^{-1} increase in flow). In addition, this mechanism by itself does not explain the increase in mechanical function during increased coronary flow.

Fourth, there may be an increase in myocardial fiber length in the mid or outer layer of the ventricular wall when wall thickness increases during increased coronary flow at a constant chamber volume. In fact, an increase in ventricular wall thickness or epicardial circumference has been observed during increased coronary flow. In addition, Poche et al. reported an increase in sarcomere length with increases in coronary perfusion pressure and flow. This increase in sarcomere length should result in increases in both generated force and myocardial oxygen consumption via the Frank-Starling mechanism even at a constant inotropic state. To determine the potential role of this effect, we hypothesized a midwall fiber length model, assuming spherical geometry, which is presented in the “Appendix.” This midwall model, by itself, explains about 71% of the increase in VO\text{2} at maximal measured left ventricular volume during increased coronary flow with adenosine but may not completely explain the parallelism of the two VO\text{2}–PVA relation lines. Therefore, additional mechanisms may be needed to completely account for our results.

In this regard, Krams et al. recently proposed that the time-varying elastance concept for the left ventricle also applies to the lumen of the coronary vasculature. If so, the left ventricular E\text{max} measured could be a composite of the end-systolic elastance of both the left ventricular myocardium and the coronary vasculature. Adenosine preferentially dilates coronary microvessels (between arterioles <170 μm in diameter and venules <150 μm in diameter), that is, capacitance vessels. Therefore, it is conceivable that adenosine increases the elastance of the coronary vasculature, leading to an increase in resultant left ventricular E\text{max}. This hypothesis merits further study.

Finally, Kitakaze and Marban recently reported an increase in the intracellular calcium transient during systole when coronary perfusion pressure and flow were elevated in isolated ferret hearts. A positive inotropic effect caused by increased intracellular calcium could explain both the parallel upward shift of the VO\text{2}–PVA relation and the close agreement of the oxygen cost of E\text{max} with increased coronary flow and calcium, which were observed in the present study. However, Kitakaze and Marban used a crystalloid-perfused heart preparation, in which coronary flow was unphysiologically high (3–7 ml·g^{-1}·min^{-1}), coronary autoregulation was absent, and the magnitude of the increase in developed pressure (approximately 100% increase for doubled coronary flow) was remarkably larger than that observed in our blood-perfused heart preparation (only a 19% increase in peak systolic pressure for doubled coronary flow). More importantly, the primary mechanism of the increase in intracellular calcium transients during increased coronary flow remains obscure. For example, an increase in fiber length in the mid to outer layer might result in an increase in intracellular calcium transients. Alternatively, there could be a direct, flow-induced increase in intracellular calcium concentration, such as that observed in endothelial cells.

Further study will be needed to determine the primary mechanism responsible for the increase in mechanical and metabolic function during increased coronary blood flow under more physiological conditions.

**Appendix**

Figure A1 illustrates our hypothesis with a midwall fiber length model of the left ventricle in the pressure–volume (upper panel) and VO\text{2}–PVA diagrams (lower panel). In the present study, peak isovolumic pressure and E\text{max} increased by approximately 20% during increased coronary blood flow with adenosine, and the end-systolic point moved from point C to A in the pressure–volume diagram, resulting in a 20% increase in PVA (Figure A1, upper panel). In contrast, with the midwall fiber length model, we considered a ventricle in which all myocytes have the same fiber length (equal to that of the midwall layer fiber of the real ventricle at a ventricular lumen volume V\text{c}) and assumed that the contractility of individual myocytes remains unchanged during adenosine. In this model, ventricular lumen volume in the control state is the same as that of the real ventricle (V\text{c}). During adenosine administration, midwall fiber length would increase because of an increased ven-
Assuming that the radius (r) to wall thickness (h) ratio is 1 (i.e., r=h), an increase in wall thickness (h') during a 3.8% increase in midwall circumference can be calculated from the equation \( \frac{r+(h+h')}{2r} = 1.038 \), yielding an incremental ratio h'/h = 0.114. This value is very close to the reported value of an increase in wall thickness (9-11%) during increased coronary blood flow. Thus, it seems realistic that an 11.4% increase in wall thickness results in a 3.8% increase in midwall circumference, a 20% increase in \( E_{\text{max}} \), and a 44% increase in PVA in the midwall fiber length model.

In the VO2-PVA diagram (Figure A1, lower panel), PVA of the real ventricle increased by approximately 20% from PVA_{C} to PVA_{A}, and VO2 increased from VO2_{C} to VO2_{A} during increased coronary blood flow. Thus, the operating point moved from point C to A, resulting in an upward shift of the VO2-PVA relation line. However, with the midwall model, the operating point would move from point C to B along the control VO2-PVA relation line with some additional increase in VO2 for basal metabolism.

Because control PVA at maximal measured left ventricular volume was 563 mm Hg·ml·beat\(^{-1}\)·100 g\(^{-1}\), and the slope of the control VO2-PVA relation was 2.2×10\(^{-5}\) ml O\(_2\)·mm Hg\(^{-1}\)·ml\(^{-1}\) in the present study, a 44% increase in PVA amounts to 0.0055 ml O\(_2\)·beat\(^{-1}\)·100 g\(^{-1}\). Thus, the midwall model can explain 71% of the total increase in VO2 (0.0077 ml O\(_2\)·beat\(^{-1}\)·100 g\(^{-1}\)) observed during increased coronary blood flow with adenosine. The remaining portion of the increase in VO2 cannot be explained by this midwall model and, hence, can be attributed to other factors, for instance, an increase in basal metabolism or an error caused by the assumption of spherical geometry.

One confounding point of this hypothesis is that at a low ventricular volume range, the amount of increase in midwall PVA would not be large enough to explain the increase in VO2 during adenosine. If the increase in PVA is smaller in a low ventricular volume range, the resultant VO2-PVA relation line during increased coronary blood flow would have a higher slope value than the control line. Thus, some other mechanism may be required to explain the parallelism of the two VO2-PVA relations.

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