O₂ cost of contractility but not of mechanical energy increases with temperature in canine left ventricle

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Mikane, Takeshi, J unichi Araki, Shunsuke Suzuki, J u Mizuno, J uichiro Shimizu, Satoshi Mohri, Hiromi Matsubara, Masahisa Hirakawa, Tohru Ohe, and Hiroiuki Suga. O₂ cost of contractility but not of mechanical energy increases with temperature in canine left ventricle. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H65–H73, 1999.—We investigated the effects of myocardial temperature on left ventricular (LV) mechanoenergetics in the excised, cross-circulated canine heart. We used the framework of the LV contractility (Eₘₐₓ)-pressure-volume area (PVA; a measure of total mechanical energy)-myocardial oxygen consumption (VO₂) relationship. We have shown this framework to be useful to integrative analysis of the mechanoenergetics of a beating heart. In isovolumic contractions at a constant pacing rate, increasing myocardial temperature from 30 to 40°C depressed Eₘₐₓ and increased the oxygen cost of Eₘₐₓ, which was enhanced by dobutamine, in a linear manner. However, the slope of the VO₂-PVA relation (reciprocal of contractile efficiency) and its VO₂ intercept remained constant. Q₁₀ values of Eₘₐₓ the oxygen cost of Eₘₐₓ and the oxygen cost of PVA were 0.4, 2.1 and 1.0, respectively, around normothermia. We conclude that the temperature-dependent processes of cross-bridge cycling and Ca²⁺ handling integratively depress Eₘₐₓ and augment its oxygen cost without affecting the oxygen cost of PVA as myocardial temperature increases by 10°C around normothermia.

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Although there are many reports about myocardial temperature effects on left ventricular (LV) contractility, almost all of them seem to be partly problematic because of inadequate heart rate control (10, 22). LV contractility measurement by load-dependent indexes (4), preparations and materials (e.g., papillary muscle rather than whole heart; Refs. 3, 22), and temperature ranges (10, 22). In these respects, it is mandatory and urgent to elucidate the quantitative effects of hypothermia and hyperthermia on cardiac mechanoenergetics. To assess cardiac mechanoenergetics more reliably, we have used the following integrative analysis method in the excised, cross-circulated canine heart preparation (2, 8, 14). This method is based on the framework of the Eₘₐₓ (a load-independent contractile index)-LV pressure-volume area (PVA, a measure of total mechanical energy)-LV myocardial oxygen consumption per beat (VO₂) relation (21, 25, 29). We previously studied mechanoenergetic effects of cardiac cooling and warming separately at two different heart rates and by different analysis methods (20, 27). Therefore, these studies were not enough to examine the linearity of these mechanoenergetic effects in a wider temperature range around normothermia.

The purpose of the present study was to assess how myocardial temperature would affect LV mechanoenergetics during changes between 30 and 40°C that the heart would encounter in situ. By using our methodological framework (Eₘₐₓ-PVA-VO₂), we successfully quantified these thermal effects. We obtained results showing that Eₘₐₓ changed reciprocally and its oxygen cost changed proportionally with temperature, whereas oxygen cost of PVA remained unchanged.

MATERIALS AND METHODS

Surgical Preparation

All procedures in this study conformed to institutional and National Institutes of Health animal care guidelines. Experiments were performed in the excised, cross-circulated (blood perfused) canine heart preparation that has consistently been used in our laboratory (2, 6, 8, 14, 16, 20, 27–31). Briefly, two mongrel dogs (body wt 8–35 kg) were anesthetized with pentobarbital sodium (25 mg/kg iv) and fentanyl (0.1–0.2 mg/h iv) after premedication with ketamine hydrochloride (20 mg/kg im). They were intubated, ventilated with respirators, and heparinized (10,000 U iv). The larger dog was used as the metabolic supporter; the common carotid arteries and right external jugular vein were cannulated and connected to the arterial and venous cross-circulation tubes, respectively. The cross-circulation tubes from the support dog were cannulated into the left subclavian artery and the right...
ventricle via the right atrial appendage of the smaller heart donor dog. The heart-lung section was isolated from the systemic and pulmonary circulation. The beating heart, supported by cross circulation, was then excised from the chest without interruption of coronary circulation.

The left atrium was opened, and all the LV chordae tendineae were cut. Complete atrioventricular block was made by chemical (0.2- to 0.5-ml injection of 36% formaldehyde solution) or electrical (direct current of 20–30 J) ablation of the His bundle. A bipolar pacing electrode was placed on the upper portion of the ventricular septal endocardium via the left atrium.

A thin latex balloon with an unstressed volume of ∼50 ml was fitted into the LV, primed with water, and connected to a volume servo-pump (Air-Brown, Tokyo, Japan). LV volume was accurately controlled and measured with the servo-pump. LV pressure was measured with a miniature pressure gauge (model P-7, Königsb erg Instruments) placed inside the apical end of the balloon. The pressure gauge was calibrated against the fluid-filled pressure transducer connected to the balloon. The LV epicardial electrocardiogram (ECG) was recorded with a pair of screw-in electrodes to trigger data acquisition.

The systemic arterial blood pressure of the support dog served as the coronary perfusion pressure of the excised heart. It was maintained stable at 116 ± 10 mmHg (mean ± SD) in each experiment by transfusing whole blood reserved from the heart donor dog, by infusing 6% of hydroxyethyl starch solution, or by continuously infusing methoxamine (5–30 mg/h), as needed. Arterial pH, Po₂, and Pco₂ of the support dog were repeatedly measured and maintained within their physiological ranges with supplemental oxygen, intravenous sodium bicarbonate, or appropriate adjustment of the ventilator setting, as needed.

Myocardial Temperature

Myocardial temperature was gradually changed from 36 to 30 and 40°C and maintained by cooling or warming the cooled portion of the arterial cross-circulation tube placed in a thermostat bath (NCB-1000, Tokyo Rika, Tokyo, Japan). The arterial blood temperature was measured with a thermistor (MTS-40030, Respiratory Support Products). Myocardial temperature was also measured with a soft and flexible thermistor (6-F Foley catheter with thermistor, Respiratory Support Products) placed between the endocardium and the LV balloon via the left atrium.

Preliminary experiments showed that cardiac cooling below 30°C induced incomplete relaxation and sustained pulsus alternans in LV contractions under ventricular pacing. It was difficult to maintain cardiac warming over 40°C without elevating arterial blood temperature to 45–46°C, which may induce protein degeneration and deterioration of myocardial metabolism (19).

Pacing

To exclude the effect of temperature-dependent variation of heart rate on LV contractility (20, 27), we fixed the heart rate at all temperatures in this study. The LV was electrically paced at a fixed heart rate (120 beats/min) throughout the experiment. Our preliminary experiments showed 120 beats/min as an optimal heart rate to avoid ventricular tachyarrhythmias during hyperthermia and both incomplete relaxation and sustained pulsus alternans during hypothermia.

Oxygen Consumption

Coronary blood flow (CF) was continuously measured with an electromagnetic flowmeter by placing an in-line flow probe in the coronary venous drainage tube from the right ventricle (RV). We neglected LV thebesian flow because of its very small fraction (<3%) in the CF (25, 28). Coronary arteriovenous oxygen content difference was continuously measured with an oximeter (PWA-2005, Shoe Technica, Chiba, Japan; Ref. 26). The oximeter was calibrated against a blood oxygen content analyzer (IL-382 CO-oximeter, Instrumentation Laboratory) in each experiment.

Cardiac oxygen consumption per minute was obtained as the product of CF and arteriovenous oxygen content difference. It was divided by heart rate to obtain LV myocardial oxygen consumption per beat (VO₂) in the steady state in the same way as in previous studies (2, 6, 8, 14, 16, 20, 25, 29–31). RV VO₂ was minimized by collapsing the RV by continuous hydrostatic drainage of the coronary venous return. The collapsed RV was assumed to have virtually zero PVA and hence no PVA-dependent VO₂. Therefore, RV PVA-independent VO₂ was calculated by multiplying biventricular PVA-independent VO₂ in each contractile state by (RV wt/LV + RV wt) (25). The RV PVA-independent VO₂ was subtracted from the total VO₂ to yield LV VO₂. At the end of each experiment, we weighed the LV, including the septum, and the RV free wall separately. They were 81.4 ± 20.4 and 32.6 ± 11.2 g, respectively.

Contractility

We assessed LV contractility by Emax, which was determined as the maximal ratio of P(t)/V(t) – V0 (2, 8, 14, 25, 29–31), where P(t) and V(t) are instantaneous LV pressure and volume, respectively. We determined V0 as the volume at which LV peak isovolumic pressure was zero. In the volume loading run (see Experimental Protocols), we determined Emax as the slope of the end-systolic P-V relation (ESPVR) obtained as a regression line from isovolumic contractions at five to eight different LV volumes, including V0 (Fig. 1A; Refs. 2, 8, 14, 21). Emax was normalized for 100 g LV volume [presented as mmHg/ml(100 g) = mmHg·ml−1·100 g]. Tmax was determined as the time to Emax from the onset of the R wave of ECG and served as a measure of the duration of systole.

Pressure-Volume Area

We calculated PVA of each isovolumic contraction from the digitized P(t) and V(t) data in the same way as in previous studies (2, 8, 14, 25, 29–31). Briefly, PVA was calculated as the area in the P-V diagram surrounded by the end-systolic and end-diastolic P-V relations and the systolic P-V trajectory (Fig. 1A). PVA was also normalized for LV weight (presented as mmHg·ml·beat−1·100 g−1).

Experimental Protocols

In the present study, we used isovolumic contraction in the same way as in our previous studies (8, 14). We considered that the contraction mode did not substantially affect the results because the VO₂-PVA relationship proved to be largely independent of the mode of contraction within physiological loading conditions (29). After LV pressure, CF, and arteriovenous oxygen content difference had stabilized, we started experimental measurements. We measured Emax, PVA, VO₂, and other data two or three times to obtain a single set of mean data for each loading and inotropic condition.

Myocardial Temperature

Myocardial temperature was gradually changed from 36 to 30 and 40°C and maintained by cooling or warming the cooled portion of the arterial cross-circulation tube placed in a thermostat bath (NCB-1000, Tokyo Rika, Tokyo, Japan). The arterial blood temperature was measured with a thermistor (MTS-40030, Respiratory Support Products). Myocardial temperature was also measured with a soft and flexible thermistor (6-F Foley catheter with thermistor, Respiratory Support Products) placed between the endocardium and the LV balloon via the left atrium.

Preliminary experiments showed that cardiac cooling below 30°C induced incomplete relaxation and sustained pulsus alternans in LV contractions under ventricular pacing at a reasonably high rate. It was difficult to maintain cardiac warming over 40°C without elevating arterial blood temperature to 45–46°C, which may induce protein degeneration and deterioration of myocardial metabolism (19).

Pacing

To exclude the effect of temperature-dependent variation of heart rate on LV contractility (20, 27), we fixed the heart rate at all temperatures in this study. The LV was electrically paced at a fixed heart rate (120 beats/min) throughout the experiment. Our preliminary experiments showed 120 beats/min as an optimal heart rate to avoid ventricular tachyarrhythmias during hyperthermia and both incomplete relaxation and sustained pulsus alternans during hypothermia.
Control volume run and dobutamine inotropism run in normothermia. First, to obtain a control relation between VO₂ and PVA in normothermia at 36°C, we produced isovolumic contractions at five to eight different LV volumes including V₀. We waited 2–3 min after each change in LV volume until cardiac variables reached a new steady state.

We then fixed LV volume at a moderate level at which peak isovolumic pressure was 60–80 mmHg before dobutamine infusion in normothermia. Dobutamine (0.01 mg/ml) was continuously infused into the arterial tube with an infusion pump (STC-521 Terumo, Tokyo, Japan). The infusion rate was increased in steps every 3–5 min until E_max was nearly doubled, to obtain five to seven sets of E_max, PVA, VO₂, and other data at the preset LV volume.

We adopted dobutamine rather than calcium as a positive inotropic agent because catecholamine and calcium have similar oxygen costs of contractility (17). Furthermore, repeated calcium infusions may easily induce irreversible deterioration of myocardial function (2).

Volume run and dobutamine run in hyperthermia. After myocardial temperature rose to 39–40°C and cardiac variables reached a new steady state, we obtained the data in a similar way. To exclude any influence of the elapsed time factor, the order of hypothermia and hyperthermia was reversed in five of nine hearts.

Fig. 1. Framework of left ventricular (LV) contractility (E_max)-LV systolic pressure-volume area (PVA)-myocardial oxygen consumption per beat (VO₂) relation utilized in present study. A: E_max and PVA in pressure-volume (P-V) diagram. Slope of end-systolic P-V relation (ESPVR) line is defined as E_max. PVA is area surrounded by ESPVR line, end-diastolic P-V relation curve, and systolic P-V trajectory. PVA consists of potential energy alone in an isovolumic contraction. V₀, V at which LV peak isovolumic P = 0. B: volume-loaded VO₂-PVA relation in a baseline contractile state (solid line). Slope a of this relation means oxygen cost of PVA. VO₂ consists of 2 components, PVA-dependent and PVA-independent VO₂ fractions; the latter is equal to VO₂ intercept b, which is sum of VO₂ for excitation-contraction (EC) coupling and basal metabolism. C: upward or downward deviation of a VO₂-PVA data point (●) from a data point on baseline VO₂-PVA relation (○) with an increase or decrease in E_max, respectively, at a constant LV volume (LVV) in inotropism run with inotropic agents such as catecholamine, calcium, and propranolol. We called this steeper relation “composite VO₂-PVA relation” (solid line). VO₂ of this point can be divided into 2 components: 1) PVA-dependent VO₂ corresponding to PVA of contraction (see B) and 2) PVA-independent VO₂, which is sum of same baseline PVA-independent VO₂ (equal to b in B) and change in PVA-independent VO₂. D: relation between PVA-independent VO₂ and E_max. Slope of this relation is oxygen cost of contractility (or E_max).

Volume run and dobutamine run in hyperthermia. After myocardial temperature rose to 39–40°C and cardiac variables reached a new steady state, we obtained the data in a similar way. To exclude any influence of the elapsed time factor, the order of hypothermia and hyperthermia runs was reversed in five of nine hearts.

Volume run and dobutamine run in normothermia as back control. After myocardial temperature returned to 36°C and cardiac variables reached a new steady state, we obtained data as a back control in a similar way to the first control run. This was done to test the reproducibility of the control data despite the elapsed time and the temperature variation and especially to confirm whether hyperthermia had induced irreversible myocardial damage.

KCl arrest run in hypothermia and hyperthermia. Finally, the KCl arrest run was performed in steady state at both 30 and 40°C. We arrested the heart by infusing KCl (continuous infusion at 1–1.5 ml/min after a 15- to 20-ml bolus infusion of 0.17 M solution) into the coronary arterial tube at a constant moderate LV volume. After both CF and arteriovenous oxygen content difference were stabilized during cardiac arrest, VO₂ was measured as basal metabolic VO₂. To exclude any influence of the elapsed time factor, the order of hypothermia and hyperthermia was reversed in four of eight hearts.
Data Sampling and Analyses

LV pressure, volume, and ECG signals were recorded continuously on a strip-chart recorder. These signals were also sampled at 2-ms intervals, digitized with an analog-digital converter (Lab-NB, National Instruments) and processed with a Power Macintosh computer.

$\text{VO}_2$-PVA relation. $\text{VO}_2$ and PVA data in the volume run at each temperature were subjected to linear regression analysis to obtain the regression equation $\text{VO}_2 = a \cdot \text{PVA} + b$ (2, 8, 14, 25). Here, $a$ is the slope of the regression line that represents the oxygen cost of PVA, $a$, which represents the PVA-dependent VO2, and $b$ is the VO2 intercept that represents PVA-independent VO2 (Fig. 1B; dimensions of oxygen cost of PVA are ml O2·mmHg·L^{-1}). The reciprocal (1/a) of the slope of the VO2-PVA relation is the contractile efficiency, which represents the efficiency of energy conversion from the PVA-dependent VO2 to PVA (25). The VO2 intercept of the VO2-PVA relation (b), PVA-independent VO2, corresponds to the nonmechanical oxygen consumption for both excitation-contraction (EC) coupling and basal metabolism (Fig. 1B; Refs. 16, 25). Here, the PVA-independent VO2 is constant independent of LV volume at a given contractility (25, 29). An enhancement of contractility with either calcium or catecholamine elevates the linear VO2-PVA relation in a parallel manner without changing the contractile efficiency (Fig. 1C; Refs. 16, 25).

Oxygen cost of contractility. $\text{VO}_2$ and PVA data in the dobutamine inotropic run at each temperature were also subjected to linear regression analysis to obtain a composite VO2-PVA relation (Fig. 1C; Ref. 25). We calculated PVA-independent VO2 as $\text{VO}_2$ minus PVA dependent VO2 for the respective PVA at each $E_{\text{max}}$ level during the enhancement of contractility with dobutamine. We also calculated the PVA-dependent VO2 as the product of the same slope value a as the baseline a and PVA of the contraction. Thus PVA-independent VO2 at each $E_{\text{max}}$ level was calculated as VO2 − a·PVA. In this calculation, we assumed that a remained constant at all $E_{\text{max}}$ levels, on the basis of the parallelism of the VO2-PVA relation (2, 8, 14, 25). The parallelism has been validated in either the hyperthermic or hypothermic condition in separate studies (20, 27).

The relation between PVA-independent VO2 and the corresponding $E_{\text{max}}$ in the inotropic run at each temperature was obtained by linear regression analysis in each heart. The linear regression has been confirmed during the enhancement of contractility with catecholamine or calcium (16, 25). The slope of the regression line, or the incremental ratio of PVA-independent VO2 to $E_{\text{max}}$ was identified as the oxygen cost of contractility (or $E_{\text{max}}$) dimensions: ml O2·ml·mmHg^{-1}·beat^{-1}·100 g^{-2}$ (Fig. 1D; Refs. 16, 25).

Statistics

We compared the VO2-PVA regression lines of the volume runs at three different temperatures in each heart by analysis of covariance (ANCOVA). We tested the significance of the differences in their slopes and elevations by F-test (24). ANCOVA was also used to compare the regression lines of PVA-independent VO2 on $E_{\text{max}}$ among the three temperatures. Comparison of the mean values at three different temperatures was performed by repeated-measures ANOVA followed by Bonferroni’s correction for multiple comparisons. Student's paired t-test was applied to compare paired mean values between the control and back control runs and between KCl arrest runs in hypothermia and hyperthermia. A value of $P < 0.05$ was considered statistically significant. All data are presented as means ± SD.

RESULTS

Myocardial Temperature

Mean values of myocardial temperature in normothermia, hyperthermia, and hypothermia (n = 9), and back-control normothermia (n = 7) were 35.9 ± 0.3, 30.7 ± 0.7, 39.8 ± 0.3, and 35.8 ± 0.3°C, respectively. We maintained the respective temperatures stably within a variation of ±0.1°C. In three of the nine hearts, we did not reduce myocardial temperature below 31.5°C, to prevent sustained pulsus alternans. In the KCl-arrest run, mean values of myocardial temperature in hypothermia and hyperthermia (n = 8) were 30.1 ± 0.2 and 39.3 ± 0.5°C, respectively.

LV Mechanics

Figure 2A depicts a representative set of LV isovolumic pressure (LVP) and LV volume (LVV) tracings in hyperthermia, normothermia, and hypothermia at a constant LV volume in one heart. Figure 2B shows a representative set of ESPVR at the three different temperatures. Increasing myocardial temperature from 30 to 40°C decreased $E_{\text{max}}$ from 8.4 to 4.2 mmHg·ml^{-1}·100 g. All the other hearts showed similar results.

Table 1 shows mean values of cardiac variables in the three different temperature protocols at a constant moderate LV volume. LV contractility ($E_{\text{max}}$) significantly decreased with increasing myocardial temperature over the entire range by 58%. $T_{\text{max}}$ also decreased simultaneously by 35%. $Q_{10}$ (increasing rate for a 10°C temperature rise) values of $E_{\text{max}}$ and $T_{\text{max}}$ calculated from the present results were 0.44 ± 0.13 and 0.62 ± 0.06, respectively, both significantly smaller than unity.

LV Energetics

Figure 2C shows a representative set of $\text{VO}_2$-PVA relations in hyperthermia, normothermia, and hyperthermia in the same heart as in Fig. 2B. The three regression lines (see equations in Fig. 2C) were virtually superimposable. ANCOVA showed significant differences in neither the slope nor the VO2 intercept among these three regression lines. Neither the mean values of the slope nor those of the VO2 intercept of the $\text{VO}_2$-PVA relations at the respective temperatures showed significant differences by repeated-measures ANOVA (Table 2). The results indicate that the oxygen cost of PVA, or reciprocally, the contractile efficiency, was constant despite the variation of myocardial temperature, although myocardial temperature considerably affected LV contractility. Therefore, $Q_{10}$ of the oxygen cost of PVA from the present results was virtually unity (0.98 ± 0.07).

Figure 3A shows a representative set of the PVA-independent VO2-$E_{\text{max}}$ relations at the three different temperatures in one heart. The slope of each regression line indicates the oxygen cost of $E_{\text{max}}$ enhanced by dobutamine at each temperature. The slopes of these three regression lines were significantly different from each other by ANCOVA ($P < 0.05$). Similar results were observed in all the other hearts (Fig. 3B). The mean
**Fig. 2.** A: representative set of simultaneous recordings of LV pressure (LVP) and LVV in hypothermia (30°C), normothermia (36°C), and hyperthermia (40°C) in a heart. B: representative set of ESPVR in hypothermia (30°C; ○), normothermia (36°C; □), and hyperthermia (40°C; ●). ESP, end-systolic pressure; EDV, end-diastolic volume. C: representative set of relations between LV VO₂ and PVA in hypothermia (30°C; ○), normothermia (36°C; □), and hyperthermia (40°C; ●) in same heart as in B. Equations indicate regression lines. NS, not significant; ANCOVA, analysis of covariance.

**Table 1.** Cardiac mechanoenergetics at three different temperatures at a constant LV volume

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Hypothermia</th>
<th>Normothermia</th>
<th>Hyperthermia</th>
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<tbody>
<tr>
<td>EDV, ml/100 g</td>
<td>20.4 ± 6.3</td>
<td>20.4 ± 6.3</td>
<td>20.4 ± 6.3</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>5.4 ± 7.5†</td>
<td>-4.9 ± 5.6</td>
<td>-5.6 ± 6.7</td>
</tr>
<tr>
<td>ESP, mmHg</td>
<td>102.0 ± 20.0 †</td>
<td>69.4 ± 13.7</td>
<td>44.8 ± 11.0*</td>
</tr>
<tr>
<td>Emax, mmHg·ml⁻¹·100 g</td>
<td>20.1 ± 6.6†</td>
<td>167.4 ± 19.5</td>
<td>133.7 ± 14.6*</td>
</tr>
<tr>
<td>T_max, ms</td>
<td>612 ± 272†</td>
<td>486 ± 248</td>
<td>304 ± 123*</td>
</tr>
<tr>
<td>PVA, mmHg·ml·beat⁻¹·100 g⁻¹</td>
<td>0.032 ± 0.009</td>
<td>0.029 ± 0.009</td>
<td>0.025 ± 0.008*</td>
</tr>
<tr>
<td>VO₂, ml·beat⁻¹·100 g⁻¹</td>
<td>0.012 ± 0.009</td>
<td>0.013 ± 0.009</td>
<td>0.014 ± 0.009</td>
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Values are means ± SD; n = 9 hearts. LV, left ventricular; EDV, end-diastolic volume; EDP, end-diastolic pressure; ESP, end-systolic pressure; Emax, end-systolic maximal pressure-to-volume ratio [=ESP/(EDV – VD); elastance] but not slope of end-systolic pressure-volume relation; T_max, duration of contraction; PVA, pressure-volume area; VO₂, myocardial oxygen consumption per beat. *Statistically significant (P < 0.05) compared with normothermia; † statistically significant (P < 0.05) compared with hyperthermia.

**Table 2.** Cardiac mechanoenergetics at three different temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Hypothermia</th>
<th>Normothermia</th>
<th>Hyperthermia</th>
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<tbody>
<tr>
<td>Emax, mmHg·ml⁻¹·100 g</td>
<td>9.8 ± 5.2†</td>
<td>6.7 ± 2.7</td>
<td>4.3 ± 1.8*</td>
</tr>
<tr>
<td>PVA-VO₂ relation</td>
<td>Slope or O₂ cost of PVA, ×10⁻³ ml O₂·mmHg⁻¹·ml⁻¹</td>
<td>1.78 ± 4.9</td>
<td>1.80 ± 4.9</td>
</tr>
<tr>
<td>Contractile efficiency, %</td>
<td>39.6 ± 9.4</td>
<td>39.1 ± 8.8</td>
<td>40.2 ± 7.7</td>
</tr>
<tr>
<td>Intercept, ×10⁻³ ml O₂·beat⁻¹·100 g⁻¹</td>
<td>2.06 ± 0.69</td>
<td>2.04 ± 0.68</td>
<td>1.97 ± 0.70</td>
</tr>
<tr>
<td>O₂ cost of Emax, ×10⁻³ ml O₂·ml·mmHg⁻¹·beat⁻¹·100 g⁻¹</td>
<td>1.09 ± 0.53†</td>
<td>1.57 ± 0.68</td>
<td>1.98 ± 0.84*</td>
</tr>
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</table>

Values are means ± SD; n = 9 hearts. Emax, slope of end-systolic pressure-volume relation. *Statistically significant (P < 0.05) compared with normothermia; † statistically significant (P < 0.05) compared with hyperthermia.
values of the oxygen cost of E\textsubscript{max} at the respective temperatures also showed significant differences by repeated-measures ANOVA (P < 0.05; Table 2); that is, increasing myocardial temperature significantly increased the oxygen cost of E\textsubscript{max} by 82%. These results indicate that increasing myocardial temperature increased the oxygen demand for a given increment in contractility. Q\textsubscript{10} of the oxygen cost of E\textsubscript{max} was 2.11 \pm 0.95, significantly greater than unity.

Basal Metabolism

Table 3 compares VO\textsubscript{2} for basal metabolism measured during KCl arrest in hypothermia and hyperthermia. Basal metabolic VO\textsubscript{2} in hyperthermia was significantly but slightly greater than that in hypothermia (P < 0.05). Q\textsubscript{10} of the basal metabolic VO\textsubscript{2} was only 1.14 \pm 0.12, significantly greater than unity.

Back Control

We were afraid of a possible deterioration of the reliability of the data near the end of each experiment. Therefore, we verified the reproducibility of control data. Table 4 compares the mean values of mechanoeenergetics variables in normothermia and those in back-control normothermia. There were no significant differences between the mean values of any mechanoeenergetic variable in the two normothermia runs. These results indicate that the control data were reproducible even near the end of the experiments in this study; the reliability of the data in this study was verified despite the elapsed time factor. These results also indicate that the temperature variation, especially hyperthermia to the extent used in this study, did not induce irreversible myocardial damage.

Linearity of Effects

Figure 4 shows the temperature-dependent changes of the two major mechanoeenergetic variables, E\textsubscript{max} and its oxygen cost. E\textsubscript{max} decreased and its oxygen cost increased with temperature in a linear fashion between 30 and 40°C (Fig. 4A). As shown in Fig. 4B, their variations were reciprocal to each other.

DISCUSSION

We compared LV mechanoeenergetics at three different temperatures around normothermia in the isolated, blood-perfused canine heart. The major findings of the present study are the following. In the range of myocardial temperature between 30 and 40°C, 1) increasing temperature linearly depresses LV contractility (E\textsubscript{max}) and shortens the duration of contraction (T\textsubscript{max}); 2) despite a considerably affected myocardial contractility, both the slope of the VO\textsubscript{2}-PVA relations (oxygen cost of PVA, or reciprocally, contractile efficiency) and their VO\textsubscript{2} intercept remain almost constant; 3) increasing temperature linearly increases the oxygen cost of E\textsubscript{max}; 4) these mechanoeenergetic changes with temperature are completely reversible.

### Table 4. Reproducibility of control data

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<th>Normothermia</th>
<th>Normothermia as Back Control</th>
<th>Difference</th>
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<tbody>
<tr>
<td>E\textsubscript{max}, mmHg·ml\textsuperscript{-1}·100 g\textsuperscript{-1}</td>
<td>7.3 \pm 3.0</td>
<td>7.2 \pm 3.3</td>
<td>NS</td>
</tr>
<tr>
<td>PVA-VO\textsubscript{2} relation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slope of VO\textsubscript{2} cost of PVA, \times 10\textsuperscript{-5}</td>
<td>1.61 \pm 5.2</td>
<td>1.63 \pm 5.2</td>
<td>NS</td>
</tr>
<tr>
<td>Intercept, \times 10\textsuperscript{-2} ml O\textsubscript{2}</td>
<td>43.5 \pm 13.4</td>
<td>42.9 \pm 12.4</td>
<td>NS</td>
</tr>
<tr>
<td>\textsuperscript{O}2 cost of E\textsubscript{max}, \times 10\textsuperscript{-2} ml O\textsubscript{2}·beat\textsuperscript{-1}·100 g\textsuperscript{-1}</td>
<td>1.92 \pm 0.74</td>
<td>1.85 \pm 0.64</td>
<td>NS</td>
</tr>
<tr>
<td>Contractile efficiency, %</td>
<td>36.5 \pm 13.4</td>
<td>35.8 \pm 12.4</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means \pm SD; n = 7 hearts. E\textsubscript{max}, slope of end-systolic pressure-volume relation; NS, not significant. Difference by paired t-test.
Contractility

The relation between myocardial temperature and LV contractility remains controversial. Many investigators reported positive inotropy of hypothermia in the papillary muscle, especially in moderate or deep hypothermia (20–25°C) at an extremely low heart rate (12–30 beats/min) (10, 22). Mattheussen et al. (12) exceptionally reported negative inotropism of hypothermia (>30°C) at a moderate or relatively high heart rate (>90 beats/min) in the isolated, crystalloid-perfused rabbit heart. Most reports consistently supported the negative inotropism of hypothermia in both papillary muscle and whole heart (3, 4, 15). In the blood-perfused excised heart, we also have revealed the positive inotropism of hypothermia (27) and the negative inotropism of hyperthermia (20) separately under different experimental conditions. The present finding that LV contractility \( \left( E_{\text{max}} \right) \) significantly decreases reversibly with increasing myocardial temperature from 30 to 40°C supports those previous results (20, 27). The present study is the first that compares cardiac mechanoenergetics at the same heart rate in both hypothermia and hyperthermia alternately in the same cross-circulated heart. This gave us the advantage of obtaining reliable \( Q_{10} \) values of the major mechanoenergetic variables around normothermia.

Oxygen Cost of PVA

The unchanged slope of the \( \text{VO}_2\text{-PVA} \) relation means an unchanged oxygen cost of PVA or, reciprocally, contractile efficiency in the range of myocardial temperature between 30 and 40°C. These results confirm those of the separate studies in the blood-perfused, excised heart (20, 27). It has been reported that the contractile efficiency remains unchanged during ordinary inotropic interventions such as administration of propranolol (28), epinephrine (17, 30), and calcium (17, 30) in the normal canine heart. On the contrary, contractile efficiency decreases in the hyperthyroid rabbit heart (5) and increases in the postischemic stunned (16) and acidic (6) canine heart. Contractile efficiency has been considered to reflect the product of the efficiency from \( \text{VO}_2 \) to ATP (mitochondrial oxidative phosphorylation) and ATP to PVA [cross-bridge (CB) cycling] (25). Therefore, the unchanged contractile efficiency indicates that the overall myocardial efficiency of energy utilization from oxygen to mechanical energy via ATP remains constant in a myocardial temperature range from 30 to 40°C. However, temperature variation affects myosin ATPase activity and hence the CB cycling rate at \( Q_{10} \approx 2–3 \) (1). Therefore, we had expected a change in contractile efficiency. Why is the contractile efficiency unchanged despite the varied contractility and varied CB cycling rate caused by myocardial temperature variation?

Recently, we have theoretically analyzed the possible mechanisms of the temperature-independent contractile efficiency in a simulation study (13). In that study, cooling primarily decelerates both sarcoplasmic reticulum (SR) \( \text{Ca}^{2+} \) pump ATPase and myosin ATPase for CB detachment. The cooling suppression of ATPase-dependent SR \( \text{Ca}^{2+} \) uptake decreases the oxygen cost of PVA and hence improves the contractile efficiency, whereas the suppression of ATPase-dependent CB detachment increases the oxygen cost of PVA and hence impairs the contractile efficiency oppositely (13); namely, when ATPase-dependent SR \( \text{Ca}^{2+} \) uptake and CB detachment rate constants change appropriately during temperature changes, oxygen cost of PVA and the contractile efficiency remain consequently unchanged (13). Therefore, the simulation has suggested that, in not only hypothermia but also hyperthermia, the concomitant changes in the temperature-dependent \( \text{Ca}^{2+} \) handling and CB cycling processes are responsible for the unchanged oxygen cost of PVA and contractile efficiency. These mechanisms may underlie our present finding.

Basal Metabolism

The intercept of the \( \text{VO}_2\text{-PVA} \) relation, namely PVA-independent \( \text{VO}_2 \), did not change significantly despite
myocardial temperature variation. Myocardial basal metabolism increases with temperature at a Q10 of 1.4 (11). Its Q10 was 1.1 in the present study. This finding suggests that the VO2 for EC coupling only slightly decreases reciprocally with temperature within the temperature-independent PVA-independent VO2.

Therefore, EC coupling VO2 within PVA-independent VO2 decreased only slightly with temperature despite the considerable decrease in Emax. This contrasts with the mechanoenergetic effects of epinephrine, calcium, and propranolol (25, 28, 31). We consider that the change in EC coupling VO2 with temperature is too small to be responsible for the temperature-dependent changes in Emax.

Oxygen Cost of Emax

The oxygen cost of Emax has increased with temperature elevation from 30 to 40°C. This cost increases by 110% in stunning (16) and by 53% in acidosis (6), both halving Emax. Therefore, the hyperthermic heart seems to be similar to the stunned and acidic hearts. In other words, hyperthermia induces a failing and oxygen-wasting heart similar to postischemic stunning and acidosis.

In contrast, hypothermia increases Emax and decreases its oxygen cost. This resembles the alkalotic heart (18). This mechanoenergetics means oxygen saving for Emax and seems advantageous in enhancing contractility under a limited oxygen supply.

Although the mechanisms of the temperature-dependent oxygen cost of Emax remain unclear, there seem to be two possibilities. One possibility is a decreased Ca2+ sensitivity (responsiveness) of contractility, and the other is a decreased coupling ratio of Ca2+ to ATP in the SR as temperature increases. In stunned myocardium, both of these mechanisms seem responsible for the increased oxygen cost of Emax (16). In acidosis, a decreased Ca2+ sensitivity seems to be more responsible (6).

A temperature-dependent Ca2+ sensitivity (responsiveness) is most likely to be responsible for the temperature-dependent oxygen cost of Emax. There is a study indicating increased maximal Ca2+-activated contractility with lowered temperature, namely, an increased Ca2+ responsiveness in hypothermia (9). We also have supported the idea of increased Ca2+ responsiveness in hypothermia by our simulation study (13). In hypothermia, the accelerated CB detachment rate decreases the maximal number of attached CB and the maximal force, and hence the Ca2+ responsiveness of contractility decreases (13) and then more Ca2+ and more Ca2+-handling energy are necessary to activate contractility to the same force level. As the result, the PVA-independent VO2 either increases for a given Emax or remains unchanged despite a decreased Emax, both increasing the oxygen cost of Emax. These phenomena are consistent with our present results.

We consider that our present findings basically hold for the in situ beating heart, because we have confirmed the reproducibility of the same mechanoenergetic relationship regardless of the contraction mode and heart rate (29, 31). However, cardiac mechanics and heart rate would be affected by neural and hormonal factors and cardiovascular loading conditions, all of which are influenced by body temperature. The secondary temperature-dependent changes in contractility and heart rate would modify the primary changes in cardiac mechanoenergetics of the in situ pumping heart.

In summary, the present results indicate that hyperthermia depresses LV contractility and shortens the duration of contraction and hypothermia enhances LV contractility and lengthens the duration of contraction. Although LV contractility is varied by myocardial temperature, neither PVA-independent VO2 nor contractile efficiency is varied. However, the oxygen cost of contractility linearly increases with myocardial temperature. The present study has revealed that LV contractility is a linearly decreasing function and the oxygen cost of contractility is a linearly increasing function of temperature. This is the first study to elucidate the existence of a variable oxygen cost of contractility around the control value in an individual LV. We conclude that these mechanoenergetics changes are an integrative expression of the temperature-dependent processes of CB cycling and Ca2+ handling.

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