Cardiac Cooling Increases E_{max} Without Affecting Relation Between O_2 Consumption and Systolic Pressure-Volume Area in Dog Left Ventricle

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We studied the effects of cardiac cooling by 7 ± 2° C (SD) from 36° C on both contractility index (E_{max}) and the relation between O_2 consumption per beat ($\dot{V}O_2$) and systolic pressure-volume area (PVA) of the left ventricle in the excised cross-circulated dog heart preparation. PVA represents the total mechanical energy generated by a contraction. The $\dot{V}O_2$-PVA relation divides measured $\dot{V}O_2$ into unloaded $\dot{V}O_2$ and excess $\dot{V}O_2$. The slope of the $\dot{V}O_2$-PVA relation represents inversely the efficiency of the contractile machinery to convert chemical energy from the excess $\dot{V}O_2$ to total mechanical energy. Cooling is known to decrease myosin ATPase activity (Q_{10} of 2–3), which in turn is expected to increase the chemomechanical efficiency of cross bridges. Therefore, we expected an increase in the efficiency and hence a decreased slope of the $\dot{V}O_2$-PVA relation with cooling. The cooling increased E_{max} by 46 ± 13% and the time to E_{max} by 45 ± 27%. Pacing rate was constant or had to be slightly decreased to avoid arrhythmias with cooling. We found that neither the slope of the $\dot{V}O_2$-PVA relation nor unloaded $\dot{V}O_2$ significantly ($p>0.05$) changed with the cooling. This result contradicts the expected increase in the efficiency with cooling. We conclude that cardiac cooling by 7° C from 36° C does not increase the efficiency of the contractile machinery in excised cross-circulated dog left ventricle. (*Circulation Research* 1988;63:61–71)

We have shown that the total mechanical energy generated by each contraction of the ventricle can be quantified by the systolic pressure-volume (P-V) area (PVA).[1,2] PVA is the area circumscribed by the end-systolic (ESPVR) and end-diastolic P-V relation (EDPVR) curves and the systolic P-V trajectory in the P-V diagram as shown schematically in Figure 1A. PVA is the sum of external mechanical work performed during systole and mechanical potential energy stored at end-systole as shown in Figure 1A. Our previous studies have shown that O_2 consumption ($\dot{V}O_2$) of the left ventricle per beat correlates closely and linearly with PVA regardless of ventricular loading conditions at a stable level of contractility index, E_{max}.[1,2] This is shown schematically in Figure 1B. The $\dot{V}O_2$-PVA relation allows us to divide $\dot{V}O_2$ into two parts: the unloaded $\dot{V}O_2$ represented by the $\dot{V}O_2$-axis intercept, and the excess $\dot{V}O_2$ linearly correlated with PVA.[1,2] When E_{max} increases with epinephrine or Ca^{2+}, the $\dot{V}O_2$-PVA relation considerably shifted upward without changes in its slope,[1,2] as shown in Figure 1C.

In interpreting these findings, we have introduced the efficiency of contractile machinery (EFF_{cm}) in converting the excess $\dot{V}O_2$ into PVA.[1,2] This efficiency is equal to the reciprocal of the slope of the $\dot{V}O_2$-PVA relation when both $\dot{V}O_2$ and PVA are expressed in Joules per beat per 100 grams to make the slope dimensionless,[1,2] as shown by a family of isoefficiency lines in Figure 1D. Our previous studies have shown that this efficiency remains largely unchanged despite the increases in E_{max} by epinephrine or Ca^{2+} and the simultaneous increases in unloaded $\dot{V}O_2$,[1,2] as shown schematically by the parallel elevation of the $\dot{V}O_2$-PVA relation in Figure 1C. EFF_{cm} is physiologically meaningful and useful

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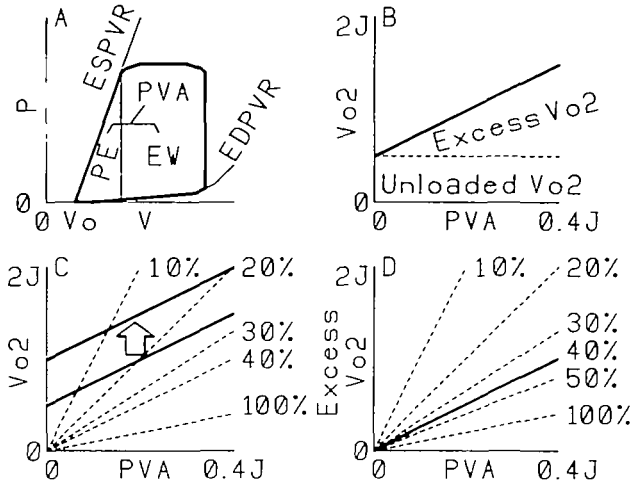

FIGURE 1. Panel A: *Schematic drawing of left ventricular pressure-volume (PV) diagram, end-systolic PV relation (ESPVR) line, end-diastolic PV relation (EDPVR) curve, and systolic PV area (PVA). PVA is the area circumscribed by the thick lines. PVA consists of elastic potential energy (PE) and external work (EW). Vo is volume at which peak isovolumic pressure is zero, and serves as the volume-axis intercept of ESPVR.* Panel B: *Schematic drawing of the relation (heavy diagonal line) between myocardial oxygen consumption ($\dot{V}O_2$) per beat and systolic PV area (PVA) of variously loaded contractions in a stable contractile state with relatively constant E_{max}. Unloaded $\dot{V}O_2$, or the Vo-axis intercept of the $\dot{V}O_2$-PVA relation, is assumed to exist commonly in all contractions in a given contractile state. Excess $\dot{V}O_2$ is equal to the fraction of $\dot{V}O_2$ above the unloaded $\dot{V}O_2$.* Panel C: *Schematic drawing of $\dot{V}O_2$-PVA relations in control contractile state and enhanced contractile state with epinephrine. The arrow indicates parallel elevation of the relation with epinephrine. Family of dashed diagonal lines indicates isoefficiency lines from $\dot{V}O_2$ to PVA as labelled by percent values.* Panel D: *Schematic drawing of the relation between excess $\dot{V}O_2$ and PVA, which is drawn by shifting down the two $\dot{V}O_2$-PVA relations in Panel C by their unloaded $\dot{V}O_2$. Family of dashed diagonal lines indicates isoefficiency lines from excess $\dot{V}O_2$ to PVA as labelled by percent values. Efficiency corresponds to EFF_{cm} focused on in this study.*

when we consider the excess $\dot{V}O_2$ and PVA to represent myocardial energy input to the contractile machinery and total mechanical energy output from it, respectively.[1,2]

This EFF_{cm} must be differentiated from the following three efficiencies: 1) the conventional mechanical efficiency of the ventricle as a pump to perform external mechanical work (EW) (EFF_{ew}); 2) the efficiency from the total $\dot{V}O_2$ to PVA (EFF_t); and 3) the efficiency of myofibrillar chemomechanical energy transduction from ATP to PVA (EFF_{mf}). Briefly, EFF_{ew} is defined as either $EW/\dot{V}O_2$ or EW/(excess $\dot{V}O_2$) and is a complex function of ventricular preload, afterload, heart rate, and contractile state.[3] EFF_t is defined as $PVA/\dot{V}O_2$, which is shown as a family of isoefficiency lines in Figure 1C. This efficiency changes with changes in unloaded $\dot{V}O_2$ even when EFF_{cm} remains unchanged,[1-3] as can be seen in Figure 1C. EFF_{mf} is defined as PVA/(ATP used by crossbridge cycling) and is greater than EFF_{cm} by not including the efficiency (60–70%) of ATP production in the oxidative phosphorylation.[2,4]

In the present study, we investigated the effect of cardiac cooling on both the unloaded $\dot{V}O_2$ and the slope of the $\dot{V}O_2$-PVA relation or the EFF_{cm}. Cooling (>20° C) generally increases peak contractile force, slows down the speed of contraction, and prolongs the duration of myocardial and ventricular contraction.[5-11] Although cardiac cooling augments left ventricular systolic pressure and $\dot{V}O_2$ in excised cross-circulated dog hearts,[6] it remains unknown whether and how cooling affects the unloaded $\dot{V}O_2$ and EFF_{cm}. EFF_{mf} (but not EFF_{cm}) seems to be inversely related to the myosin ATPase activity and crossbridge cycling rate,[12-14] and cooling decreases myosin ATPase activity at a Q_{10} of 2–3.[15-18] Therefore, in the present study, we expected EFF_{cm} to increase and then the slope of the $\dot{V}O_2$-PVA relation to decrease with cooling. Instead, we found that cardiac cooling by 7° C from 36° C, which increased E_{max} significantly, did not significantly change the slope of the $\dot{V}O_2$-PVA relation.

Materials and Methods

Preparation

We made an excised cross-circulated heart preparation from two adult mongrel dogs in each experiment as previously described.[1,2] Briefly, the dogs were anesthetized with a mixture of urethan (500 mg i.v.) and α-chloralose (50 mg i.v.) after premedication with ketamine hydrochloride (8 mg i.m.). The dogs were heparinized (1,000 IU/kg). Arterial and venous cross-circulation tubes were cannulated into the common carotid arteries and jugular vein in the larger dog (support). The smaller dog (heart donor, 11±1 [SD] kg) was thoracotomized midsternally, and the cross-circulation tubes from the support dog were cannulated into the left subclavian artery and the right ventricle via the right atrial appendage. Then, the heart was isolated from the systemic and pulmonary circulation by ligating the azygos vein, descending aorta, inferior and superior venae cavae, brachiocephalic artery, and bilateral pulmonary hili. Cross circulation was then started. The supported beating heart was excised from the chest.

The left atrium was opened widely and all chordae tendineae were cut. A thin latex balloon (unstressed volume>50 ml) was placed in the left ventricle, and its mouth was fixed at the mitral anulus.[1,2] The cable of a Konigsberg P-7 miniature pressure gauge in the apical end of the balloon was pulled out through an apical stab. The balloon was connected to the same servo-controlled pump used in previous studies,[1,2] and the balloon and pump were primed with water. The servo pump precisely

controlled and accurately measured left ventricular volume.[1,2]

The temperature of the left ventricle was measured with a small thermistor probe (model TF-DNP-1, Terumo, Tokyo, Japan) placed between the endocardium and the balloon via the apical stab wound. The heart temperature was controlled by gradually cooling or warming the arterial cross-circulation tube coiled in a thermostat bath. The rectal temperature of the support dog was measured with a thermometer and maintained at 36–38° C with the venous cross-circulation tube coiled in another thermostat bath. Heart rate was paced electrically with electrodes on the left atrium.

The systemic arterial blood pressure of the support dog served as the coronary perfusion pressure. Occurrence of systemic hypotension under cross circulation was minimized with diphenhydramine hydrochloride (30–60 mg i.m.). Phenylephrine (5–10 mg i.m.) was given when hypotension occurred. The mean perfusion pressure level was constant (>70 mm Hg and with an allowance of 5 mm Hg) in each experiment. When it decreased by more than the allowance, 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 at each time. When the mean perfusion pressure was overcompensated, we waited for data collection until it fell within the allowance.

After the experiment, the left ventricle including the septum (LV) and the right ventricular free wall (RV) were weighed. LV weighed 67±8 g and RV weighed 22±3 g.

E_{max} and T_{max}

We obtained E_{max} as an index of ventricular contractile state as previously described.[1,2] Briefly, we first determined Vo as the ventricular volume at which peak isovolumic pressure was zero (Figure 1A). Then, we calculated the slope of the line connecting Vo and an instantaneous P-V point drawing the P-V trajectory of a given contraction. We identified end systole as the P-V point at which the slope of the line became maximum. This line and its maximum slope were identified as the ESPVR line and E_{max}, respectively, of this contraction. The time from the onset of contraction identified as the rising limb of the R wave of left ventricular ECG to E_{max} was determined as T_{max}. We determined both E_{max} and T_{max} from ventricular pressure and volume signals of individual contractions on-line with a signal processing computer (model 7T17, NEC Sanei, Tokyo, Japan) at a sampling rate of 500 Hz.

We also determined the maximum rate of ventricular pressure rise (dP/dt_{max}) and its time (τ_{max}) of individual contractions from the same pressure signal used for determinations of E_{max} and T_{max} with the same signal processor.

Pressure-Volume Area

PVA is the area circumscribed by the ESPVR line, the EDPVR curve, and the systolic P-V trajectory,[1,2] as shown schematically in Figure 1A. We determined PVA from 500 Hz sampled ventricular pressure and volume data on-line with the signal processing computer. The algorithm of PVA computation was described elsewhere.[1,2] Briefly, it was obtained as the area swept by the line (mentioned above) connecting Vo and an instantaneous P-V point drawing the systolic P-V trajectory in individual contractions. PVA was expressed in mm Hg · ml/beat or in J/beat, where 1 mm Hg · ml is physically equivalent to 1.33×10^{-4} J.[1,2] PVA was normalized for 100 g left ventricle.

O_2 Consumption

The total coronary flow through the heart preparation was measured with an electromagnetic flowmeter (model MVF-2100, Nihon Kohden, Tokyo, Japan) by placing an in-line probe (FF-050T) in the cross-circulation venous tube, which continuously drained all coronary venous blood from the right heart. We neglected the left ventricular thebesian venous blood flow because of its small fraction (less than 3%) in the total coronary flow.[1,2] Coronary arteriovenous O_2 content difference was measured with a continuous arteriovenous O_2 difference analyzer[19] (A-VOX Systems, San Antonio, Texas), which was calibrated against a Lex-O_2-Con O_2 content analyzer in each experiment. The transit time of coronary venous blood from the right heart to the A-VOX cuvette was only 10–20 seconds.

O_2 consumption of the heart was determined as the product of coronary flow and arteriovenous O_2 content difference with the signal processor. $\dot{V}O_2$ per beat was obtained by dividing O_2 consumption per minute by heart rate in a steady state and was expressed in milliliters oxygen per beat or Joules per beat, where we assumed 1 ml O_2 biochemically equivalent to 20 J.[1,2] It was also normalized for 100 g left ventricle after eliminating the right ventricular free wall fraction of $\dot{V}O_2$ by the following method.

We minimized the contribution of right ventricular free wall $\dot{V}O_2$ to the measured total $\dot{V}O_2$ by keeping the right ventricle collapsed by continuous hydrostatic drainage of the coronary venous blood in the right heart. We assumed that $\dot{V}O_2$ of the unloaded right ventricular free wall was equal to (RV free wall weight)/(LV weight including septum weight + RV free wall weight) times the total unloaded $\dot{V}O_2$ measured when both right and left ventricles were collapsed. The weight fraction of RV/(LV + RV) was 0.25±0.03 (SD). We assumed that $\dot{V}O_2$ of the unloaded right ventricular free wall was constant independent of left ventricular loading conditions in a given heart in a given contractile state.[1,2] This constant right ventricular unloaded $\dot{V}O_2$ was subtracted from the measured total $\dot{V}O_2$ to determine left ventricular $\dot{V}O_2$ in each contractile

state in individual hearts. This correction of $\dot{V}O_2$ was performed in each temperature run (see below). Hereafter, $\dot{V}O_2$ represents $\dot{V}O_2$ of the left ventricle. It will be divided into the excess $\dot{V}O_2$ and the unloaded $\dot{V}O_2$ of the left ventricle, as seen in Figure 1B.

Experimental Protocol

We paced the heart at a constant rate slightly above the natural sinus rhythm observed at the beginning of each experiment in all 11 hearts. Pacing rate was fixed constant at 142±11 beats/min throughout the experiment in five of 11 hearts. However, in the other six hearts, pacing rate had to be decreased by 5–30% with cardiac cooling to avoid either pulsus alternans or incomplete atrioventricular block. On the average, heart rate after cooling was lower by 15±13% than before cooling. The pacing rate after warming was returned to the same level as before cooling in nine of these 11 hearts. In the other two hearts, which were warmed to 40–41° C, the sinus rhythm exceeded the pacing rate by 4–12%, and the pacing was stopped to avoid arrhythmias.

High-temperature run. At first, heart temperature was 36–37° C (36.2±0.4° C). Ventricular volume of ejecting contraction was set to a middle volume range (10–30 ml). We waited for left ventricular pressure, coronary flow, and O_2 content difference to stabilize under this condition. Then, we obtained $\dot{V}O_2$ and PVA of steady-state contractions under 5–10 different preload and afterload conditions including unloaded contraction at Vo. Between adjacent conditions, we waited 2–3 minutes until steady state was reached. We obtained the data twice (or exceptionally, three times) at an interval of 0.5–1 minute under each loading condition to confirm reproducibility of the data under each given loading condition and also to increase the number of data within a short period (15–30 minutes) for each run. We used all the sampled $\dot{V}O_2$ and PVA data except for those in arrhythmic contractions. In each heart, 10~25 data were successfully sampled. Eleven hearts were subjected to this run.

Low-temperature run. Following the high-temperature run, we gradually cooled the heart for 10–30 minutes to an arbitrary constant level between 26 and 33° C (29.6±2.1° C). The cooling amounted to 6.6±2.1° C. We then obtained $\dot{V}O_2$ and PVA from steady-state contractions under 5–10 different preload and afterload conditions including unloaded contraction at Vo in a manner similar to those in the high-temperature run. We obtained the data twice (or exceptionally, three times) at an interval of 0.5–1 minute under each loading condition. We also used all the sampled $\dot{V}O_2$ and PVA data except for those in arrhythmic contractions. It also took 15–30 minutes. In each of the 11 hearts, 10~20 samples were successfully obtained.

Re-high-temperature run. Finally, we warmed the heart gradually over 10–30 minutes to 36–37° C in nine hearts and to 40–41° C in two hearts (37.2±1.8° C). The warming amounted to 7.2±2.2° C. We obtained $\dot{V}O_2$ and PVA from steady-state contractions under 5–10 different preload and afterload conditions including unloaded contraction at Vo in a manner similar to those in both high- and low-temperature runs. We also obtained the data twice (or exceptionally, three times) at an interval of 0.5–1 minute under each loading condition. We also used all the sampled $\dot{V}O_2$ and PVA data except for those in arrhythmic contractions. It also took 15–30 minutes. In each of the 11 hearts, 10~20 samples were successfully obtained.

Data Analysis

Similar to our previous studies,[1,2] we studied correlation between $\dot{V}O_2$ and PVA and determined a linear regression line of $\dot{V}O_2$ on PVA in each of the high-, low-, and re–high-temperature runs in individual hearts. We subjected all steady-state data for each correlation coefficient and regression line, assuming mutual independence of individual data because two adjacent sampled data under a given loading condition were not exactly the same even in apparently the same steady state.

We compared regression lines between three temperature runs in each heart by the analysis of covariance (ANCOVA).[20] We used this test because the ranges of PVA were comparable in the three temperature runs. Homogeneity of variances was first tested, and then statistical significance of the differences of the slopes or elevations of the regression lines was tested by F test in individual hearts,[20] as in our previous study.[1] Probability values smaller than 0.05 indicate statistical significance.

Mean±SD of the slope and the $\dot{V}O_2$-axis intercept in all hearts were calculated assuming that their values of each $\dot{V}O_2$-PVA regression line were their reliable estimates because of the high correlation coefficient (see "Results") as in our previous study.[1,2]

E_{max} and T_{max} were obtained in individual contractions. Their mean values were calculated from the contractions under different loading conditions in each of the high-, low-, and re–high-temperature runs in each heart. Mean±SD of the means in all hearts were then obtained. Mean±SD of cardiac temperature and heart rate in all hearts were also obtained.

Differences of mean values for temperature, heart rate, E_{max}, and T_{max} among the high-, low-, and re–high-temperature runs were tested by two-way analysis of variance (ANOVA).[20] We also judged $p<0.05$ by F test to be statistically significant. When ANOVA was statistically significant, we compared the mean values among the three runs by the least significance difference method.[20]

dP/dt_{max} and τ_{max} of individual contractions in the three temperature runs were also calculated. We picked up dP/dt_{max} and τ_{max} values at the same end-diastolic volume (18.3±3.4 ml) in individual hearts. Changes in dP/dt_{max} and τ_{max} with cooling

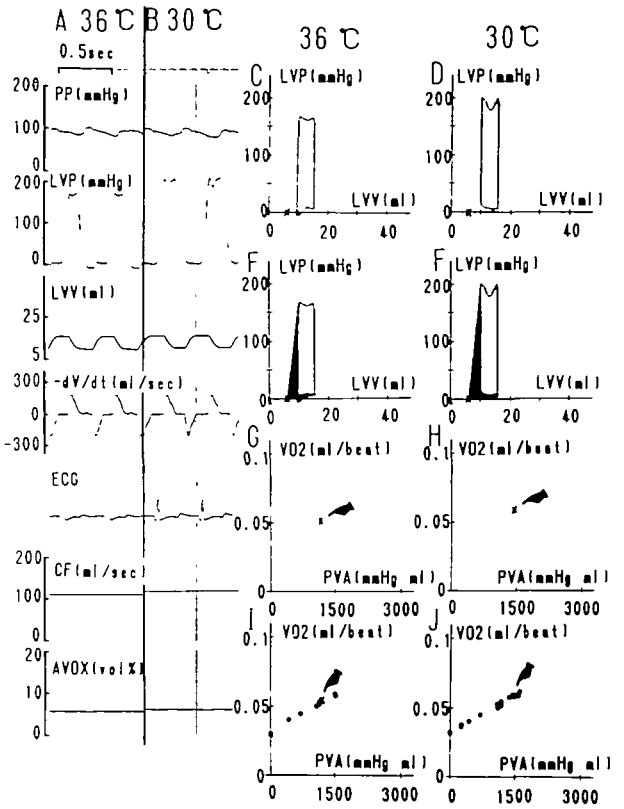

FIGURE 2. *Simultaneous tracings of coronary perfusion pressure (PP), left ventricular pressure (LVP), left ventricular volume (LVV), time-derivative of volume (−dV/dt), left ventricular surface electrocardiogram (ECG), coronary flow (CF), and coronary arteriovenous O_2 content difference (AVOX) signals from top down in the high-temperature run (36° C) in* Panel A *and in the low-temperature run (30° C) in* Panel B. *Pressure-volume loops of these contractions are shown in* Panels C–F. *Ordinates indicate LVP (0–200 mm Hg), and the abscissae indicate LVV (0–40 ml). In Panels E and F, the black triangular areas represent mechanical potential energy. The sum of this area and the area within the loop represents PVA. In* Panels G–J, *ordinates indicate $\dot{V}O_2$ (0–0.1 ml/beat), and the abscissae indicate PVA (0–3,000 mm Hg · ml), both directly measured and not yet normalized for 100 g left ventricle. $\dot{V}O_2$-PVA points at the index finger marks in Panels G and H correspond to the contractions in Panels C and D. Panel I plots multiple $\dot{V}O_2$-PVA points (retouched) in the high-temperature run. Panel J plots multiple $\dot{V}O_2$-PVA points (retouched) in the low-temperature run on top of those points in the high-temperature run.*

were correlated with those in E_{max} and T_{max} by multiple correlation analysis.[20]

Results

Figures 2A and 2B compare coronary perfusion pressure, left ventricular pressure, volume, its time derivative (−dV/dt), ECG, mean coronary flow, and coronary arteriovenous O_2 content difference tracings of steady-state contractions in the high-temperature run (Panel A, 36° C) and the low-temperature run (Panel B, 30° C) in one heart. Pacing rate was fixed constant in these two runs. These contractions had the same end-diastolic and stroke volumes. The cooling increased ventricular end-systolic pressure from 163 to 202 mm Hg at the same end-systolic volume of 10.2 ml, and hence increased E_{max} by 24% from 24.8 mm Hg/(ml/100 g) to 30.7 mm Hg/(ml/100 g). The cooling also increased T_{max} by 17% from 150 to 176 msec. Simultaneously, dP/dt_{max} at an end-diastolic volume of 15.5 ml increased by 31% from 3,230 to 4,240 mmHg/sec and τ_{max} increased slightly from 50 to 54 msec. Coronary flow increased from 115 to 126 ml/min and coronary arteriovenous O_2 content difference increased from 6.1% to 6.4%, simultaneously.

Figures 2C–2J are hard copies (with labels and symbols retouched) of the computer display obtained in the high and low temperature runs in the same heart as in Figures 2A and 2B. Figures 2C, 2E, 2G, and 2I correspond to the contraction at 36° C shown in Figure 2A. Figures 2D, 2F, 2H, and 2J correspond to the contractions at 30° C shown in Figure 2B. Figures 2C and 2D compare P-V trajectories, and Figures 2E and 2F compare PVAs between the high and low temperature runs. Crosses on the volume axes in Figures 2C–2F indicate $\dot{V}O$. Despite cooling, $\dot{V}O$ was reproducible within 1 ml in this heart as well as in other hearts. The open rectangle within the P-V loop represents external mechanical work, and the black triangle represents mechanical potential energy. The sum of these two areas is PVA. With the cooling, external mechanical work increased by 16% from 870 mm Hg · ml to 1,007 mm Hg · ml and potential energy increased by 47% from 297 mm Hg · ml to 437 mm Hg · ml. As the result, PVA increased by 24% from 1,167 mm Hg · ml to 1,444 mm Hg · ml. With the increases in both coronary flow and arteriovenous O_2 content difference, $\dot{V}O_2$ increased by 16% from 0.051 ml to 0.059 ml. Figures 2G–2H plot these $\dot{V}O_2$s against PVAs of the respective contractions in the two runs. The $\dot{V}O_2$-PVA point was moved slightly right and upward by the cooling.

Figure 2I plots $\dot{V}O_2$-PVA data points of several steady-state contractions in this high-temperature run, and Figure 2J superimposes $\dot{V}O_2$-PVA data points of several steady-state contractions in this low-temperature run on those in the high-temperature run in the same heart. These correlograms show that the $\dot{V}O_2$-PVA relation was linear with a small scatter of the data, and the linear relation did not shift despite the cooling by 6° C in this heart. Although not shown here, all $\dot{V}O_2$-PVA points in the re–high-temperature run in this heart were close to those shown in Figure 2J. Similar to this heart, the $\dot{V}O_2$-PVA relations in the three temperature runs were closely superimposable in each individual heart.

Figures 3A and 3B show representative examples of the $\dot{V}O_2$-PVA data points and the regression lines

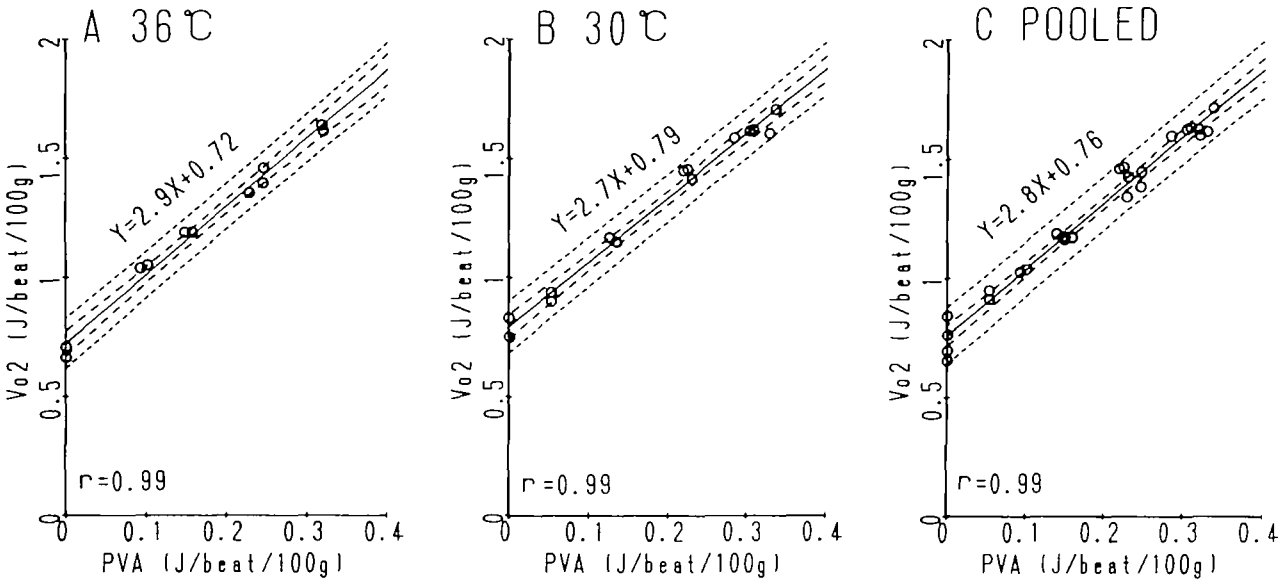

FIGURE 3. *Representative set of $\dot{V}O_2$-PVA correlation and regression data in the high-* (Panel A) *and low-* (Panel B) *temperature runs.* Panel C *pools the same data shown in Panels A and B. Inner pair of dashed curves indicate 95% confidence limits of the regression line. Outer pair of dashed curves indicate 95% confidence limits of the sampled data. Equation above the regression line indicates the regression equation.* r, *correlation coefficient.*

of $\dot{V}O_2$ on PVA. Figure 3C pools these data in both Figures 3A and 3B. Both $\dot{V}O_2$ and PVA were normalized for 100 g left ventricle and their units (ml O_2 and mm Hg · ml, respectively) were unified to Joule (see "Materials and Methods"). We found $\dot{V}O_2$ to correlate linearly and closely with PVA with a correlation coefficient (r) close to unity in both high- and low-temperature runs. The regression equation has the same form as in our previous studies[1,2]: $\dot{V}O_2 = A \times PVA + B$, where A (dimensionless) is the regression coefficient for the slope and B (in Joules per beat per 100 g) is the regression constant for the $\dot{V}O_2$-axis intercept at zero PVA. The $\dot{V}O_2$-axis intercept was close to the directly determined unloaded $\dot{V}O_2$, as shown in Figure 3. We call $A \times PVA$ "excess $\dot{V}O_2$" and constant B either "unloaded $\dot{V}O_2$" or "$\dot{V}O_2$-axis intercept" as shown in Figure 1B. The excess $\dot{V}O_2$ and the unloaded $\dot{V}O_2$ can also be called "PVA-dependent $\dot{V}O_2$" and "PVA-independent $\dot{V}O_2$, respectively."

When both $\dot{V}O_2$ and PVA were expressed in Joules per beat per 100 g, A was 2.87 (dimensionless) at 36° C in Figure 3A and 2.70 (dimensionless) at 30° C in Figure 3B, and B was 0.72 J/beat/100 g at 36° C in Figure 3A and 0.79 J/beat/100 g at 30° C in Figure 3B. Thus, A decreased little (by 5.9%) and B increased little (by 9.7%). After pooling all data in Figure 3A and 3B into Figure 3C, A was 2.80 (dimensionless) and B was 0.76 J/beat/100 g. The sample standard deviation from regression ($S_{y \cdot x}$)[20] was 0.039 J/beat/100 g in the high-temperature run in Figure 3A, 0.043 J/beat/100 g in the low-temperature run in Figure 3B, and 0.053 J/beat/100 g when both were pooled in Figure 3C. These $S_{y \cdot x}$ values were small (6–8%) compared with the $\dot{V}O_2$-axis intercepts in the individual runs in this heart. The small change (0.06 J/beat/100 g) in the $\dot{V}O_2$-axis intercept with the cooling was comparable to these $S_{y \cdot x}$ values. Although not shown in Figure 3, in the re–high-temperature run, $S_{y \cdot x}$ was 0.042 J/beat/100 g and comparable to the small change (0.05 J/beat/100 g) in the $\dot{V}O_2$-axis intercept with the warming in this heart.

The 95% confidence limits[20] of both regression lines and sampled data were narrow as seen in Figures 3A–3C in both the high- and low-temperature runs. The linear regression line from the pooled data in Figure 3C was almost the same in the slope and elevation as those in Figures 3A and 3B. The 95% confidence limits of the regression line of the pooled data were as narrow as those in Figures 3A and 3B. The 95% confidence limit of sampled data at the $\dot{V}O_2$-axis intercept was 0.095 J/beat/100 g in Figure 3A, 0.107 J/beat/100 g in Figure 3B, and 0.118 J/beat/100 g in Figure 3C. These values were approximately 50% greater than the change (0.07 J/beat/100 g) in $\dot{V}O_2$-axis intercept with cooling described above. This indicates that the change in the $\dot{V}O_2$-axis intercept with the cooling fell within the 95% confidence limits of the $\dot{V}O_2$-axis intercept per se.

ANCOVA (see "Materials and Methods") showed no statistically significant difference in the slope ($p > 0.25$) or the elevation ($p > 0.1$) of the regression line between Figures 3A and 3B. This indicates that the two regression lines in Figures 3A and 3B are practically the same. Although not shown here, the $\dot{V}O_2$-PVA regression line remained practically unchanged in the re–high-temperature run from those in the high- and low-temperature runs in this heart. We interpreted the statistical results as indi-

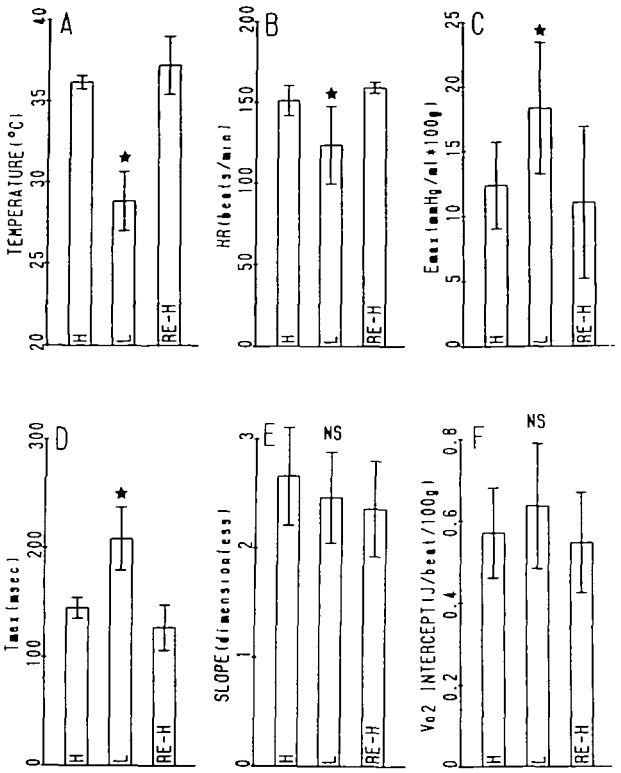

FIGURE 4. *Comparison of temperature, heart rate, E_{max} (slope of end-systolic PV line), T_{max} (time to E_{max}), slope of the $\dot{V}O_2$-PVA regression line, and $\dot{V}O_2$-axis intercept (or unloaded $\dot{V}O_2$) of the $\dot{V}O_2$-PVA regression line in the high-(H), low-(L), and re-high-(RE-H) temperature runs from left to right in each panel. Bars indicate mean values and ticks indicate SD values. Asterisks indicate statistical significance ($p<0.05$) of the low-temperature data as compared with the high-temperature data by the least-significant difference method. NS, statistically insignificant ($p<0.05$) by ANCOVA.*

cating no significant shift of the $\dot{V}O_2$-PVA relation with the cooling despite the 24% increased E_{max} in this left ventricle.

In any other hearts, $\dot{V}O_2$ linearly correlated with PVA in the high-, low-, and re-high-temperature runs in a manner similar to the heart shown in Figures 2 and 3. The correlation coefficient (r) between $\dot{V}O_2$ and PVA was always close to unity, ranging between 0.962 and 0.997 (mean 0.987 after z transformation[20]) in the high-temperature runs; ranging between 0.940 and 0.991 (mean 0.971) in the low temperature runs; and ranging between 0.937 and 0.996 (mean 0.979) in the re-high-temperature runs. The coefficient of determination[20] ($CD=r^2$) was therefore 0.974, 0.943, and 0.958 on the average in the high-, low-, and re-high-temperature runs, respectively. These CD values indicate that as much as 94–97% of the changes in $\dot{V}O_2$ were attributable to the changes in PVA and that the remaining 3–6% should be attributed to factors other than PVA. $S_{y\cdot x}$ (see above) was 0.046 ± 0.017 J/beat/100 g in the high-temperature runs, 0.071 ± 0.020 J/beat/100 g in the low-temperature runs, and 0.059 ± 0.022 J/beat/100 g in the re-high-temperature runs. These $S_{y\cdot x}$ values were $8.7\pm3.3\%$, $11.9\pm4.4\%$, and $10.5\pm3.7\%$ of the $\dot{V}O_2$-axis intercepts in the corresponding high-, low-, and re-high-temperature runs. The 95% confidence limit of the $\dot{V}O_2$-axis intercept was 0.122 ± 0.031 J/beat/100 g in the high-temperature runs, 0.135 ± 0.037 J/beat/100 g in the low-temperature runs, and 0.128 ± 0.035 J/beat/100 g in the re-high-temperature runs. These values were as small as $21\pm7\%$, $20\pm6\%$, and $23\pm7\%$ of the $\dot{V}O_2$-axis intercepts in the high-, low-, and re-high-temperature runs, respectively. These statistical data indicate that the $\dot{V}O_2$ was closely related to PVA in all three temperature runs.

ANCOVA (see "Materials and Methods") in all hearts showed that neither the slope nor the elevation of the $\dot{V}O_2$-PVA regression line was significantly different between any two of the three temperature runs. Therefore, we interpreted these results as indicating no significant shift of the $\dot{V}O_2$-PVA relation with the cooling and warming by 7° C in the present study.

Figures 4A–4F summarize mean ± SD of temperature, heart rate, E_{max}, T_{max}, slope of the $\dot{V}O_2$-PVA regression line, and its $\dot{V}O_2$-axis intercept in all 11 hearts. Asterisks in Figures 4A–4D indicate statistical significance of the changes in the respective variables in the low-temperature run relative to the high-temperature run by ANOVA and least-significant difference method ("Materials and Methods"). On the average, heart rate decreased by $15\pm4\%$, E_{max} increased by $46\pm13\%$, and T_{max} increased by $45\pm27\%$ in the low-temperature run. These changes were statistically significant ($p<0.05$). All these parameters returned to their control levels in the re-high-temperature run. Despite these changes, neither the $\dot{V}O_2$-axis intercept nor the slope of the $\dot{V}O_2$-PVA regression line significantly changed in any heart by ANCOVA as mentioned above.

There were some interindividual variations of the slope and elevation of the $\dot{V}O_2$-PVA relation. The coefficients of variation (CV = SD/mean) of the slope and the elevation were 18.7% and 19.3% in the high-temperature run, 16.9% and 23.9% in the low-temperature run, and 19.2% and 22.3% in the re-high-temperature run, respectively. These CV values are comparable to those in our previous studies.[1-3]

Directly determined unloaded $\dot{V}O_2$ of the left ventricle per minute was 4.32 ± 1.00 ml O_2/min/100 g, 4.08 ± 1.68 ml O_2/min/100 g, and 4.46 ± 1.22 ml O_2/min/100 g, respectively, in the high-, low-, and re-high-temperature runs in all 11 hearts. ANOVA showed no significant difference between these mean values for unloaded $\dot{V}O_2$ per minute.

We calculated the ratios of both slope and $\dot{V}O_2$-axis intercept in the low-temperature run to those in the high-temperature run, assuming that both the slope and the $\dot{V}O_2$-axis intercept of the $\dot{V}O_2$-PVA regression line were reliably estimated in each run

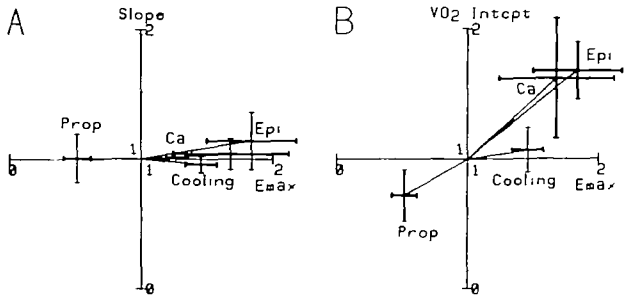

FIGURE 5. *Relative changes in the slope and the $\dot{V}O_2$-axis intercept ($\dot{V}O_2$ Intcpt) of the $\dot{V}O_2$-PVA relation with changes in E_{max} by cooling superimposed on those by epinephrine (Epi), calcium (Ca), and propranolol (Prop) in our previous study. Thick bars indicate mean±SD significantly different from unity (paired t test, p<0.05). Thin bars indicate mean±SD insignificantly different from unity (p>0.05). See text for details.*

as shown by the high correlation. The slope ratio was 0.95±0.05, and the $\dot{V}O_2$-axis intercept ratio was 1.07±0.17 in 11 hearts. Neither was significantly different from unity. Figure 5 plots these ratios against the ratio of E_{max} in the low-temperature run to that in the high-temperature run (1.46±0.13). This E_{max} ratio was significantly greater than unity. For comparison, Figure 5 also plots the slope and $\dot{V}O_2$-axis intercept ratios when E_{max} was increased with epinephrine and Ca^{2+} in our previous study[1-3] and decreased with propranolol.[21] The $\dot{V}O_2$-axis intercept ratios for these agents were significantly different from unity. The response of the $\dot{V}O_2$-axis intercept to E_{max} by cooling was different from that by epinephrine or Ca^{2+}.

We also calculated the sensitivity of changes in unloaded $\dot{V}O_2$ to simultaneous changes in E_{max} by cooling as the slope of the regression line of unloaded $\dot{V}O_2$ on E_{max} in the high- and low-temperature runs in 11 hearts as previously described.[1-3] Correlation coefficient between these unloaded $\dot{V}O_2$ and E_{max} values was 0.65 ($p<0.05$). The sensitivity was 0.01 J/beat/mm Hg (ml × 100 g^{-2}), and its 95% confidence range is 0.0046–0.0154 J/beat/mm Hg (ml × 100 g^{-2}). This sensitivity was only one tenth to one fourth of the sensitivity [0.04–0.06 J/beat/mm Hg (ml × 100 g^{-2})] for epinephrine and Ca^{2+} obtained in our previous study.[1-3] This comparison also contrasts the effect of cooling against epinephrine and Ca^{2+}.

Mean±SD of the reciprocal of the slope of the $\dot{V}O_2$-PVA regression line were 0.38±0.06 (dimensionless), 0.41±0.07, and 0.44±0.09 in the high-, low-, and re–high-temperature runs, respectively. Therefore, mean±SD of EFF_{cm} were 38±6%, 41±7%, and 44±9% in the high-, low-, and re–high-temperature runs, respectively. ANOVA (see "Materials and Methods") showed no significant difference between these mean values.

Changes in dP/dt_{max} and τ_{max} with cooling at the same end-diastolic volumes were obtained in individual left ventricles. dP/dt_{max} increased by 27±24% ($p<0.05$) from 1,790±1,010 mm Hg/sec to 2,200±1,430 mm Hg/sec, and τ_{max} increased by 40±33% from 55±9 msec to 75±11 msec by the 7° C cooling. Both returned to the control levels by warming. The multiple correlation analysis of the relative changes of dP/dt_{max}, τ_{max}, E_{max}, T_{max}, and E_{max}/T_{max} ratio[22] in all runs and hearts showed that dP/dt_{max} correlated best with E_{max}/T_{max} ratio ($r=0.850$, $p<0.001$) and next best with E_{max} (0.761, $p<0.01$) but did not significantly correlate with T_{max} and τ_{max}. Multiple correlation coefficient of dP/dt_{max} with all four other parameters together was 0.896, which was only slightly greater than the correlation (0.850) with E_{max}/T_{max} ratio alone. τ_{max} correlated significantly only with T_{max} (0.625, $p<0.05$).

Discussion

The most important new finding in this study using dog left ventricles is that the $\dot{V}O_2$-PVA relation is largely independent of cooling by 7° C from 36° despite the increase in E_{max}. This is an unexpected result in light of the sensitive elevation of the $\dot{V}O_2$-PVA relation with E_{max} under such positive inotropic interventions as catecholamines and Ca^{2+}.[1-3]

Our observation of the increased E_{max} and T_{max} with the cooling in the dog left ventricle is consistent with the previous findings that cooling enhances the strength of myocardial and ventricular contraction but prolongs its duration.[5-11] For example, excised cross-circulated dog left ventricles beating at a constant pacing rate increased peak isovolumic pressure by 40% and the time to peak isovolumic pressure by 37% (our determination on their tracings) with cooling from 38° C to 32° C.[6] Papillary muscles also increased peak isometric force by 30–160% and the time to the peak force by 70–140% with cooling by about 10° C.[7,9] Therefore, the enhanced E_{max} and prolonged T_{max} of the left ventricle seem to be based on the effects of cooling on the strength and duration of myocardial contraction.

Although cooling is considered a positive inotropic intervention,[5-11] it is different from the positive inotropism of catecholamines because the latter increases E_{max} and shortens T_{max}.[22] The changes in the strength and duration of contraction and their relative change are well represented by E_{max}, T_{max}, and E_{max}/T_{max} ratio.[22] dP/dt_{max} also reflects the relative change of the strength and duration of contraction.[22] Cooling increased both E_{max} and T_{max} by comparable percentages and dP/dt_{max} by a smaller percentage in this study. This result is consistent with the smaller percent change (25%) in d(force)/dt_{max} than the changes in peak force (160%) and time to peak force (135%) in rabbit papillary muscle with cooling by 10° C from 30° C.[7] Although this and our results are inconsistent with the 17% decrease in d(force)/dt_{max} despite the 33% increase in peak force with cooling by 8° C from 37° C in cat papillary muscle,[6] this decrease in d(force)/dt_{max} can be accounted for by the simultaneous 76% increase in the time to peak force.[6] These parallel changes

between dP/dt_{max} and E_{max}/T_{max} ratio in the ventricle or between d(force)/dt_{max} and (peak force)/(time to peak force) ratio in myocardium are reasonable if the basic pattern of mechanical contraction as a function of time remains unchanged.[22]

We consider that the increased E_{max} and T_{max} with cooling could be a manifestation of cooling-induced slowing of the rates of various physical and chemical processes taking place in myocardium.[7] They include a decreased cross-bridge cycling rate (Q_{10} of 2–3),[15–18] an increased duration of excitation and contraction,[7,23] a decreased active transport of Na^+ (Q_{10} of 1.6),[7] a decreased Ca^{2+} efflux (Q_{10} of 1.35),[24] a decreased release and sequestration of Ca^{2+} by the sarcoplasmic reticulum,[7,25] a decreased reaction of Ca^{2+} with contractile proteins,[7] an increased sarcoplasmic $[Ca^{2+}]_i$ for contraction,[7] and a decreased compliance of series elasticity (Q_{10} of 1.4).[9] Variable changes of these factors may be responsible for the different changes in the strength and duration of contraction and hence dP/dt_{max} or d(force)/dt_{max} with cooling in different preparations.[6,7,9]

From the decreased cross-bridge cycling by cooling rate,[15–18] we expected that the slope of the $\dot{V}O_2$-PVA regression line would decrease with cooling, assuming this slope to be inversely related to EFF_{cm} (i.e., the efficiency of contractile machinery) to convert energy from the excess $\dot{V}O_2$ to total mechanical energy.[1–3] However, to our surprise, the present result does not support this expectation. The cooling by 7° C probably decreased the myosin ATPase activity in the present heart preparation at the same Q_{10} of 2–3 as in myocardium[15,18] and other striated muscles,[16] thereby decreasing the cross-bridge cycling rate by 35–50%. In relation to this, the decreased myosin ATPase activity in the hypothyroid state is associated with a higher heat economy of force development, reflected in a decreased slope of the heat-force relation line.[13,14,26] Since the inverse relation between the myosin ATPase activity and the heat economy is considered to hold in general,[12,13] it seems reasonable to assume that the same relation would hold with cooling. Therefore, we expected in vain an increased EFF_{cm} and, hence, a decreased slope of the $\dot{V}O_2$-PVA relation with cooling.

Although the heat economy[13,14,27] is related to EFF_{cm} or more closely to EFF_{mf} (i.e., efficiency of myofibrillar chemomechanical energy transduction from ATP to PVA), it is different from these efficiencies in both definition and dimensions in that the heat economy is the ratio of (peak isometric force)/(excess heat) with dimensions of force/energy (Newton/Joule). In contrast, the reciprocal of the slope of the $\dot{V}O_2$-PVA relation line indicates a physically sound efficiency in both definition and dimensions (energy/energy, Joule/Joule, or dimensionless).[1–3] Since the $\dot{V}O_2$-PVA relation did not significantly change its slope with cooling in the present study, we could not find any evidence that EFF_{cm} increased with cooling by 7° C from 36° C in the excised cross-circulated dog left ventricle. Our present result, therefore, appears contradictory to the higher heat economy of force development predictable from the decreased ATPase activity with cooling.

However, the unchanged slope of the $\dot{V}O_2$-PVA relation with cooling in this study seems consistent with the unchanged slope of the heat-force relation of rabbit, rat, and cat myocardium with cooling from 27–32° C to 19–20° C.[8,10,28] Since the slope of the heat-force relation line is inversely related to the heat economy of force development by the cross-bridge cycling, the unchanged slope of the heat-force relation implies that the heat economy of the contractile machinery remains unchanged with cooling. Although this result seems inconsistent with the expectation from the decreased myosin ATPase activity with cooling,[13,14] the unchanged slope[10] suggests that EFF_{mf} is insensitive to cooling. In this respect, the unchanged slope of the $\dot{V}O_2$-PVA relation seems consistent with the experimentally observed unchanged slope of the heat-force relation or unchanged heat economy despite cooling.[8,10,28]

The unloaded $\dot{V}O_2$ or $\dot{V}O_2$-axis intercept either per beat or per minute did not significantly increase with cooling by 7° C in this study. This $\dot{V}O_2$ component is primarily used for the basal metabolism and excitation-contraction coupling (EC) and secondarily for residual mechanical energy of unloaded contraction.[1–3] We assume that this residual mechanical energy is negligibly small because average circumferential force of the ventricular wall at zero transmural pressure is calculated to be zero and mechanical work of shortening against this zero force is zero as discussed in our previous study.[1]

Myocardial basal metabolism per minute is known to increase with temperature at a Q_{10} of 1.4.[29,30] Therefore, $\dot{V}O_2$ per minute for basal metabolism probably decreased by approximately 20% with cooling in our experiment. Since pacing rate was decreased by 15 ± 4% on average simultaneously, $\dot{V}O_2$ for basal metabolism per beat probably remained almost unchanged. On the other hand, the cooling probably increased sarcoplasmic $[Ca^{2+}]_i$[7] also in our preparation. The constant stoichiometry between the numbers of sequestrated Ca^{2+} and hydrolyzed ATP by the sarcoplasmic Ca^{2+}-dependent ATPase[31] may have required an increased $\dot{V}O_2$ for EC per beat. However, the cooling probably decreased the sarcoplasmic ATPase activity at a Q_{10} of 2–3[25] and simultaneously slowed contraction process at a similar rate.[12] In fact, T_{max} increased by 45 ± 27% in the present study. Therefore, it is likely that these two factors reciprocally affecting the temperature-dependent changes in $\dot{V}O_2$ for EC produced no net change in $\dot{V}O_2$ for EC per beat in our study. The statistically insignificant change in the unloaded $\dot{V}O_2$ per beat as well as per minute may have resulted from the net effect of all these temperature-dependent changes in $\dot{V}O_2$ for basal metabolism and EC. Experimental analysis of the relative contributions of these changes was beyond the scope of the present study.

No significant elevation of the $\dot{V}O_2$-PVA relation, or no significant increase in the PVA-independent $\dot{V}O_2$, with the cooling in this study is different from the elevation of the heat-force relation, or the increased force-independent heat, with cooling of myocardium.[8,10,28] The different response of energetics to cooling may be due to a species difference (dog vs. rabbit, cat, and rat) or differences in experimental conditions such as a whole heart vs. excised myocardium, blood vs. artificial perfusate, 26–41° C vs. 19–32° C, and 130–180 beats/min vs. 10–30 beats/min between our study and those other studies.[8,10,28] Nevertheless, our preparation is more physiological than the excised myocardium preparations. Therefore, we consider that our present finding indicates a more physiological effect of 7° C cooling from 36° C on the energetics of the blood-perfused dog left ventricle.

No significant change in the slope of the $\dot{V}O_2$-PVA relation with an increase in E_{max} by cooling is similar to that by epinephrine or Ca^{2+},[1-3] as shown in Figure 5A. In other words, EFF_{cm} remains practically unchanged despite increases in E_{max} by cooling, epinephrine, and Ca^{2+}. The slope also did not change with decreases in E_{max} by propranolol.[21] The general constancy of the slope of the $\dot{V}O_2$-PVA relation seems consistent with that of the slope of the heat-force relation.[8,10,26,28,32] What can then change EFF_{cm} or EFF_{mf} is an interesting question remaining to be answered.

No elevation of the $\dot{V}O_2$-PVA relation with an increase in E_{max} by cooling contrasts with the considerable elevation of the $\dot{V}O_2$-PVA relation with epinephrine or Ca^{2+} in our previous study,[1-3] as shown in Figure 5B. Different from cooling, E_{max} increased by 84% and 68% and the $\dot{V}O_2$-axis intercept increased by 67% and 63% with epinephrine and Ca^{2+}, respectively.[1,2] Propranolol decreased E_{max} by 48% and the $\dot{V}O_2$-axis intercept by 25%.[21] Many positive inotropic interventions (ouabain, catecholamines, and Ca^{2+}) elevate the heat-force relation by increasing the force-independent heat for EC.[8,32] This is probably because they commonly increase $[Ca^{2+}]_i$ as indicated by the enhanced calcium transient detected by aequorin.[33] Energy utilization for EC per beat will then increase because of the constant stoichiometry of sarcoplasmic ATPase.[4,31] Since cooling does not increase the unloaded $\dot{V}O_2$ and hence does not elevate the $\dot{V}O_2$-PVA relation, it is likely that cooling increases E_{max} by a mechanism different from catecholamines and Ca^{2+} given to coronary circulation.

No elevation of the $\dot{V}O_2$-PVA relation despite increases in E_{max} as observed in this study may be advantageous to the ventricle as a pump. The $\dot{V}O_2$-PVA relation can be expressed as $\dot{V}O_2 = A \times PVA + C \times E_{max} + D$, where A, C, and D are coefficients and constant.[3] $A \times PVA$ represents excess $\dot{V}O_2$, and $C \times E_{max} + D$ represents unloaded $\dot{V}O_2$. Of this, D represents $\dot{V}O_2$ for basal metabolism $C \times E_{max}$ increases with E_{max}, and this increase accompanies an increment in $\dot{V}O_2$, independent of both PVA and $A \times PVA$. Therefore, an increment in $\dot{V}O_2$ per a given increment in E_{max} will be smaller with a smaller C if PVA remains unchanged. Therefore, the smaller sensitivity of the $\dot{V}O_2$-axis intercept to changes in E_{max} with cooling than with epinephrine or Ca^{2+} as shown in Figure 5B suggests that the increment in $\dot{V}O_2$ per an increment in E_{max} is smaller with cooling when PVA is kept unchanged.

EFF_{cm} is the product of the efficiency of oxidative phosphorylation (EFF_{ox}) and EFF_{mf} (i.e., myofibrillar efficiency). EFF_{ox} is known to be 60–70%, slightly varying depending on metabolic substrates.[2,4,32] Part of ATP is used for EC and basal metabolism. The rest of ATP is used for myofibrils to generate total mechanical energy. EFF_{mf} is calculated to be $EFF_{cm}/EFF_{ox} \fallingdotseq 40\%/65\% = 60\%$.[2,4] The relatively constant EFF_{ox}[2,4,32] suggests that EFF_{mf} like EFF_{cm} is insensitive to cooling as well as to epinephrine and Ca^{2+}. How EFF_{mf} is unchanged despite the probably decreased myosin ATPase activity with cooling remains to be elucidated.

In this study, we focused our analysis to the relation of $\dot{V}O_2$ only with PVA without considerations of many other indexes and determinants of $\dot{V}O_2$.[34,35] We did so because we have elucidated that PVA can predict $\dot{V}O_2$ better than peak systolic pressure, peak ventricular wall force, and their systolic time integrals can when ejection fraction varies widely.[35] Moreover, these indexes are inconvenient in studying efficiency because they are not quantities of energy or work and do not have dimensions of energy. We did not use the new pressure-work index[34] for the following reasons, although it seems a clinically useful predictor of $\dot{V}O_2$. 1) This index is a weighted sum of external work and the pressure-rate product, which per se is not a quantity of energy. Therefore, this index does not allow us to assess the efficiency of contractile machinery of our present interest. 2) This index cannot deal with any changes in $\dot{V}O_2$ of unloaded contraction because this index is always equal to 1.43 ml O_2/min/100 g (a constant for arrested heart) regardless of changes in contractile state when systolic pressure and stroke volume are zero.[34]

To summarize, we studied the effect of cardiac cooling by 7° C from 36° C on the relation between O_2 consumption per beat ($\dot{V}O_2$) and the total mechanical energy generated by contraction in terms of PVA in the excised cross-circulated dog left ventricle. Despite the increased E_{max} by 46% and T_{max} by 45%, the cooling did not significantly change the slope and elevation of the $\dot{V}O_2$-PVA relation. This response of the $\dot{V}O_2$-PVA relation is different from the elevation of the $\dot{V}O_2$-PVA relation with catecholamines and Ca^{2+}. The unchanged slope indicates practically no change in the efficiency of contractile machinery from the excess $\dot{V}O_2$ above unloaded $\dot{V}O_2$ to the total mechanical energy. The unchanged elevation indicates practically no change in the unloaded $\dot{V}O_2$ for basal metabolism plus excitation-contraction coupling.

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