Negative inotropism of hyperthermia increases oxygen cost of contractility in canine hearts

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Departments of Cardiovascular Dynamics and Medicine, National Cardiovascular Center, 5 Fujishirodai, Suita, Osaka, 565-8565; and 2Department of Physiology II, Okayama University Medical School, 2 Shikatacho, Okayama, 700-8558 Japan

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Saeki, Akio, Yoichi Goto, Katsuya Hata, Toshiyuki Takasago, Takehiko Nishioka, and Hiroyuki Suga. Negative inotropism of hyperthermia increases oxygen cost of contractility in canine hearts. Am J Physiol Heart Circ Physiol 279: H2855–H2864, 2000.—Heart temperature affects left ventricular (LV) function and myocardial metabolism. However, how and whether increasing heart temperature affects LV mechanoenergetics remain unclear. We designed the present study to investigate effects of increased temperature by 5°C from 36°C on LV contractility and energetics. We analyzed the LV contractility index ($E_{\text{max}}$) and the relation between the myocardial oxygen consumption (MV$\dot{O}_2$) and the pressure-volume area (PVA; a measure of LV total mechanical energy) in isovolumetrically contracting isolated canine hearts during normothermia (NT) and hyperthermia (HT). HT reduced $E_{\text{max}}$ by 38% ($P < 0.01$) and shortened time to $E_{\text{max}}$ by 20% ($P < 0.05$). HT, however, altered neither the slope nor the unloaded MV$O_2$ of the MV$O_2$-PVA relation. HT increased the oxygen cost of contractility (the incremental ratio of unloaded MV$O_2$ to $E_{\text{max}}$) by 49%. When Ca$^{2+}$ infusion restored the reduced LV contractility during HT to the NT baseline level, the unloaded MV$O_2$ in HT exceeded the NT value by 36%. We conclude that HT-induced negative inotropism accompanies an increase in the oxygen cost of contractility.

Hypothermia (HT) (<41°C) increases cardiac output and myocardial oxygen consumption (MV$O_2$) due to increasing heart rate and cardiac work in the isolated and in situ heart (4, 6, 10, 13, 16). A few investigators (5, 35), in contrast, reported that HT adversely reduced cardiac function. Although these hemodynamic effects suggest that HT may be unfavorable for patients with cardiovascular diseases, the exact cardiac effect of HT remains controversial. These controversies might have resulted from the uncontrolled heart rate and cardiac loading and the evaluation of left ventricular (LV) contractility by load-dependent indexes. To clarify this issue, a precise control of cardiac loading conditions and appropriate utilization of a load-independent index of contractility would be necessary.

In addition, variation of temperature has been reported to change the activities of many enzymes in the myocardium (14–15, 24). This effect of varied temperature on the myocardium suggests that the key processes in myocardial contraction [i.e., basal metabolism, excitation-contraction (E-C) coupling, and cross-bridge cycling] are also affected. In previous studies (10, 16, 24), however, these fractions in cardiac energetics have not been differentiated. Moreover, it is still unclear whether and how HT influences the cardiac chemomechanical efficiency and the oxygen cost of contractility.

To assess the mechanoenergetic effects of HT, we used the LV contractility index [$E_{\text{max}}$: the slope of the end-systolic pressure-volume relation (ESPVR)] as a relatively load-independent index of LV contractility (26) and LV pressure-volume (PV) area (PVA) as a measure of the total mechanical energy generated by the LV (26). The relation between MV$O_2$ and PVA is proven to be linear and independent of various ventricular loading conditions in a stable contractile state (26). The reciprocal of the slope of the linear MV$O_2$-PVA relation is considered to reflect the contractile efficiency of the energy conversion from MV$O_2$ to PVA via ATP (26). The MV$O_2$ intercept of this relation reflects the MV$O_2$ fraction of E-C coupling and basal metabolism (26). With the use of this mechanoenergetic framework of the $E_{\text{max}}$-PVA-MV$O_2$ relation, we could precisely analyze and characterize the relation between changes in LV contractility and mechanoenergetic cost during HT.

MATERIALS AND METHODS

Surgical Preparation

Experiments were performed on the excised cross-circulated canine heart preparation as previously described in

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EFFECT OF HYPERTERMIA ON CARDIAC ENERGETICS

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In each experiment, two mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg iv) after premedication with ketamine hydrochloride (5 mg/kg im) and heparinized (10,000 U/dog iv). The common carotid arteries and external jugular vein were cannulated in the support dog, and the arterial and venous cross-circulation tubes from the support dog were inserted into the left subclavian artery and the right ventricle (RV) in the heart donor dog, respectively. The heart was isolated from the systemic and pulmonary circulation and was excised after cross circulation was started. There was no interruption of the coronary circulation during the surgical procedure.

A thin latex balloon with a miniature pressure gauge (P-6, Konigsberg) was placed in the LV. The balloon was primed with water and connected to a volume servo pump, which accurately controlled and measured LV volume. A LV epicardial electrocardiogram was recorded to trigger the volume command signal of the servo pump. Heart rate was held constant by left atrial pacing throughout the experiments.

Coronary blood flow was measured with an electromagnetic flowmeter (MVF-2100, Nihon Kohden) in the coronary venous drainage tube from the right heart. We neglected LV thebesian venous blood flow because of its small fraction (<3%) in the total coronary flow (20). Coronary arteriovenous oxygen content difference (AVO2D) was measured continuously with a custom-made oxygen content difference analyzer (PWA-2000S, Erma) (27). This analyzer was calibrated against an IL-286 CO-oximeter in each experiment.

The temperature of the heart was measured with a thermoster probe (TF-DNP-1, Termo) placed between the endocardium and the balloon via the apical stab wound. The heart temperature was gradually increased from 36–37 to 40–42°C by warming the arterial cross-circulation tube in a thermostat bath.

Mean arterial blood pressure of the support dog served as the coronary perfusion pressure of the heart preparation. This pressure was maintained above 80 mmHg by infusing either fresh blood, which had been collected from the heart donor dog, or 10% Dextran 40 solution as needed. Arterial pH, PO2, and PCO2 were maintained within their physiological ranges by using supplemental oxygen and intravenous sodium bicarbonate if necessary.

Experimental Protocol

In the present study, the experimental protocol consisted of the following eight runs.

Volume run under normothermia (normothermia-volume run; n = 12). In a stable, baseline contractile state under normothermia (NT) (heart temperature 36.3 ± 0.3°C), we produced isovolumic contractions at 6–10 different LV volumes to obtain a baseline relation between MVO2 and PVA. We waited 2–3 min after each change in LV volume until the cardiac variables reached a new steady state. In the present study, we used isovolumic contractions to avoid confounding effects of ejection on the cardiac contractility and energetics (26).

Calcium run under NT (NT-Ca2+ run; n = 8). In this run, LV volume was fixed at a moderate level (23.0 ± 1.5 ml), where peak isovolumic pressure was ∼100 mmHg before calcium (1% CaCl2) infusion. Calcium was continuously infused into the coronary arterial perfusion tube with an infusion pump (STC-521, Termo) at a starting rate of 0.01 meq·kg⁻¹·min⁻¹. The infusion rate was increased in steps every 5 min until Emax was nearly doubled to obtain six to eight sets of mechanoenergetic data at the preset volume. The calcium infusion was then stopped, and 10–15 min was allowed to elapse for the return of contractility to the precalcium baseline level.

We used calcium rather than catecholamine as a positive inotropic agent. Calcium-induced inotropy is in part mediated by protein kinase C, which regulates phosphorylation processes (17). However, calcium-induced inotropy involves fewer complex phosphorylation processes than catecholamine-induced inotropy (23, 32).

HT run (n = 8). We fixed LV volume at the same LV volume as in the NT-Ca2+ run under NT. Heart temperature was then gradually increased from 36 to 41°C. During this procedure, all data were repeatedly obtained.

Volume run under HT (HT-volume run; n = 12). After the heart temperature reached the target temperature (40–42°C), we obtained the MVO2-PVA relation and other variables in a manner similar to the NT-volume run.

Calcium run under HT (HT-Ca2+ run; n = 8). At the highest heart temperature (40–42°C), LV volume was fixed at the same LV volume as in the NT-Ca2+ run. We then repeated the calcium infusion in a manner similar to the NT-Ca2+ run until Emax was enhanced to the baseline level observed in the NT-volume run.

Calcium volume run under HT (HT-Ca2+ volume run; n = 6). When Emax was steadily enhanced to the NT level during the HT-Ca2+ run, we obtained another MVO2-PVA relation in a manner similar to the NT-volume and HT-volume runs.

Post-HT volume run (n = 6). After the hyperthermic protocols, we readjusted the heart temperature to 36–37°C. In a stable contractile state, we obtained the MVO2-PVA relation during NT.

KCl arrest under NT and HT (n = 6). Six hearts were arrested at the volume at which peak isovolumic pressure was zero (V0) by injecting KCl (5- to 6-ml bolus dose of 0.75 mol/l) into the coronary arterial tube. After both coronary blood flow (CBF) and AVO2D were stabilized under NT, we determined MVO2 as the basal metabolic MVO2 under NT. As heart temperature was then increased to the same temperature as in the previous HT run, we obtained another basal metabolic MVO2 under KCl arrest during HT.

Data Analysis

Data were analog-to-digital converted at sampling intervals of 2 ms and analyzed with a signal processing computer (7T-18, NEC San-ei).

Contractility Index

LV contractility was assessed by Emax and maximum rate of LV pressure rise (dP/dtmax) at the same LV volume in each experiment. Emax was computed as the maximal value of the instantaneous ratio of P(t)(V(t) – V0), where P(t) and V(t) are the instantaneous LV pressure and volume, respectively, in each contraction. Emax was normalized for 100 g of LV. [The dimensions of Emax are measured in mmHg·ml⁻¹·100 g LV but not mmHg·ml⁻¹·100 g LV⁻¹, because ml but not mmHg was normalized for LV weight (31)]. The time to Emax (Tmax) was determined as the time from the beginning of the QRS interval of the electrocardiogram to Emax.

Pressure-Volume Area

PVA is a specific area in the P-V diagram that is circumscribed by the end-systolic P-V relation line, the end-diastolic P-V relation curve, and the systolic P-V trajectory (Fig. 1A). PVA represents the total mechanical energy generated by each contraction of LV. We calculated PVA of each beat from the digitized P(t) and V(t) data in the same way as previously.
described (30). PVA was normalized for 100 g of LV. (The dimensions are measured in standard units as \( \text{mmHg} \times \text{ml} \times \text{beat}^{-1} \times 100 \text{ g LV}^{-1} \)).

Myocardial Oxygen Consumption

\( \text{MVO}_2 \) was determined as the product of CBF and AVO$_2$D. The \( \text{MVO}_2 \) per beat (ml O$_2$/beat) was obtained by dividing \( \text{MVO}_2 \) per minute by heart rate in a steady state. It was normalized for 100 g LV after subtracting the unloaded RV free wall \( \text{MVO}_2 \) from the measured total \( \text{MVO}_2 \). The unloaded RV \( \text{MVO}_2 \) was determined as the following: total unloaded \( \text{MVO}_2 \times \text{RV free wall weight} + \text{total ventricular weight} \) (30).

Oxygen Cost of Contractility

The \( \text{MVO}_2 \) of a contraction at an enhanced \( E_{\text{max}} \) consists of the following three components: an increased \( \text{PVA-independent MVO}_2 \) with calcium inotropism, an increased \( \text{PVA-dependent MVO}_2 \) with an augmented contraction, and the same \( \text{PVA-independent MVO}_2 \) as the baseline value. We calculated \( \text{PVA-independent MVO}_2 \) for each enhanced \( E_{\text{max}} \) in the NT-Ca$^{2+}$ and HT-Ca$^{2+}$ runs on the basis of the previous finding that the enhancement of contractility with calcium elevates the \( \text{MVO}_2 \)-PVA relation in a parallel manner. The \( \text{MVO}_2 \)-PVA data points (●) of these \( \text{MVO}_2 \)-PVA relations, which indicate \( \text{PVA-independent MVO}_2 \) for 4 different \( E_{\text{max}} \) levels, also increase. D: relation between \( E_{\text{max}} \) and \( \text{PVA-independent MVO}_2 \) derived from C. Slope of this relation is designated as oxygen cost of contractility.

We also calculated the ratio of the change in (Δ) \( \text{PVA-independent MVO}_2 \) to \( \Delta E_{\text{max}} \) from a set of data in the HT, NT-Ca$^{2+}$, and HT-Ca$^{2+}$ runs. In these calculations, \( \Delta E_{\text{max}} \) was matched among the three runs.

Statistics

We tested the significance of the effects of HT on the ESPVR and \( \text{MVO}_2 \)-PVA and \( \text{PVA-independent MVO}_2 \)-\( E_{\text{max}} \) relationships in each heart using an analysis of covariance (ANCOVA). We compared the paired variables between the NT and HT runs by paired \( t \)-test. In addition, the slope and intercept of the \( \text{MVO}_2 \)-PVA relation were also compared between the two temperatures by paired \( t \)-test; we assumed that the slope and intercept values of the individual regression lines reasonably represented their true values because the correlation coefficients were close to unity in every heart (26).

A value of \( P < 0.05 \) was considered statistically significant. Data are presented as means ± SD.

RESULTS

Effect of HT on LV function

Figure 2 shows representative recordings of LV pressure and volume, CBF, and AVO$_2$D and the electrocardiogram obtained during the NT (36.8°C) and HT (41.0°C) conditions in the same heart. During HT condition, LV systolic pressure strikingly decreased by 84.7 mmHg (NT: 193.1 vs. HT: 108.4 mmHg) at a
constant LV volume (23.6 ml). In this heart, CBF and AVO₂D slightly decreased during HT (CBF: from 81 to 79 ml/min and AVO₂D: from 10.8 to 9.0% vol).

Table 1 compares the mean values of cardiac variables between NT and HT conditions at the fixed LV volume. Heart rate was not significantly different between both conditions; in three experiments, heart rate under HT conditions exceeded the cardiac pacing rate.

During HT, LV peak pressure at the same LV volume, and hence \( E_{\text{max}} \), significantly decreased by 36 and 38%, respectively (both \( P < 0.001 \)), whereas \( \frac{dP}{dt}_{\text{max}} \) decreased by 24%. Both CBF and AVO₂D slightly decreased in HT, but these were not significantly different between the two conditions. The two energetic variables, PVA and MV˙O₂, significantly decreased during HT by 38 and 19%, respectively (both \( P < 0.001 \)).

LV diastolic function was assessed by the LV end-diastolic pressure and relaxation time constant. Both the LV end-diastolic pressure and time constant decreased during HT, indicating that HT augmented the rate of relaxation in LV.

Figure 3A shows a representative example of ESPVR during NT and HT in a heart. Both ESPVRs were linear over a wide range (both \( r = 0.98 \)). The slope of ESPVR (\( E_{\text{max}} \)) was decreased in HT (NT: 10.8 vs. HT: 6.1 mmHg·ml⁻¹·100 g LV), indicating that LV contractility decreased in the HT condition over the full range of LV volume. In 12 hearts, \( E_{\text{max}} \) was significantly decreased in HT by 35.2 ± 6.10.7% (\( P < 0.001 \) by paired \( t \)-test; Table 2).

Effect of HT on Cardiac Energetics

Figure 3B shows an example of the MV˙O₂-PVA relations during NT and HT in the same heart as in Fig. 3A. Despite the depressed LV contractility during HT, neither the slope (NT: 1.27 x 10⁻⁵ vs. HT: 1.32 x 10⁻⁵ ml O₂·mmHg⁻¹·ml⁻¹, \( P = \) not significant (NS) by ANCOVA) nor the MV˙O₂ intercept (NT: 0.024 vs. HT: 0.024 ml O₂·beat⁻¹·100 g LV⁻¹) of the MV˙O₂-PVA relation was changed from the NT condition.

Table 1. Effects of hyperthermia on mechanoenergetics

<table>
<thead>
<tr>
<th></th>
<th>Normothermia</th>
<th>Hyperthermia</th>
<th>Difference (P Value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>155.2 ± 16.9</td>
<td>161.8 ± 16.9</td>
<td>NS</td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>36.3 ± 0.3</td>
<td>41.0 ± 0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVV, ml</td>
<td>23.1 ± 1.6</td>
<td>23.1 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>125.1 ± 37.5</td>
<td>80.5 ± 26.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( E_{\text{max}} ), mmHg·ml⁻¹·100 g</td>
<td>6.9 ± 2.0</td>
<td>4.3 ± 1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( T_{\text{max}} ), ms</td>
<td>155.7 ± 14.5</td>
<td>124.0 ± 10.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \frac{dP}{dt}_{\text{max}} ), mmHg/s⁻¹</td>
<td>1,517.3 ± 517.4</td>
<td>1,178.8 ± 442.9</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LVDP, mmHg</td>
<td>4.9 ± 3.7</td>
<td>2.4 ± 3.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( \tau ), ms</td>
<td>34.2 ± 5.5</td>
<td>25.4 ± 5.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CBF, ml·min⁻¹·100 g⁻¹</td>
<td>73.6 ± 30.6</td>
<td>71.3 ± 26.5</td>
<td>NS</td>
</tr>
<tr>
<td>AVO₂,D, %vol</td>
<td>10.8 ± 2.9</td>
<td>10.0 ± 3.1</td>
<td>NS</td>
</tr>
<tr>
<td>PVA, mmHg·ml·beat⁻¹</td>
<td>1,111.6 ± 360.5</td>
<td>722.1 ± 270.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MV˙O₂, ml O₂·beat⁻¹·100 g⁻¹</td>
<td>0.0421 ± 0.0084</td>
<td>0.0349 ± 0.0083</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are means ± SD. LVV, left ventricular (LV) volume; LVSP, LV systolic pressure; \( E_{\text{max}} \), maximum elastance; \( T_{\text{max}} \), time to \( E_{\text{max}} \); \( \frac{dP}{dt}_{\text{max}} \), maximum change in pressure over time; LVDP, LV diastolic pressure; \( \tau \), time constant; CBF, coronary blood flow; AVO₂,D, arteriovenous oxygen content difference; PVA, pressure-volume area; MV˙O₂, myocardial oxygen consumption; NS, not significant.
In all hearts, both relations were highly linear under both the NT and HT conditions, and there was no significant difference in the slope of the $\text{MV}_\text{O}_2$-PVA relation by ANCOVA. In 4 of 12 hearts, there was a significant elevation difference by ANCOVA between the NT and HT conditions, but these differences in the $\text{MV}_\text{O}_2$ intercept were practically small. Averaged data for the slope and $\text{MV}_\text{O}_2$ intercept of the $\text{MV}_\text{O}_2$-PVA relation were not significantly different by paired $t$-test, whereas the elevation difference was significant. In all six hearts, the paired $t$-test indicated no significant difference in the slope of the $\text{MV}_\text{O}_2$-PVA relation between the HT-volume and HT-$\text{Ca}^{2+}$ volume runs (1.64 ± 0.32 vs. 1.66 ± 0.25 × 10⁻⁵ ml O₂·mmHg⁻¹·ml⁻¹, $P = \text{NS}$), but a significant difference in the $\text{MV}_\text{O}_2$ intercept (0.022 ± 0.005 vs. 0.028 ± 0.006 ml O₂·beat⁻¹·100 g LV⁻¹, $P < 0.01$) was found. The $\text{MV}_\text{O}_2$-PVA data points in the HT-$\text{Ca}^{2+}$ run shifted in the same manner as those in the NT-$\text{Ca}^{2+}$ run (HT-$\text{Ca}^{2+}$: $\text{MV}_\text{O}_2 = 3.6 \times 10⁻⁵ \text{PVA} + 0.018$, $r = 0.995$).

The amount of infused calcium to achieve the same $E_{\text{max}}$ was greater in the HT than NT conditions ($\Delta\text{calcium}/\Delta E_{\text{max}}$: NT 4.8 ± 1.7 vs. HT 5.8 ± 2.7 mg·ml⁻¹·mmHg⁻¹, $P < 0.05$ by paired $t$-test). This result demonstrates that, in the HT condition, a greater calcium delivery is required to enhance LV contractility to the same extent.

**Effect of HT on Oxygen Cost of Contractility**

Figure 5A demonstrates the relation between PVA-independent $\text{MV}_\text{O}_2$ and $E_{\text{max}}$ in the NT-$\text{Ca}^{2+}$ and HT-$\text{Ca}^{2+}$ runs in the same heart as shown in Fig. 4. PVA-independent $\text{MV}_\text{O}_2$ increased linearly with increases in $E_{\text{max}}$ in both runs (both $r = 0.99$). The slope (oxygen cost of contractility) was greater in the HT-$\text{Ca}^{2+}$ run than in the NT-$\text{Ca}^{2+}$ run (HT-$\text{Ca}^{2+}$: 0.0013 vs. NT-$\text{Ca}^{2+}$: 0.0010 ml O₂·ml⁻¹·mmHg⁻¹·beat⁻¹·100 g LV⁻², $P < 0.05$ by ANCOVA), whereas the intercepts of the PVA-independent $\text{MV}_\text{O}_2$ axis (PVA-independent $\text{MV}_\text{O}_2$ at zero $E_{\text{max}}$) in both relations were similar (NT-$\text{Ca}^{2+}$: 0.0182 vs. HT-$\text{Ca}^{2+}$: 0.0178 ml O₂·beat⁻¹·100 g LV⁻¹). The same tendency was observed in the other hearts (Fig. 6). On average, the oxygen cost of contractility increased by 48.8% in the HT condition (NT-$\text{Ca}^{2+}$: 0.0015 ± 0.0003 vs. HT-$\text{Ca}^{2+}$: 0.0023 ± 0.0007 ml O₂·ml⁻¹·mmHg⁻¹·beat⁻¹·100 g LV⁻², $P < 0.001$ by paired $t$-test, Fig. 6A). PVA-independent $\text{MV}_\text{O}_2$ at zero $E_{\text{max}}$ was not significantly different between the NT and HT conditions (NT-$\text{Ca}^{2+}$: 0.014 ± 0.004 vs. HT-$\text{Ca}^{2+}$: 0.014 ± 0.005 ml O₂·beat⁻¹·100 g LV⁻¹, $P = \text{NS}$ by paired $t$-test, Fig. 6B). Figure 5B shows a representative example of the relation between PVA-independent $\text{MV}_\text{O}_2$ and $E_{\text{max}}$ in the HT run. The slope of this relation in the HT run was smaller than those in the NT and HT-$\text{Ca}^{2+}$ runs.

Figure 7 shows average responses of $\Delta \text{MV}_\text{O}_2 / \Delta \text{PVA}$ (i.e., the oxygen cost of PVA) and $\Delta \text{PVA}$-independent $\text{MV}_\text{O}_2$ (i.e., the oxygen cost of $E_{\text{max}}$) among the HT, NT-$\text{Ca}^{2+}$, and HT-$\text{Ca}^{2+}$ runs. The slope of $\Delta \text{MV}_\text{O}_2 / \Delta \text{PVA}$ was greater in the HT-$\text{Ca}^{2+}$ run than in the
These responses of $\Delta$MV$_{O2}/\Delta$PVA in both runs shifted right and upward from the average regression line in the HT-volume run. In contrast, $\Delta$MV$_{O2}/\Delta$PVA in the HT run changed along the average regression line of the HT-volume run (Fig. 7A). The slope of $\Delta$PVA-independent MV$_{O2}/\Delta$E$_{max}$ was greater in the HT-Ca$^{2+}$ run than in the NT-Ca$^{2+}$ run (NT-Ca$^{2+}$: 0.0013 ± 0.0002 vs. HT-Ca$^{2+}$: 0.0025 ± 0.0008 ml O$_2$·mmHg$^{-1}$·beat$^{-1}$·100 g LV$^{-2}$). The slope in the HT run showed a near-zero oxygen cost of contractility (HT: $-0.5 \times 10^{-5} \pm 0.0009$ ml O$_2$·mmHg$^{-1}$·beat$^{-1}$·100 g LV$^{-2}$; Fig. 7B). These results in the HT condition (i.e., the HT and HT-Ca$^{2+}$ runs) indicated that the oxygen cost of contractility under the HT condition was greater than that in the NT condition.

**Recovery From HT to NT**

To test whether the effect of temporary HT on mechanoenergetics is reversible or not, the post-HT volume run was performed because HT was reported to bring about irreversible myocardial damage (3, 22). Compared with the pre-HT condition, $E_{max}$ (6.4 ± 1.7 vs. 5.9 ± 1.6 mmHg·ml$^{-1}$·100 g LV, $P = \text{NS}$), the slope (1.64 ± 0.28 vs. 1.59 ± 0.28 × 10$^{-5}$ ml O$_2$·mmHg$^{-1}$·ml$^{-1}$, $P = \text{NS}$), and the MV$_{O2}$ intercept (0.023 ± 0.002 vs. 0.024 ± 0.002 ml O$_2$·beat$^{-1}$·100 g LV$^{-1}$, $P = \text{NS}$) of the MV$_{O2}$-PVA relation under the post-HT condition did not significantly change, indicating that temporary HT in the present study did not result in irreversible myocardial damage.

**Effect of HT on Basal Metabolism**

Basal metabolic MV$_{O2}$ under KCl arrest was significantly greater by 18% in the HT condition (40.7 ± 0.8°C) than in the NT condition (36.5 ± 0.3°C) (NT: 0.0092 ± 0.0013 vs. HT: 0.0109 ± 0.0013 ml O$_2$·beat$^{-1}$·100 g LV$^{-1}$, $P < 0.05$ by paired $t$-test).

### Table 2. Effect of hyperthermia on the VO$_2$-PVA relation

<table>
<thead>
<tr>
<th>Experiment Number</th>
<th>$E_{max}$, mmHg·ml$^{-1}$·100 g</th>
<th>Slope, ml O$_2$·mmHg$^{-1}$·ml$^{-1}$</th>
<th>Intercept, ml O$_2$·beat$^{-1}$·100 g$^{-1}$</th>
<th>$r$ Value</th>
<th>$P$-Test</th>
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<tbody>
<tr>
<td>1</td>
<td>N 6.4</td>
<td>1.36</td>
<td>0.027</td>
<td>0.996</td>
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<tr>
<td></td>
<td>H 4.5</td>
<td>1.23</td>
<td>0.026</td>
<td>0.997</td>
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</tr>
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<td>2</td>
<td>N 10.2</td>
<td>1.55</td>
<td>0.024</td>
<td>0.994</td>
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<tr>
<td></td>
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<td>1.46</td>
<td>0.022</td>
<td>0.982</td>
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<td>3</td>
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<td>0.024</td>
<td>0.995</td>
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<td>4</td>
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<td></td>
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<tr>
<td>5</td>
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Means ± SD

| N | 7.2 ± 2.1 | 1.71 ± 0.31 | 0.023 ± 0.004 | 0.992 ± 0.007 |
| H | 4.7 ± 1.6 | 1.65 ± 0.30 | 0.023 ± 0.004 | 0.983 ± 0.011 |

Paired $t$-test for means

$P < 0.001$ NS NS NS NS

All values are means ± SD. N, normothermia; H, hyperthermia; $r$, correlation coefficient.
DISCUSSION

This study investigates the effects of HT on LV mechanoenergetics in the isolated, blood-perfused canine heart. Our findings demonstrate that HT depresses LV contractility and shortens time to peak contraction. Despite the depressed LV contractility, PVA-independent MV˙O2 remains unchanged in HT. When the depressed contractility under HT is restored to the baseline level (NT condition) by calcium infusion, the PVA-independent MV˙O2 under HT is markedly higher than that under NT. Moreover, the oxygen cost of contractility under HT is significantly higher than that under NT.

Effect of HT on LV Contractility

LV function assessed by cardiac output or work is well known to be enhanced by HT in both animal and human studies (4, 6, 10, 13, 16). However, these indexes are influenced by LV loading conditions and heart rate. Therefore, to clarify effect of HT on LV contractility, we use $E_{\text{max}}$ to assess LV contractility. Recently, $E_{\text{max}}$ has been reported to be a load-independent index for measure of LV contractility (26).

D’ambra et al. (5) reported that the regional LV function assessed by the shortening fraction depressed by 42% when myocardial temperature was increased from 38 to 42°C; the pressure-length loop area was inversely related with myocardial temperature. Templeton et al. (35) showed that HT from 37 to 40°C depressed LV stiffness, assessed by sinusoidal forcing function, by 17% in a heart-lung bypass preparation. Our present results, assessed by $E_{\text{max}}$ and $dP/dt_{\text{max}}$ at the same LV volume, clearly demonstrate that HT reduces LV contractility by itself. Our results have confirmed that HT depresses LV contractility, as was found in those previous studies (5, 35).

Effect of HT on Cardiac Energetics

No previous study has directly assessed the effects of HT on cardiac mechanoenergetics. To the best of our knowledge, the present study is the first to have precisely determined the mechanoenergetic effect of HT. In our observations, HT does not change the contractile efficiency (reciprocal of the slope of the MV˙O2-PVA relation) and the MV˙O2 intercept. As previously reported, acute inotropic interventions by conventional inotropic agents (e.g., Ca$^{2+}$, catecholamines, β-blockers, and calcium antagonists) changed the MV˙O2 intercept but not the slope of the MV˙O2-PVA relation (26, 29, 30).

Fig. 4. A: MV˙O2 and PVA data points and the regression line obtained from the NT-Ca$^{2+}$ run and NT-volume run. B: MV˙O2 and PVA data points and the regression lines obtained in the HT-volume, HT-Ca$^{2+}$, and HT-Ca$^{2+}$ volume runs. Note that the regression line in the HT-Ca$^{2+}$ volume run shifts upward in a parallel manner from that in the HT-volume run.

Fig. 5. A: relations between the PVA-independent MV˙O2 and $E_{\text{max}}$ obtained in the NT-Ca$^{2+}$ and HT-Ca$^{2+}$ runs in the same heart as in Fig. 3. The slope of each regression line indicates oxygen cost of contractility. Note that the slope of the regression line is greater in HT than in NT. B: relations between the PVA-independent MV˙O2 and $E_{\text{max}}$ obtained in the HT run. Both regression lines in the NT-Ca$^{2+}$ and HT-Ca$^{2+}$ runs are the same as shown in A.
32). In contrast, mechanical vibration (21) did not change the slope and MV˙O2 intercept of the MV˙O2-PVA relation, despite a substantial depression of LV contractility. This response of cardiac vibration on the LV mechanoenergetics is similar with HT. However, there is a clearly difference between vibration and HT; mechanical vibration instantly depressed $E_{\text{max}}$ and unaltered $T_{\text{max}}$ (21).

Varied heart temperature affects myosine ATPase activity and the cross-bridge cycling rate (both the reported rates of change with a 10°C increase in temperature, $Q_{10} = 2–3$) (1, 7). This finding suggests that HT increases the oxygen consumption in cross-bridge cycling and hence the PVA-dependent MV˙O2. The present study, however, shows that HT does not change the PVA-dependent MV˙O2 and contractile efficiency. Simultaneously, we observe that HT shortens the time to peak contraction (i.e., $T_{\text{max}}$) and time constant despite the negative inotropic effect, suggesting that HT enhances the attachment and detachment rates of cross-bridge cycling. Thus the increased myosine ATPase activity and cross-bridge cycling rate by HT seems to cause the reduced $T_{\text{max}}$ and time constant rather than affecting the PVA-dependent MV˙O2 and contractile efficiency.

In the present study, the most interesting point is the unchanged PVA-independent MV˙O2 despite the depressed contractility in HT. The MV˙O2 component for basal metabolism under KCl arrest increases during HT. This suggests that the unchanged PVA-independent MV˙O2 results from a combined effect of the increased basal metabolism and a decreased E-C coupling energy, such as those under propranolol. In our previous observations (29), propranolol lowered the PVA-independent MV˙O2 by 25% without a change in basal metabolism when $E_{\text{max}}$ decreased by 48% in the same experimental preparation. In contrast, HT does not change the PVA-independent MV˙O2 but increases the basal metabolic MV˙O2 by only 7% of the PVA-independent MV˙O2, whereas HT decreases $E_{\text{max}}$ by 38%. Therefore, this small increment of basal metabolic MV˙O2 cannot explain the unchanged PVA-independent MV˙O2 despite the marked decrease in contractility during HT. Thus HT should have substantially increased the MV˙O2 component for E-C coupling for a given LV contractility.

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**Fig. 6.** Comparison of the slope (oxygen cost of contractility; $A$) and intercept ($B$) of the relation between PVA-independent MV˙O2 and $E_{\text{max}}$ in NT-Ca²⁺ and HT-Ca²⁺ runs. Group data are presented as means ± SD. NS, not significant.

**Fig. 7.** Graphs showing the changes in ($\Delta$) MV˙O2 and PVA ($\Delta$MV˙O2/ $\Delta$PVA; $A$) and the changes in PVA-independent MV˙O2 and $E_{\text{max}}$ ($\Delta$PVA-independent MV˙O2/$\Delta E_{\text{max}}$; $B$) from their respective baseline values in HT, NT-Ca²⁺, and HT-Ca²⁺ runs. In $A$, the dashed line indicates averaged data of the MV˙O2-PVA relation in the HT-volume run.
In the HT-Ca\(^{2+}\) run, the oxygen cost of contractility was higher by 49% than that in the NT-Ca\(^{2+}\) run. Recently, Mikane et al. (19) observed that the oxygen cost of contractility obtained by dobutamine infusion increased in increasing myocardial temperature at 40°C. In the present study, the oxygen cost of contractility during HT is obtained from both HT and HT-Ca\(^{2+}\) runs. In the HT run, the oxygen cost of contractility is near zero with increased heart temperature (Figs. 5 and 7). This response in the HT run clearly demonstrates that the oxygen cost of contractility during the course of increasing myocardial temperature is greater than conventional negative inotropic agents.

In the stunned myocardium (22) and during acidosis (8–9), the oxygen cost of contractility has been shown to be higher by 120 and 50%, respectively, than in the normal heart. Therefore, HT is similar to the acidic hearts. This similarity may predict that intracellular acidosis is one of the important mechanisms of the negative inotropism in HT. Hypothermia has been reported to result in intracellular alkalosis in cardiac muscle (25). In contrast, Kusuoka et al. (11) demonstrated that hypothermia (31–38°C) did not alter intracellular pH and energy-related phosphorus compounds. The shortened \(T_{\text{max}}\) and time constant in the present study are not observed in the acidic heart. Therefore, intracellular acidosis may not be the substantial mechanism of the negative inotropism in HT.

Thus the mechanism of the negative inotropism in HT remains unclear. Our observation demonstrates that, in a given LV contractility, the HT condition needs calcium more than the NT condition. This suggests that the mechanism of the negative inotropism is either a decreased responsiveness of the contractile protein to Ca\(^{2+}\), a decreased intracellular Ca\(^{2+}\) level, or both.

The first possible mechanism of the negative inotropism in HT is a decreased responsiveness of the contractile protein to Ca\(^{2+}\). Kusuoka et al. (11) demonstrated that the maximal Ca\(^{2+}\)-activated pressure was inversely related with decreased temperature (30–37°C), indicating that the responsiveness of the contractile protein to Ca\(^{2+}\) decreased at a high temperature range. In contrast, Brandt et al. (2) observed that an increased temperature (20–29°C) enhanced calcium sensitivity in ventricular muscle. However, the temperature ranges in this study are low and not physiological. No study in muscle and skinned preparation has been performed around 38–40°C. Recently, Mikane et al. (18) reported that HT decreased the Ca\(^{2+}\) responsiveness of cross-bridge force development and shifted the force-pCa\(^{2+}\) curve to the right in a simulation model. Therefore, a decreased responsiveness of the contractile protein to Ca\(^{2+}\) should play an important role in the negative inotropism in HT.

The second possible mechanism of the negative inotropism in HT is a decreased intracellular Ca\(^{2+}\) level. In hypothermic hearts, decreases in the active transport of Na\(^+\), Ca\(^{2+}\) efflux (33), and release and sequestration of Ca\(^{2+}\) by the sarcoplasmic reticulum (12) have been reported. These findings indicate that HT reduces the intracellular Ca\(^{2+}\) level and handling. In the present study, however, little change in the MV\(_{\text{O2}}\) components for E-C coupling was observed during HT, indicating that HT does not influence the intracellular Ca\(^{2+}\) handling. Thus this mechanism is considered to be a small part of the negative inotropism in HT.

The third possible mechanism is that a reduced amount of released Ca\(^{2+}\) during systole is due to the dysfunction of the Ca\(^{2+}\) release channel of the sarcoplasmic reticulum. In our previous observations (34), low-dose ryanodine (30–40 nM) decreased \(E_{\text{max}}\) but disproportionately induced high MV\(_{\text{O2}}\) for E-C coupling by increasing the open probability of the Ca\(^{2+}\) release channel. Inappropriate leak of Ca\(^{2+}\) from the sarcoplasmic reticulum should result in similar mechanoenergetic findings to the present study. However, the time to peak contraction and time constant were prolonged by ryanodine, whereas they were shortened by HT. Therefore, this mechanism induced by ryanodine is different from that of HT.

**Limitations**

Our present study did not directly measure the cross-bridge activity and Ca\(^{2+}\) handling in the sarcoplasmic reticulum. We used their mechanoenergetic manifestations on the LV level. Therefore, we have no direct evidence as to how HT affects the cross-bridge activity and Ca\(^{2+}\) handling. To clarify the mechanism of HT-induced inotropy, further study will be needed.

In summary, we have assessed the direct effect of HT by 5°C from 36°C on LV mechanoenergetics, fully utilizing the MV\(_{\text{O2}}\)-PVA-\(E_{\text{max}}\) framework in the isolated cross-circulated canine heart. HT depresses \(E_{\text{max}}\), shortens \(T_{\text{max}}\), and decreases both PVA and MV\(_{\text{O2}}\). The MV\(_{\text{O2}}\)-PVA relation in HT is superiorimposable to that in NT, and the apparent oxygen cost of contractility obtained during the course of an increase in heart temperature is virtually zero, like that in hypothermia (28) but unlike the conventional negative inotropism. However, the true oxygen cost of contractility obtained with calcium is 1.5 times greater in HT than in NT. These results suggest that the depressed LV contractility during HT is largely due to a decreased Ca\(^{2+}\) responsiveness of the contractile protein.

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EFFECT OF HYPERTHERMIA ON CARDIAC ENERGETICS


