Cardiac Cooling Increases $E_{\text{max}}$ 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 ($E_{\text{max}}$) and the relation between $O_2$ consumption per beat ($O_2$) 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 $O_2$-PVA relation divides measured $O_2$ into unloaded $O_2$ and excess $O_2$. The slope of the $O_2$-PVA relation represents inversely the efficiency of the contractile machinery to convert chemical energy from the excess $O_2$ to total mechanical energy. Cooling is known to decrease myosin ATPase activity ($Q_o$ 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 $O_2$-PVA relation with cooling. The cooling increased $E_{\text{max}}$ by 46 ± 13% and the time to $E_{\text{max}}$ 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 $O_2$-PVA relation nor unloaded $O_2$ 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). 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 ($O_2$) of the left ventricle per beat correlates closely and linearly with PVA regardless of ventricular loading conditions at a stable level of contractility index, $E_{\text{max}}$. This is shown schematically in Figure 1B. The $O_2$-PVA relation allows us to divide $O_2$ into two parts: the unloaded $O_2$ represented by the $O_2$-axis intercept, and the excess $O_2$ linearly correlated with PVA. When $E_{\text{max}}$ increases with epinephrine or Ca$^{2+}$, the $O_2$-PVA relation considerably shifted upward without changes in its slope, as shown in Figure 1C.

In interpreting these findings, we have introduced the efficiency of contractile machinery ($EFF_{\text{cm}}$) in converting the excess $O_2$ into PVA. This efficiency is equal to the reciprocal of the slope of the $O_2$-PVA relation when both $O_2$ and PVA are expressed in Joules per beat per 100 grams to make the slope dimensionless 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 $E_{\text{max}}$ by epinephrine or Ca$^{2+}$ and the simultaneous increases in unloaded $O_2$, as shown schematically by the parallel elevation of the $O_2$-PVA relation in Figure 1C. $EFF_{\text{cm}}$ is physiologically meaningful and useful...
Potential energy (PE) and external work (EW). Vo is potential energy (PE) and external work (EW). Vo is equal to the fraction of Vo above the unloaded Vo. Panel C: Schematic drawing of Vo2-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 Vo2 to PVA as labeled by percent values. Panel D: Schematic drawing of the relation between excess Vo2 and PVA, which is drawn by shifting down the two Vo2-PVA relations in Panel C by their unloaded Vo2. Family of dashed diagonal lines indicates isoefficiency lines from excess Vo2 to PVA as labeled by percent values. Efficiency corresponds to EFFcm focused on in this study.

When we consider the excess Vo2 and PVA to represent myocardial energy input to the contractile machinery and total mechanical energy output from it, respectively.1,2 This EFFcm 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) (EFFcm); 2) the efficiency from the total Vo2 to PVA (EFFf); and 3) the efficiency of myofilibril chemomechanical energy transduction from ATP to PVA (EFFcm). Briefly, EFFcm is defined as either EW/Vo2 or EW/(excess Vo2) and is a complex function of ventricular preload, afterload, heart rate, and contractile state.3 EFFf is defined as PVA/Vo2, which is shown as a family of isoefficiency lines in Figure 1C. This efficiency changes with changes in unloaded Vo2 even when EFFcm remains unchanged,1,2 as can be seen in Figure 1C. EFFf is defined as PVA/(ATP used by crossbridge cycling) and is greater than EFFcm 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 Vo2 and the slope of the Vo2-PVA relation or the EFFcm. Cooling (>20°C) generally increases peak contractile force, slows down the speed of contraction, and prolongs the duration of myocardial and ventricular contraction.3–11 Although cardiac cooling augments left ventricular systolic pressure and Vo2 in excised cross-circulated dog hearts,6 it remains unknown whether and how cooling affects the unloaded Vo2 and EFFcm. EFFf (but not EFFcm) seems to be inversely related to the myosin ATPase activity and crossbridge cycling rate,12–14 and cooling decreases myosin ATPase activity at a Q10 of 2–3.15–19 Therefore, in the present study, we expected EFFcm to increase and then the slope of the Vo2-PVA relation to decrease with cooling. Instead, we found that cardiac cooling by 7°C from 36°C, which increased Emax significantly, did not significantly change the slope of the Vo2-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 urethane (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, 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 annulus.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.  

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.

\( E \text{ max} \) and \( T \text{ max} \)

We obtained \( E \text{ max} \) as an index of ventricular contractile state as previously described. \(^1\) \(^2\) Briefly, we first determined \( V_0 \) as the ventricular volume at which peak isovolumic pressure was zero (Figure 1A). Then, we calculated the slope of the line connecting \( V_0 \) 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 \( E \text{ max} \), 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 \( E \text{ max} \) was determined as \( T \text{ max} \). We determined both \( E \text{ max} \) and \( T \text{ max} \) from ventricular pressure and volume signals of individual contractions on-line with a signal processing computer (model 7T17, NEC San-ei, Tokyo, Japan) at a sampling rate of 500 Hz.

We also determined the maximum rate of ventricular pressure rise (dP/dt\text{max}) and its time (t\text{max}) of individual contractions from the same pressure signal used for determinations of \( E \text{ max} \) and \( T \text{ max} \) 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 \( V_0 \) 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 \( 1.33 \times 10^{-4} \) J. \(^1\) \(^2\) PVA was normalized for 100 g left ventricle.

**O₂ 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 O₂ content difference was measured with a continuous arteriovenous O₂ difference analyzer \(^19\) (A-VOX Systems, San Antonio, Texas), which was calibrated against a Lex-O₂-Con O₂ 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. \( V_{O_2} \) 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 \( V_{O_2} \) by the following method.

We minimized the contribution of right ventricular free wall \( V_{O_2} \) to the measured total \( V_{O_2} \) by keeping the right ventricle collapsed by continuous hydrostatic drainage of the coronary venous blood in the right heart. We assumed that \( V_{O_2} \) 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 \( V_{O_2} \) 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 \( V_{O_2} \) 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 \( V_{O_2} \) was subtracted from the measured total \( V_{O_2} \) to determine left ventricular \( V_{O_2} \) 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 \( \dot{V}_o \). 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 \( \dot{V}_o \) 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 \( \dot{V}_o \) 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, 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). 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, as in our previous study. 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.
were correlated with those in $E_{\text{max}}$ and $T_{\text{max}}$ by multiple correlation analysis.\textsuperscript{20}

**Results**

Figures 2A and 2B compare coronary perfusion pressure, left ventricular pressure, volume, its time derivative ($-\frac{dV}{dt}$), 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 $E_{\text{max}}$ by 24% from 24.8 mm Hg/ml/100 g to 30.7 mm Hg/ml/100 g. The cooling also increased $T_{\text{max}}$ by 17% from 150 to 176 msec. Simultaneously, $\frac{dP}{dt}_{\text{max}}$ at an end-diastolic volume of 15.5 ml increased by 31% from 3,230 to 4,240 mmHg/sec and $T_{\text{mM}}$ 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 $V_o$. Despite cooling, $V_o$ 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, $V_o$ increased by 16% from 0.051 ml to 0.059 ml. Figures 2G–2H plot these $V_o$ against PVA data points of the respective contractions in the two runs. The $V_o$-PVA point was moved slightly right and upward by the cooling.

Figure 2I plots $V_o$-PVA data points of several steady-state contractions in this high-temperature run, and Figure 2J superimposes $V_o$-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 $V_o$-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 $V_o$-PVA points in the re–high-temperature run in this heart were close to those shown in Figure 2J. Similar to this heart, the $V_o$-PVA relations in the three temperature runs were closely superimposable in each individual heart.

Figures 3A and 3B show representative examples of the $V_o$-PVA data points and the regression lines.
of VO₂ on PVA. Figure 3C pools these data in both Figures 3A and 3B. Both VO₂ and PVA were normalized for 100 g left ventricle and their units (ml O₂ and mm Hg · ml, respectively) were unified to Joule (see "Materials and Methods"). We found VO₂ 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 studies1-2: VO₂ = A x 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 VO₂-axis intercept at zero PVA. The VO₂-axis intercept was close to the directly determined unloaded VO₂, as shown in Figure 3. We call A x PVA "excess VO₂" and constant B either "unloaded VO₂" or "VO₂-axis intercept" as shown in Figure 1B. The excess VO₂ and the unloaded VO₂ can also be called "PVA-dependent VO₂" and "PVA-independent VO₂, respectively.

When both VO₂ 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 (Sₓ.y) 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 Sₓ.y values were small (6-8%) compared with the VO₂-axis intercepts in the individual runs in this heart. The small change (0.06 J/beat/100 g) in the VO₂-axis intercept with the cooling was comparable to these Sₓ.y values. Although not shown in Figure 3, in the re-high-temperature run, Sₓ.y was 0.042 J/beat/100 g and comparable to the small change (0.05 J/beat/100 g) in the VO₂-axis intercept with the warming in this heart.

The 95% confidence limits²⁰ 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 VO₂-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 VO₂-axis intercept with cooling described above. This indicates that the change in the VO₂-axis intercept with the cooling fell within the 95% confidence limits of the VO₂-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 VO₂-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-
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Figure 4. Comparison of temperature, heart rate, Eₘᵦₓ (slope of end-systolic PV line), Tₘᵦₓ (time to Eₘᵦₓ), slope of the VO₂-PVA regression line, and VO₂-axis intercept (or unloaded VO₂) of the VO₂-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.

Eating no significant shift of the VO₂-PVA relation with the cooling despite the 24% increased Eₘᵦₓ in this left ventricle.

In any other hearts, VO₂ 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 VO₂ and PVA always close to unity, ranging between 0.962 and 0.997 (mean 0.987 after z transformation²⁰) 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 determination²⁰ (CD = r²) 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 VO₂ were attributable to the changes in PVA and that the remaining 3–6% should be attributed factors other than PVA. Sₚᵥ × (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 Sₚᵥ × values were 8.7 ± 3.3%, 11.9 ± 4.4%, and 10.5 ± 3.7% of the VO₂-axis intercepts in the corresponding high-, low-, and re-high-temperature runs. The 95% confidence limit of the VO₂-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 ± 7%, 20 ± 6%, and 23 ± 7% of the VO₂-axis intercepts in the high-, low-, and re-high-temperature runs, respectively. These statistical data indicate that the VO₂ 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 VO₂-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 VO₂-PVA relation with the cooling and warming by 7°C in the present study.

Figures 4A–4F summarize mean±SD of temperature, heart rate, Eₘᵦₓ, Tₘᵦₓ, slope of the VO₂-PVA regression line, and its VO₂-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±4%, Eₘᵦₓ increased by 46±13%, and Tₘᵦₓ increased by 45±27% 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 VO₂-axis intercept nor the slope of the VO₂-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 VO₂-PVA relation. The coefficients of variation (CV = SD/mean) of the slope and the VO₂-axis intercept 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 VO₂ of the left ventricle per minute was 4.32 ± 1.00 ml O₂/min/100 g, 4.08 ± 1.68 ml O₂/min/100 g, and 4.46 ± 1.22 ml O₂/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 VO₂ per minute.

We calculated the ratios of both slope and VO₂-axis intercept in the low-temperature run to those in the high-temperature run, assuming that both the slope and the VO₂-axis intercept of the VO₂-PVA regression line were reliably estimated in each run.
as shown by the high correlation. The slope ratio was 0.95 ± 0.05, and the V̇O₂-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 V̇O₂-axis intercept ratios when E_max was increased with epinephrine and Ca²⁺ in our previous study and decreased with propranolol. 21 The V̇O₂-axis intercept ratios for these agents were significantly different from unity. The response of the V̇O₂-axis intercept to E_max by cooling was different from that by epinephrine or Ca²⁺.

We also calculated the sensitivity of changes in unloaded V̇O₂ to simultaneous changes in E_max by cooling as the slope of the regression line of unloaded V̇O₂ on E_max in the high- and low-temperature runs in 11 hearts as previously described. 1-3 Correlation coefficient between these unloaded V̇O₂ and E_max was 0.65 (p < 0.05). The sensitivity was 0.01 J/beat/mm Hg (ml x 100 g⁻²), and its 95% confidence range is 0.0046-0.0154 J/beat/mm Hg (ml x 100 g⁻²). This sensitivity was only one tenth to one fourth of the sensitivity [0.04-0.06 J/beat/mm Hg (ml x 100 g⁻²)] for epinephrine and Ca²⁺ obtained in our previous study. 1-3 This comparison also contrasts the effect of cooling against epinephrine and Ca²⁺.

Mean ± SD of the reciprocal of the slope of the V̇O₂-PVA regression line were 0.38 ± 0.06 (dimensionless), 0.41 ± 0.07, and 0.44 ± 0.09 in the high-, low-, and re-temperature runs, respectively. Therefore, mean ± SD of EFF cm were 38 ± 6%, 41 ± 7%, and 44 ± 9% in the high-, low-, and re-temperature runs, respectively. ANOVA (see “Materials and Methods”) showed no significant difference among these mean values.

Changes in dP/dt_max and T_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 T_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, T_max, E_max, T_max, and E_max/T_max ratio22 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 E_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. T_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 V̇O₂-PVA relation is largely independent of cooling by 7°C from 36°C despite the increase in E_max. This is an unexpected result because of the sensitivity of the V̇O₂-PVA relation with E_max under such positive inotropic interventions as catecholamines and Ca²⁺. 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 and duration of myocardial 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. 7,8 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, this decrease in d(force)/dt_max can be accounted for by the simultaneous 76% increase in the time to peak force. 9 These parallel changes
between \(\frac{dP}{dt_{\text{max}}}\) and \(\frac{E_{\text{max}}}{T_{\text{max}}}\) ratio in the ventricle or between \(\frac{d(\text{force})}{dt_{\text{max}}}\) 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.\(^2\)

We consider that the increased \(E_{\text{max}}\) and \(T_{\text{max}}\) 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,\(^2,23\) a decreased active transport of \(\text{Na}^+\) (\(Q_{10}\) of 1.6),\(^7\) a decreased \(\text{Ca}^{2+}\) efflux (\(Q_{10}\) of 1.35),\(^24\) a decreased release and sequesteration of \(\text{Ca}^{2+}\) by the sarcoplasmic reticulum,\(^2,25\) a decreased reaction of \(\text{Ca}^{2+}\) with contractile proteins,\(^7\) an increased sarcoplasmic \([\text{Ca}^{2+}]\), for contraction,\(^7\) and a decreased compliance of series elasticity (\(Q_{10}\) of 1.4).\(^4\) Variable changes of these factors may be responsible for the different changes in the strength and duration of contraction and hence \(\frac{dP}{dt_{\text{max}}}\) or \(\frac{d(\text{force})}{dt_{\text{max}}}\) 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 \(\text{VO}_2\)-PVA regression line would decrease with cooling, assuming this slope to be inversely related to \(\text{EFF}_{\text{cm}}\) (i.e., the efficiency of contractile machinery) to convert energy from the excess \(\text{VO}_2\) 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 \(\text{EFF}_{\text{cm}}\) and, hence, a decreased slope of the \(\text{VO}_2\)-PVA relation with cooling.

Although the heat economy\(^13,14,27\) is related to \(\text{EFF}_{\text{cm}}\) or more closely to \(\text{EFF}_{\text{mf}}\) (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 \(\text{VO}_2\)-PVA relation line indicates a physically sound efficiency in both definition and dimensions (energy/energy, Joule/Joule, or dimensionless).\(^1-3\) Since the \(\text{VO}_2\)-PVA relation did not significantly change its slope with cooling in the present study, we could not find any evidence that \(\text{EFF}_{\text{cm}}\) 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 \(\text{VO}_2\)-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 \(\text{EFF}_{\text{mf}}\) is insensitive to cooling. In this respect, the unchanged slope of the \(\text{VO}_2\)-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 \(\text{VO}_2\) or \(\text{VO}_2\)-axis intercept either per beat or per minute did not significantly increase with cooling by 7°C in this study. This \(\text{VO}_2\) 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.\(^7,29,30\) Therefore, \(\text{VO}_2\) 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, \(\text{VO}_2\) for basal metabolism per beat probably remained almost unchanged. On the other hand, the cooling probably increased sarcoplasmic \([\text{Ca}^{2+}]\) also in our preparation. The constant stoichiometry between the numbers of sequestered \(\text{Ca}^{2+}\) and hydrolyzed ATP by the sarcoplasmic \(\text{Ca}^{2+}\)-dependent ATPase\(^31\) may have required an increased \(\text{VO}_2\) for EC per beat. However, the cooling probably decreased the sarcoplasmic ATPase activity at a \(Q_{10}\) of 2–3\(^2\) and simultaneously slowed contraction process at a similar rate.\(^12\) In fact, \(T_{\text{max}}\) increased by 45±27% in the present study. Therefore, it is likely that these two factors reciprocally affecting the temperature-dependent changes in \(\text{VO}_2\) for EC produced no net change in \(\text{VO}_2\) for EC per beat in our study. The statistically insignificant change in the unloaded \(\text{VO}_2\) per beat as well as per minute may have resulted from the net effect of all these temperature-dependent changes in \(\text{VO}_2\) 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 VO₂-PVA relation, or no significant increase in the PVA-independent VO₂, 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. 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. 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 VO₂-PVA relation with an increase in Eₘₐₓ by cooling is similar to that by epinephrine or Ca²⁺,¹⁻³ as shown in Figure 5A. In other words, EFFcm remains practically unchanged despite increases in Eₘₐₓ by cooling, epinephrine, and Ca²⁺. The slope also did not change with decreases in Eₘₐₓ by propranolol.²¹ The general constancy of the slope of the VO₂-PVA relation seems consistent with that of the slope of the heat-force relation.³⁸⁻⁶²⁻²⁸ What can then change EFFcm or EFFmf is an interesting question remaining to be answered.

No elevation of the VO₂-PVA relation with an increase in Eₘₐₓ by cooling contrasts with the considerable elevation of the VO₂-PVA relation with epinephrine or Ca²⁺ in our previous study.¹⁻³ as shown in Figure 5B. Different from cooling, Eₘₐₓ increased by 84% and 68% and the VO₂-axis intercept increased by 67% and 63% with epinephrine and Ca²⁺, respectively.¹² Propranolol decreased Eₘₐₓ by 48% and the VO₂-axis intercept by 25%.²¹ Many positive inotropic interventions (ouabain, catecholamines, and Ca²⁺) elevate the heat-force relation by increasing the force-independent heat for EC.³²⁻³³ This is probably because they commonly increase [Ca²⁺], as indicated by the enhanced calcium transient detected by aequorin.³¹ Energy utilization for EC per beat will then increase because of the constantly stoichiometry of sarcoplasmic ATPase.³¹⁻³² Since cooling does not increase the unloaded VO₂ and hence does not elevate the VO₂-PVA relation, it is likely that cooling increases Eₘₐₓ by a mechanism different from catecholamines and Ca²⁺ given to coronary circulation.

No elevation of the VO₂-PVA relation despite increases in Eₘₐₓ as observed in this study may be advantageous to the ventricle as a pump. The VO₂-PVA relation can be expressed as VO₂ = A × PVA + C × Eₘₐₓ + D, where A, C, and D are coefficients and constant.³ A × PVA represents excess VO₂, and C × Eₘₐₓ + D represents unloaded VO₂. Of this, D represents VO₂ for basal metabolism C × Eₘₐₓ increases with Eₘₐₓ, and this increase accompanies an increment in VO₂, independent of both PVA and A × PVA. Therefore, an increment in VO₂ per a given increment in Eₘₐₓ will be smaller with a smaller C if PVA remains unchanged. Therefore, the smaller sensitivity of the VO₂-axis intercept to changes in Eₘₐₓ with cooling than with epinephrine or Ca²⁺ as shown in Figure 5B suggests that the increment in VO₂ per an increment in Eₘₐₓ is smaller with cooling when PVA is kept unchanged.

EFFcm is the product of the efficiency of oxidative phosphorylation (EFFox) and EFFmf (i.e., myofibrillar efficiency). EFFcm is known to be 60–70%, slightly varying depending on metabolic substrates.²⁻³²⁻³³ Part of ATP is used for EC and basal metabolism. The rest of ATP is used for myofibrils to generate total mechanical energy. EFFmf is calculated to be EFFcm/EFFox = 40%/65% = 60%.²⁻³³ The relatively constant EFFmf²⁻³²⁻³³ suggests that EFFmf like EFFcm is insensitive to cooling as well as to epinephrine and Ca²⁺. How EFFmf 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 VO₂ only with PVA without considerations of many other indexes and determinants of VO₂.³₄⁻³₅ We did so because we have elucidated that PVA can predict VO₂ 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 index³⁴ for the following reasons, although it seems a clinically useful predictor of VO₂. 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 VO₂ of unloaded contraction because this index is always equal to 1.43 ml O₂/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 O₂ consumption per beat (VO₂) and the total mechanical energy generated by contraction in terms of PVA in the excised cross-circulated dog left ventricle. Despite the increased Eₘₐₓ by 46% and Tₘₐₓ by 45%, the cooling did not significantly change the slope and elevation of the VO₂-PVA relation. This response of the VO₂-PVA relation is different from the elevation of the VO₂-PVA relation with catecholamines and Ca²⁺. The unchanged slope indicates practically no change in the efficiency of contractile machinery from the excess VO₂ above unloaded VO₂ to the total mechanical energy. The unchanged elevation indicates practically no change in the unloaded VO₂ for basal metabolism plus excitation-contraction coupling.
References


**KEY WORDS** • temperature • myocardium • efficiency • heart • myosin ATPase
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