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Journal:	<i>Canadian Journal of Physiology and Pharmacology</i>
Manuscript ID	cjpp-2015-0196.R1
Manuscript Type:	Article
Date Submitted by the Author:	29-Jan-2016
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Keyword:	hyperthermia, hypothermia, inotropism, lusitropism, mono-exponential time constant

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Temperature-dependent inotropic and lusitropic indices based on half-logistic time constants for four segmental phases in isovolumic left ventricular pressure-time curve in excised, cross-circulated canine heart

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**Short Running Title: Temperature-Dependent Half-logistic Time Constant
4 Tables and 3 Figures**

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Abstract

Varying temperature affects cardiac systolic and diastolic function and the left ventricular (LV) pressure–time curve (PTC) waveform that includes information about LV inotropism and lusitropism. Our proposed half-logistic (h-L) time constants obtained by fitting using h-L functions for four segmental phases (Phases I–IV) in the isovolumic LV PTC are more useful indices for estimating LV inotropism and lusitropism during contraction and relaxation periods than the mono-exponential (m-E) time constants at normal temperature. In this study, we investigated whether the superiority of the goodness of h-L fits remained even at hypothermia and hyperthermia. Phases I–IV in the isovolumic LV PTCs in eight excised, cross-circulated canine hearts at 33°C, 36°C, and 38°C were analyzed using h-L and m-E functions and the least-squares method. The h-L and m-E time constants for Phases I–IV significantly shortened with increasing temperature. Curve fitting using h-L functions was significantly better than that using m-E functions for Phases I–IV at all temperatures. Therefore, the superiority of the goodness of h-L fit vs. m-E fit remained at all temperatures. As LV inotropic and lusitropic indices, temperature-dependent h-L time constants could be more useful than m-E time constants for Phases I–IV.

Key words: hyperthermia; hypothermia; inotropism; lusitropism; mono-exponential time constant

Introduction

It is well known that varying temperature affects cardiac systolic and diastolic functions and the left ventricular (LV) pressure–time curve (PTC) waveform that includes information about LV inotropism and lusitropism for evaluating LV performance during contraction and relaxation periods (Eucker et al. 2001, 2002). For example, hypothermia causes considerable prolongation in the LV contraction and relaxation periods, whereas hyperthermia causes considerable shortening.

Nonlinear regression analyses using the least-squares method are valuable tools for elucidating the mechanisms, summarizing information, eliminating noise, allowing speculation regarding unmeasured data, and separating the effects of multiple factors. To maximize the amount of useful information extracted from the isovolumic LV PTC, LV PTC is generally divided into two sequential phases by a boundary at the peak LV pressure (LVP): the ascending phase and descending phase, which are regarded as the contraction and relaxation phases, respectively (Mizuno et al. 2008b). The contraction phase represents the duration of the increase in the myocardial intracellular free calcium (Ca^{2+}) ($[\text{Ca}^{2+}]_i$) concentration from the sarcoplasmic reticulum (SR) by Ca^{2+} -induced Ca^{2+} release, and the relaxation phase represents the duration of the decrease in $[\text{Ca}^{2+}]_i$ concentration resulting from Ca^{2+} sequestration into the SR and Ca^{2+} removal from the cytoplasm to the extracellular space through the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. However, the cardiac cycle does not suddenly alternate its contraction phase with its relaxation phase at one particular point, for example, the peak LVP. Thus, the contraction and relaxation phases have temporarily overlapping phases. The entire ascending phase is not the simple contraction process, and the entire descending phase is not the simple relaxation process. Therefore, we need to investigate the isovolumic LV PTC more thoroughly to understand LV contraction and relaxation functions and the relationship between contraction and relaxation periods. In our previous study, we divided the isovolumic LV PTC into four segmental phases (Phases I–IV) by boundaries at three meaningful points: the maximum of the first-order time derivative of LVP ($dP/dt_{\max}$), the peak LVP, and the minimum of the first-order time derivative of LVP ($dP/dt_{\min}$).

Thereafter, we found that the half-logistic (h-L) functions, which represent half-curves of symmetrical sigmoid logistic functions by a boundary at the inflection

point, produced better fits for Phases I–IV in the isovolumic LV PTC than the mono-exponential (m-E) functions, which have been used for Phase IV, at normothermia (Mizuno et al. 2006, 2008b). Therefore, the h-L time constants obtained by fitting using h-L functions for Phases I–IV are more reliable and useful inotropic and lusitropic indices for estimating LV contraction and relaxation periods during the cardiac cycle at normothermia than the m-E time constants.

In previous studies, hypothermia-induced prolongation in the lusitropic index m-E time constant obtained by fitting using the m-E function for Phase IV in the isovolumic LV PTC and hyperthermia-induced shortness (Fischer et al. 2005; Greene et al. 1980; Lauri et al. 1996; Saeki et al. 2000; Tveita et al. 1998). However, we found that the h-L function provided better fits for Phase IV in the isovolumic LV PTC than the m-E function at any temperature (Mizuno et al. 2008a). Our proposed h-L time constant for Phase IV is more useful as a lusitropic index than the m-E time constant, regardless of temperature.

In the present study, we investigated the potential application of the h-L and m-E functions to the analyses of Phases I, II, and III in the isovolumic LV PTC at hypothermia and hyperthermia, how temperature affects the h-L and m-E time constants, and whether the superiority of the goodness of h-L fits vs. that of m-E fits remained at different temperatures.

Materials and Methods

This study protocol was approved by the Animal Investigation Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences. All procedures were conducted in conformity with the National Institutes of Health guidelines for animal care, Canadian Council on Animal Care (CCAC) Guidelines, and the Guiding Principles for Research Involving Animals and Human Beings endorsed by the American Physiological Society and the Physiological Society of Japan.

Surgical preparation

The excised, cross-circulated canine heart samples from another adult mongrel dogs were prepared as shown in Figure 1. The details of the surgical preparations are described elsewhere (Suga 1990; Suga et al. 1998).

Briefly, a metabolic support dog [20.1 ± 5.6 kg, mean $\pm$ standard deviation (SD)] and a heart donor dog (12.7 ± 1.9 kg) were anesthetized with pentobarbital sodium ($25 \text{ mg} \cdot \text{kg}^{-1}$ i.v.) and fentanyl citrate ($0.1\text{--}0.2 \text{ mg} \cdot \text{h}^{-1}$ i.v.) after premedication with ketamine

hydrochloride ($25 \text{ mg} \cdot \text{kg}^{-1}$ i.m.). Each animal was intubated tracheally, ventilated with room air, and heparinized (15,000 U i.v. per support dog and 10,000 U i.v. per donor dog).

The bilateral common carotid arteries and unilateral external jugular vein of the support dog were cannulated and connected to the arterial and venous cross-circulation tubes, respectively. The chest of the donor dog was opened midsternally. The arterial and venous cross-circulation tubes from the support dog were cannulated into the left subclavian artery and the right ventricle (RV) via the right atrial appendage of the donor dog, respectively. All systemic and pulmonary vascular connections to the heart, were ligated, including the descending aorta, inferior vena cava, brachiocephalic artery, superior vena cava, azygos vein, and bilateral pulmonary hili. The metabolically supported beating heart was excised from the chest of the donor dog under continuous cross-circulation from the support dog. The coronary perfusion of the excised donor heart was not interrupted during the surgical preparation.

The left atrium of the donor heart was opened and all the LV chordae tendineae were severed. Complete atrioventricular block was created chemically (0.2-0.5 ml injection of 36% formaldehyde solution) or electrically (direct current of 20-30 J) with ablation of the bundle of His. A bipolar pacing electrode was placed on the upper portion of the ventricular septal endocardium via the left atrium. The LV was electrically paced at 500-millisecond intervals throughout the study, thus maintaining the heart rate at $120 \text{ beats} \cdot \text{min}^{-1}$.

A thin latex balloon with an unstretched volume of approximately 50 ml was mounted on a rigid connector and placed in the LV. The connector was secured at the mitral annulus. The balloon was connected to a custom-made volume servo pump (AR-Brown, Tokyo, Japan). Both the balloon and the water housing of the servo pump were primed with water without any air bubbles. The servo pump enabled accurate measurement and precise control of the LV volume (LVV). LVP was measured by using a miniature pressure gauge (Model P-7, Konigsberg Instruments, Pasadena, CA, USA) placed inside the apical end of the balloon, processed with a DC stain amplifier, and low-pass filtered at a corner frequency of 100 Hz (Model 6M76, NEC San-ei, Tokyo, Japan). This corner frequency was high enough to avoid blunting the original pressure signals. An 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 for the excised donor heart. Systemic arterial blood pressure was maintained at a stable level by infusing whole blood obtained from the donor dog, 6% hydroxyethylated starch solution, or methoxamine ($5\text{--}30\text{ mg}\cdot\text{h}^{-1}$) as necessary. Arterial pH, P_{aCO_2} , and P_{aO_2} of the support dog were repeatedly measured with a blood gas analyzer and maintained within physiological ranges with supplemental oxygen, intravenous sodium bicarbonate, or appropriate adjustment of the ventilator setting. The LV, including the septum, weighed $81.2 \pm 12.4\text{ g}$ and the RV weighed $33.6 \pm 5.8\text{ g}$ after each experiment.

Experimental protocol

The temperature of the excised donor heart was controlled at 33°C , 36°C , and 38°C by regulating the arterial blood temperature through cooling or warming of the coiled portion of the arterial cross-circulation tube in a thermostatic bath (NCB-1000, Tokyo Rika, Tokyo, Japan). The arterial blood temperature was measured with a thermistor (MTS-40030, Respiratory Support Products). In addition, the myocardial temperature was measured with a thermistor (6-F Foley catheter with thermistor, Respiratory Support Products) that was placed between the LV endocardium and the balloon via the left atrium and kept stable at $\pm 0.2^\circ\text{C}$ during measurements. The temperature was randomly set for measurements. Hemodynamics, including LV pressure-time waveforms, was recorded after 30 min of equilibration in each temperature state.

Data acquisition

The LVP, LVV, and ECG signals were digitized at 2-millisecond intervals and processed on a LabVIEW (National Instruments, Austin, TX, USA)-installed computer. To suppress small noises in the digitized LVP data, the sampled LVP data were digitally smoothed by an 11-point, non-weighted moving average.

The time period of the isovolumic LV PTC was limited from the onset as the upstroke of the R wave of the ECG to the full end-point as the time when the decrease in the isovolumic LV PTC reached LV end-diastolic pressure (LVEDP) of the preceding diastole. This end-point corresponded to the time when LV developed pressure decreased to 0 mmHg. The first-order time derivative of LVP (dP/dt) value was obtained by differentiating digitized LVP signals.

The isovolumic LV PTC was divided into Phases I–IV during the cardiac cycle by boundaries at the dP/dt_{max} , the peak LVP, and the dP/dt_{min} as shown in Figure 2. Thus, Phase I is the first half of the ascending phase defined as the period from the time

corresponding to the onset of the isovolumic LV PTC to the time corresponding to $dP/dt_{\max}$. Phase II is the second half of the ascending phase defined as the period from the time corresponding to $dP/dt_{\max}$ to the time corresponding to peak LVP. Phase III is the first half of the descending phase defined as the period from the time corresponding to peak LVP to the time corresponding to $dP/dt_{\min}$. Phase IV is the second half of the descending phase defined as the period from the time corresponding to $dP/dt_{\min}$ to the time corresponding to LVEDP. Therefore, we suggest that Phase I corresponds to the early part of the contraction process; Phase II affects both the middle part of the contraction process and the early part of the relaxation process, and Phase III affects both the late part of the contraction process and the middle part of the relaxation process. Phase IV corresponds to the late part of the relaxation process.

Half-Logistic (h-L) and mono-Exponential (m-E) equations

The following h-L function equations (Matsubara et al. 1995) were used to fit Phases I–IV in the isovolumic LV PTC using the least-squares method with DeltaGraph 4.0 (DeltaPoint, Monterey, CA, USA):

$$P(t) = 2P_{1A}/\{1 + \exp[-(t - t_{aC})/P\tau_{1L}]\} + P_{1B} \quad (\text{Eq. 1})$$

$$P(t) = 2P_{2A}/\{1 + \exp[(t - t_{aC})/P\tau_{2L}]\} + P_{2B} \quad (\text{Eq. 2})$$

$$P(t) = 2P_{3A}/\{1 + \exp[-(t - t_{dC})/P\tau_{3L}]\} + P_{3B} \quad (\text{Eq. 3})$$

$$P(t) = 2P_{4A}/\{1 + \exp[(t - t_{dC})/P\tau_{4L}]\} + P_{4B} \quad (\text{Eq. 4})$$

, respectively. The t is the time from the onset of the isovolumic LV PTC, P_{1A} , P_{2A} , P_{3A} , and P_{4A} represent the h-L amplitude constants, $P\tau_{1L}$, $P\tau_{2L}$, $P\tau_{3L}$, and $P\tau_{4L}$ represent the h-L time constants, and P_{1B} , P_{2B} , P_{3B} , and P_{4B} represent the h-L nonzero asymptotes obtained by fitting with the h-L functions for Phases I, II, III, and IV, respectively. The t_{aC} and t_{dC} values are constants which represent the time corresponding to $dP/dt_{\max}$ and $dP/dt_{\min}$, respectively. It is noted that t_{aC} and t_{dC} are determined before the h-L function fittings. The best-fitted h-L function curves given by Eqs.1-4 change monotonically, but not in an S-shaped manner.

The following m-E function equations (Frederiksen et al. 1978; Weiss et al. 1976; Yellin et al. 1986) were used to fit Phases I–IV in the isovolumic LV PTC using the least-squares method:

$$P(t) = P_{10}\exp[(t - t_{aC})/P\tau_{1E}] + P_{1\infty} \quad (\text{Eq. 5})$$

$$P(t) = P_{20}\exp[-(t - t_{aC})/P\tau_{2E}] + P_{2\infty} \quad (\text{Eq. 6})$$

$$P(t) = P_{30}\exp[(t - t_{dC})/P\tau_{3E}] + P_{3\infty} \quad (\text{Eq. 7})$$

$$P(t) = P_{40}\exp[-(t - t_{dC})/P\tau_{4E}] + P_{4\infty} \quad (\text{Eq. 8})$$

, respectively. The t is the time from the onset of the isovolumic LV PTC, P_{10} , P_{20} , P_{30} , and P_{40} represent the m-E amplitude constants, $P\tau_{1E}$, $P\tau_{2E}$, $P\tau_{3E}$, and $P\tau_{4E}$ represent the m-E time constants, and $P_{1\infty}$, $P_{2\infty}$, $P_{3\infty}$, and $P_{4\infty}$ represent the m-E nonzero asymptotes obtained by fitting with the m-E functions for Phases I, II, III, and IV, respectively. The t_{aC} and t_{dC} values are constants which represent the time corresponding to $dP/dt_{\max}$ and $dP/dt_{\min}$, respectively. It is noted that t_{aC} and t_{dC} are determined before the m-E function fittings.

The h-L function equations (Eqs. 1-4) utilize the same number of parameters, i.e., three, as the m-E function equations (Eqs. 5-8).

Temperature coefficient

The temperature coefficient (Q_{10}) (Hak et al. 1992; Reyes et al. 2008; Suga et al. 1988) is a convenient way to examine and report the temperature dependence of a process and represents the temperature sensitivity by which the rate of a reaction increases for every 10°C rise in temperature. Q_{10} is a unitless quantity. In a typical experiment, the rate of the physiological process under investigation is measured at two different temperatures, T_1 and T_2 , thus yielding the rate measurements R_1 measured at T_1 and R_2 measured at T_2 , respectively. The Q_{10} equation

$$Q_{10} = (R_2/R_1)^{10/(T_2 - T_1)} \quad (\text{Eq. 9})$$

is then used to estimate the Q_{10} for the process. The temperature unit must be either Celsius or Kelvin and may not be any other unit, such as Fahrenheit. Note that T_1 and T_2 do not need to be exactly 10°C apart to use Eq. 9. The same unit must be used for the two temperatures T_1 and T_2 at which the rate measurements are obtained. Moreover, the rate measurements R_1 and R_2 must use the same unit.

The rate may represent any measure of the progress of a process. If the rate of the reaction is completely temperature independent, it can be seen from Eq. 9 that the resulting Q_{10} will be 1.0. If the reaction rate increases with increasing temperature, Q_{10} will be > 1 . Thus, the Q_{10} value increases with increasing temperature dependence of a process. Q_{10} is > 1 for diffusion of ions and molecules in bulk solutions. For typical chemical reactions, such as pump, channel, and exchange, Q_{10} values are > 2 . For many biological processes, particularly those that involve large-scale protein conformational changes, Q_{10} values are > 2 . Many enzymes have a Q_{10} of approximately 2, which means that the rate of reaction doubles when the temperature is increased by 10°C. Thus, Q_{10} values may be used to infer mechanisms underlying the physiological process being investigated.

Statistics analysis

The hemodynamic data, and three h-L and three m-E function parameter values were compared among 33°C, 36°C, and 38°C by using one-way repeated measures analysis of variance (RANOVA) after Shapiro-Wilk test and Levene test. When one-way RANOVA was significant (F -test, $p < 0.05$), multiple comparisons by the post hoc Scheffe's test were performed. The absolute values of the h-L and m-E amplitude constants were used for the comparison.

Goodness of h-L and m-E fits was evaluated by using the correlation coefficient (r) and residual mean square (RMS). Paired Student's t -test was applied to the value after Fisher's Z -transformation (Z) of r : $Z = 1/2[\ln(1 + r) - \ln(1 - r)]$ (Snedecor and Cochran 1971). The pressure residuals, that is, the differences between the original LVP data and the best-fitted h-L or m-E function values at all the sampling points, were analyzed. RMS was calculated as the residual sum of squares divided by the residual degrees of freedom, that is, the number of data points analyzed minus the number of parameters in the function, i.e., three (Thompson et al. 1983a, 1983b). The h-L RMS value was compared with the m-E RMS value by using Wilcoxon's signed-rank test.

33°C and 38°C were chose as the two temperatures T_1 and T_2 for the estimation of the Q_{10} values.

All data were analyzed with Microsoft Excel 98 (Microsoft-Japan, Tokyo, Japan), Statcel (OMS, Saitama, Japan), and StatView 5.0 (SAS Institute Inc, Cary, NC, USA) software programs. The observed and calculated values were presented as the means $\pm$ SD. A p value < 0.05 indicated statistical significance.

Results

Hemodynamic change

Table 1 summarizes hemodynamic data for the isovolumic LV PTCs at 33°C, 36°C, and 38°C in eight excised, cross-circulated canine hearts. LVP at $dP/dt_{\max}$, peak LVP, and LVP at $dP/dt_{\min}$ decreased significantly with increasing temperature. The $dP/dt_{\max}$, $dP/dt_{\min}$, and LVEDP did not change significantly with varying temperature. The time to peak LVP, time to $dP/dt_{\min}$, time to LVEDP, period of Phase II, period of Phase III, and period of Phase IV shortened significantly with increasing temperature. LVV was always controlled at 12.4 ± 2.4 ml at 33°C, 36°C, and 38°C.

h-L and m-E function parameters

PT_{1L} , PT_{2L} , PT_{3L} , and PT_{4L} , as well as PT_{1E} , PT_{2E} , PT_{3E} , and PT_{4E} shortened

significantly with increasing temperature, as shown in Figure 3. $P\tau_{1L}$ was the shortest among four h-L time constants at 33°C, 36°C, and 38°C. Similarly, $P\tau_{1E}$ was the shortest among four m-E time constants at 33°C, 36°C, and 38°C. $P\tau_{3E}$ at 36°C and 38°C was longer than $P\tau_{4E}$.

The absolute values of P_{1A} , P_{2A} , P_{4A} , P_{10} , P_{20} , and P_{40} decreased significantly with increasing temperature, as listed in Table 2. P_{2B} , P_{3B} , $P_{2\infty}$, $P_{3\infty}$, and $P_{4\infty}$ decreased significantly with increasing temperature.

Goodness of h-L and m-E fits

For Phases I–IV, the h-L Z values were always significantly greater than the m-E Z values at 33°C, 36°C, and 38°C, as listed in Table 3. The h-L RMS values for Phases I–IV were always significantly smaller than the m-E RMS values at 33°C, 36°C, and 38°C.

Temperature coefficient

The Q_{10} values of the reciprocal values of h-L and m-E time constants listed in Table 4. The Q_{10} values of $1/P\tau_{1L}$, $1/P\tau_{2L}$, $1/P\tau_{1E}$, and $1/P\tau_{2E}$ were between 1.0 and 2.0, and the Q_{10} values of $1/P\tau_{3L}$, $1/P\tau_{3E}$, $1/P\tau_{4L}$, and $1/P\tau_{4E}$ were >2.0 .

Discussion

The above results demonstrated that both the h-L and m-E time constants for Phases I–IV in the isovolumic LV PTC significantly shortened with increasing temperature, and the superiority of the goodness of h-L fits to that of m-E fits remained even under hypothermic and hyperthermic conditions in the excised, cross-circulated canine heart. Our main finding indicates that the h-L function is a superior model to express Phases I–IV more accurately at any temperature, and the h-L time constants for Phases I–IV are more reliable and useful temperature-dependent LV inotropic and lusitropic indices during estimation of the LV contraction and relaxation periods of the cardiac cycle.

Temperature-dependent h-L time constants

The time constant has been shown to prolong with decreasing temperature in basic and clinical cardiovascular research. For example, $P\tau_{4E}$ prolonged from 44.8 to 61.6 ms with decreasing temperature from 38°C to 34°C in the RV bypassed pig (Greene et al. 1989), from 25.8 to 41.5, 78.5 ms with decreasing temperature from 37°C to 31°C, 25°C in the unsupported *in situ* canine heart (Tveita et al. 1998), and from 63 to 87, 112 ms with decreasing temperature from $>36^\circ\text{C}$ to between 36°C and 33°C, <

33°C in the human heart (Luke et al. 1989). In our previous study, both $P\tau_{4L}$ and $P\tau_{4E}$ prolonged with decreasing temperature from 36°C to 33°C, 30°C (Mizuno et al. 2008a). In contrast, $P\tau_{4E}$ shortens with increasing temperature. For example, $P\tau_{4E}$ shortened from 34.2 to 25.4 ms with increasing temperature from 36°C to 41°C in the isolated, cross-circulated canine heart (Saeki et al. 2000). In our previous study, both $P\tau_{4L}$ and $P\tau_{4E}$ shortened with increasing temperature from 36°C to 38°C, 40°C (Mizuno et al. 2008a). Thus, $P\tau_{4E}$ prolonged linearly with cooling and recovered linearly with rewarming in the anesthetized canine (Lauri et al. 1996). A study showed that the r value between $P\tau_{4E}$ and temperature was 0.91 (Fischer et al. 2005). In the present results, the h-L and m-E time constants for Phases I–IV prolonged with decreasing temperature and shortened with increasing temperature. Therefore, hypothermia induced significant extension of both $P\tau_{4L}$ and $P\tau_{4E}$, and hyperthermia induced significant reduction.

The other time indices showing contraction and relaxation periods extended with decreasing temperature and decreased with increasing temperature. For example, the period from the beginning of the QRS interval of the ECG to the time corresponding to $dP/dt_{\max}$ prolonged from 50 to 54 ms with decreasing temperature from 36°C to 30°C and the period from the beginning of the QRS interval of the ECG to end-systolic maximal elastance ($T_{\max}$) prolonged from 150 to 176 ms (Suga et al. 1988). In contrast, $T_{\max}$ shortened from 155.7 to 124.0 ms with increasing temperature from 36°C to 41°C (Saeki et al. 2000) and shortened from 206.6 to 167.4, 133.7 ms with increasing temperature from 30°C to 36°C, 40°C (Mikane et al. 1999). In the present study, the time to peak LVP, time to $dP/dt_{\min}$, time to LVEDP, and periods of Phases II, III, and IV shortened with increasing temperature.

In the present results, the relationship among the h-L time constants for Phases I–IV was nearly similar to that among the m-E time constants at 33°C, 36°C, and 38°C. For example, both $P\tau_{1L}$ and $P\tau_{1E}$ were the smallest among the h-L and m-E time constants for Phases I–IV at all temperatures, respectively. Our study of curve fitting using the h-L function may have important implications in the analysis and evaluation for Phases I–IV in the isovolumic LV PTC at any temperature.

Goodness of h-L fit and temperature

The superiority of the goodness of h-L fit to that of m-E fit for Phase IV in the isovolumic LV PTC was found in the excised, cross-circulated canine heart in 1995 (Matsubara et al. 1995). Thereafter, $P\tau_{4L}$ has been used as a reliable and useful

lusitropic index, in the calf (Mueller et al. 2001), in vivo canine (Mohri et al. 2001), in vivo mouse (Nagayama et al. 2007; Takimoto et al. 2004), isolated ejecting rat, guinea pig, ferret (Langer 2000; Langer 2002; Langer and Schmid 2003), excised cross-circulated rat (Abe et al. 2002), Langendorff-perfused ferret (Mohri et al. 2003), failing canine (Senzaki and Kass 2010), and failing human hearts (Senzaki et al. 1999; Shmuylovich and Kovács 2008). In our previous study, we found that the superiority of the goodness of h-L fit to that of m-E fit for Phase IV in the isovolumic LV PTC was consistently shown at 30°C, 33°C, 36°C, 38°C, and 40°C because $P\tau_{4E}$ overestimated in hyperthermia and underestimated in hypothermia (Mizuno et al. 2008a).

Moreover, the goodness of h-L fits for Phases I–IV in the isovolumic LV PTC was consistently superior to that of m-E fits in normothermia (Mizuno et al. 2008b). In the present study, the superiority of the goodness of h-L fits to that of m-E fits was demonstrated even at 33°C and 38°C. The h-L functions appear to minimize systemic bias at any temperature and provide more robust assessments of LV inotropism and lusitropism. These results enhance the general superiority of the h-L time constants to the m-E time constants for Phases I–IV, regardless of temperature.

Myocardial force–time curve and temperature

The time constant obtained by fitting using a function for the segmental phase in the myocardial force–time curve (FTC) can change with varying temperature. The m-E time constant for the second half of the descending phase, which is defined as the period from the time corresponding to the minimum of the first-order time derivative of force ($dF/dt_{\min}$) to the time corresponding to the resting force level (Phase IV) in the myocardial isometric FTC ($F\tau_{4E}$), extended with cooling and decreased with rewarming in rat RV papillary muscle (Dobrunz and Berman 1994). However, the superiority of the goodness of h-L fit to that of m-E fit for Phase IV in the myocardial isometric FTC has been shown at 30°C in the papillary muscle superfused with Tyrode solution in isolated ferret RV (Mizuno et al. 2000), rabbit RV (Mizuno et al. 2007; Baudet et al. 1999), and mouse LV (Mizuno et al. 2007) papillary muscles. Therefore, the h-L time constant is more reliable than $F\tau_{4E}$ for Phase IV in the myocardial isometric FTC ($F\tau_{4L}$). In our previous studies, we also found that the h-L functions produced better fits than the m-E functions for Phases I–IV in the myocardial isometric FTC at 30°C in mouse LV and rabbit RV papillary muscles (Mizuno et al. 2009). Therefore, the h-L time constants for Phases I–IV in the myocardial isometric FTC can be more reliable and useful myocardial inotropic and lusitropic indices than the m-E time constants at any

temperature.

Myocardial intracellular Ca^{2+} concentration-time curve and temperature

Furthermore, the time constant obtained by fitting using a function for the segmental phase in the myocardial $[\text{Ca}^{2+}]_i$ -time curve (CaTC) can change with varying temperature. The m-E time constant for the second half of the descending phase, which is defined as the period from the time corresponding to the minimum of the first-order time derivative of $[\text{Ca}^{2+}]_i$ ($d\text{Ca}/dt_{\min}$) to the time corresponding to the resting $[\text{Ca}^{2+}]_i$ concentration (Phase IV) in the myocardial CaTC ($\text{Ca } \tau_{4E}$), has been used as an index for Ca^{2+} decline in the isovolumic LV in excised whole hearts of rats (Camacho et al. 1994; Chang et al. 1996) and in cardiomyocytes of mice (Su et al. 2003), rabbits (Bassani et al. 1994; Su et al. 2003), rats (Bassani and Noireaud 1994), canines (Su et al. 2003), and humans (Su et al. 2003). However, in our previous study, we found that the h-L function produced a better fit than the m-E function for Phase IV in the myocardial CaTC at 30°C in rabbit RV and mouse LV papillary muscles (Mizuno et al. 2007). Therefore, the h-L time constant is more reliable than $\text{Ca } \tau_{4E}$ for Phase IV in the myocardial CaTC ($\text{Ca } \tau_{4L}$). Moreover, the h-L functions produced better fits than the m-E functions for Phases I (Mizuno et al. 2011), II (Mizuno et al. 2013), and III (Mizuno et al. 2016) in the myocardial CaTC at 30°C in mouse LV papillary muscle. Therefore, the h-L time constants for Phases I–IV in the myocardial CaTC can be more reliable and useful indices for assessing increases and decreases in myocardial $[\text{Ca}^{2+}]_i$ than the m-E time constants at any temperatures.

Myocardial Ca^{2+} handling and temperature

The time course during myocardial contraction and relaxation periods is governed by a variety of temperature-dependent myocardial intracellular Ca^{2+} processes (Stowe et al. 1999). A rise in myocardial Ca^{2+} occurs in response to hypothermia (Aasum and Larsen 1999). Cooling is known to decrease myosin adenosine triphosphatase (ATPase) activity, the Q_{10} of which is between 2 and 3, which in turn is expected to increase the chemo-mechanical efficiency of cross-bridge (CB) cycling (Suga et al. 1988).

The Q_{10} value of reciprocal values of h-L and m-E time constants represents the rate of the physical, chemical, and biological phenomena related to inotropism and lusitropism for every 10°C rise in temperature and may be a useful index enabling speculation about whether the phenomenon depends on a physical, chemical, or biological process. Our present results are similar to the obtained Q_{10} values from our

previous studies (Mikane et al. 1999; Suga et al. 1988). These results suggest that Q_{10} values are closely related to physical processes in Phases I and II and to one or more chemical processes in Ca^{2+} handling, consisting of the SR Ca^{2+} pump, the sarcolemma Na^+/Ca^{2+} exchange coupled with the Na^+/K^+ pump, and the resultant mechanical contraction (Kappl and Hartung 1996; Shattock and Bers 1987) in Phases III and IV.

Simulation of myocardial relaxation suggests that the mechanoenergetics of the cooled heart integratively account for changes in cooling-decelerated ATPase-dependent SR Ca^{2+} sequestration and CB detachment (Mikane et al. 1997). Further, we speculate that the recirculation fraction rate of internal Ca^{2+} via SR becomes larger than Ca^{2+} extrusion with cooling and smaller than Ca^{2+} extrusion with warming by analyzing transient alternans decay (Mizuno et al. 2002). On the basis of contemporary reductionistic knowledge, the temperature sensitivities of all the Ca^{2+} transporters have been shown to be comparable among themselves.

Thus, the decreased myosin ATPase activity and CB cycling rate caused by cooling might give elongated h-L and m-E time constants. In contrast, the increased myosin ATPase activity and CB cycling rate caused by warming might give shortened h-L and m-E time constants. Therefore, the Ca^{2+} transient and mechanical processes during contraction and relaxation periods might become slower with cooling and faster with warming.

Limitations

The h-L function remains an empirical model, and many mathematically equivalent models have been used to express the isovolumic LV PTC. It remains unknown whether contraction and relaxation have an underlying h-L mechanism. However, our contention is that the isovolumic LV PTC (Matsubara et al. 1995; Mizuno et al. 2008b), myocardial isometric FTC (Mizuno et al. 2000, 2007), and CaTC (Mizuno et al. 2009, 2011, 2013, 2016) are characterized by an h-L mechanism. These time curves are thought to reflect the instantaneous number of attached and detached CB. Computer simulation of Ca^{2+} handling and CB kinetics has also shown that the cumulative CB attachment and detachment–time curves are reasonably well fitted using a sigmoid logistic function (Sakamoto et al. 1996). Therefore, we think that the h-L nature reflects overall physiological manifestations or developments of LVP, myocardial force, and $[Ca^{2+}]_i$ concentration caused by CB cycling. The physiological implication of the h-L function is that the segmental time curves reflect the interaction of contractile events during contraction and relaxation periods at different temperatures.

Conclusions

We conclude that both the h-L and m-E time constants obtained by fitting using the h-L and m-E functions for Phases I–IV in the isovolumic LV PTC shorten with increasing temperature in the excised, cross-circulated canine heart. The superiority of the goodness of h-L fit to that of m-E fit remained even under hypothermic and hyperthermic conditions. This main finding broadens the generality of the h-L functions for comprehensive assessment of Phases I–IV at any temperature. The temperature-dependent h-L time constants are more reliable and useful LV inotropic and lusitropic indices for estimating LV contraction and relaxation during the cardiac cycle with varying temperature. This approach may help gain better insight into the logic of integration in LV inotropism and lusitropism under different temperature conditions.

Authors' contributions

The work presented here was carried out in collaboration between all authors. Ju Mizuno defined the research theme, designed methods and experiments, carried out the laboratory experiments, analyzed the data, interpreted the results, and wrote the manuscript. Satoshi Mohri designed methods and experiments, and carried out the laboratory experiments. Takeshi Yokoyama, Mikiya Otsuji, Hideko Arita, and Kazuo Hanaoka discussed analyses, interpretation, and presentation. All authors have contributed to, seen and approved the manuscript.

Acknowledgments

We thank Drs. Junichi Araki, Hiromi Matsubara, Juichiro Shimizu, Shunsuke Suzuki, Takeshi Mikane, Terumasa Morita, Kuniyoshi Kohno, and Hiroyuki Suga for their excellent advice and support and thank Mr. Kimikazu Hosokawa for animal supply and care.

Conflicts of interest

The authors declare that they have no conflicts of interest in this work.

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Figure 1. Excised, cross-circulated canine heart model. ECG, electrocardiogram; LVP, left ventricular pressure; LVV, left ventricular volume.

Figure 2. Phases I–IV in the isovolumic left ventricular (LV) pressure–time curve (PTC). $dP/dt_{\max}$ is the maximum of the first-order time derivative of LV pressure (LVP). $dP/dt_{\min}$ is the minimum of the first-order time derivative of LVP. LVEDP is the LV end-diastolic pressure. Phase I is the first half of the ascending phase defined as the period from the time corresponding to the onset of the isovolumic LV PTC to the time corresponding to $dP/dt_{\max}$. Phase II is the second half of the ascending phase defined as the period from the time corresponding to $dP/dt_{\max}$ to the time corresponding to the peak LVP. Phase III is the first half of the descending phase defined as the period from the time corresponding to the peak LVP to the time corresponding to $dP/dt_{\min}$. Phase IV is the second half of the descending phase defined as the period from the time corresponding to $dP/dt_{\min}$ to the time corresponding to LVEDP.

Figure 3. Half-logistic (h-L) and mono-exponential (m-E) time constants for Phases I–IV in the isovolumic left ventricular (LV) pressure–time curves (PTCs) at 33°C, 36°C, and 38°C. Values indicate the mean $\pm$ SD in eight excised, cross-circulated canine hearts. **A**, **B**, **C**, and **D** show h-L (black squares) and m-E (white circles) time constants for Phase I, II, III, and IV, respectively. *, $p < 0.05$ and **, $p < 0.001$ vs. 33°C. †, $p < 0.05$ and ††, $p < 0.001$ vs. Phase II. ‡, $p < 0.05$, and ‡‡, $p < 0.001$ vs. Phase III. ¶, $p < 0.05$ and ¶¶, $p < 0.001$ vs. Phase IV.

Table 1. Hemodynamic data for the isovolumic left ventricular (LV) pressure–time curves (PTCs) at 33°C, 36°C, and 38°C.

	Temperature (°C)		
	33	36	38
LVP at onset (mmHg)	-0.8 ± 7.0	-1.6 ± 7.1	-1.0 ± 7.2
LVP at $dP/dt_{\max}$ (mmHg)	29.7 ± 9.9	26.5 ± 9.4	23.9 ± 7.8*
Peak LVP (mmHg)	78.0 ± 16.4	68.2 ± 14.8*	60.6 ± 18.3**
LVP at $dP/dt_{\min}$ (mmHg)	39.8 ± 16.7	31.9 ± 8.7	29.5 ± 11.3*
LVEDP (mmHg)	-0.8 ± 7.2	-1.8 ± 7.4	-1.4 ± 7.4
Time to $dP/dt_{\max}$ (ms)	80.8 ± 14.6	79.3 ± 8.5	73.8 ± 7.5
Time to peak LVP (ms)	196.3 ± 17.0	181.0 ± 11.1	165.0 ± 16.2*
Time to $dP/dt_{\min}$ (ms)	335.0 ± 28.3	293.8 ± 26.9**	260.3 ± 29.9**#
Time to LVEDP (ms)	494.5 ± 12.4	448.3 ± 36.7*	404.5 ± 44.3**#
Period of Phase I (ms)	80.8 ± 14.6	79.3 ± 8.6	75.3 ± 8.7
Period of Phase II (ms)	115.5 ± 16.8	101.8 ± 13.2*	91.3 ± 13.7**
Period of Phase III (ms)	138.8 ± 29.8	112.8 ± 17.2*	95.3 ± 18.8**#
Period of Phase IV (ms)	159.5 ± 27.0	154.5 ± 17.5	144.3 ± 33.0*
$dP/dt_{\max}$ (mmHg s ⁻¹)	752.5 ± 155.0	751.5 ± 189.0	702.9 ± 208.2
$dP/dt_{\min}$ (mmHg s ⁻¹)	-492.0 ± 124.1	-554.3 ± 170.4	-566.7 ± 206.5

Values indicate the means ± SD in eight excised, cross-circulated canine hearts. LVP is LV pressure. $dP/dt_{\max}$ is the maximum of the first-order time derivative of LVP. LVEDP is LV end-diastolic pressure. $dP/dt_{\min}$ is the minimum of the first-order time derivative of LVP.

* $p < 0.05$ vs. 33°C.

** $p < 0.001$ vs. 33°C.

$p < 0.05$ vs. 36°C.

Table 2. Half-logistic (h-L) and mono-exponential (m-E) amplitude constants and h-L and m-E nonzero asymptotes obtained by fitting using h-L and m-E functions for Phases I–IV in the isovolumic left ventricular (LV) pressure–time curves (PTCs) at 33°C, 36°C, and 38°C

	Temperature (°C)		
	33	36	38
Phase I			
P_{1A} (mmHg)	32.6 ± 10.3	29.9 ± 8.4	$26.6 \pm 6.4^*$
P_{10} (mmHg)	36.0 ± 10.9	32.7 ± 9.0	$29.2 \pm 7.0^*$
P_{1B} (mmHg)	-2.3 ± 6.7	-2.7 ± 7.0	-2.2 ± 7.0
$P_{1\infty}$ (mmHg)	-4.7 ± 6.4	-4.5 ± 6.9	-3.9 ± 6.9
Phase II			
P_{2A} (mmHg)	-54.7 ± 7.6	-45.8 ± 8.9	$-40.8 \pm 14.2^*$
P_{20} (mmHg)	-64.0 ± 10.2	-52.7 ± 10.3	$-47.2 \pm 16.3^*$
P_{2B} (mmHg)	83.8 ± 15.3	$72.1 \pm 15.5^*$	$64.4 \pm 19.4^{**}$
$P_{2\infty}$ (mmHg)	92.0 ± 15.9	$77.9 \pm 16.2^*$	$69.9 \pm 21.0^{**}$
Phase III			
P_{3A} (mmHg)	-43.4 ± 9.4	-40.7 ± 9.9	-26.7 ± 26.0
P_{30} (mmHg)	-50.8 ± 11.8	-47.6 ± 11.5	-41.1 ± 10.3
P_{3B} (mmHg)	82.8 ± 16.4	$72.3 \pm 15.9^*$	$64.2 \pm 19.2^{**}$
$P_{3\infty}$ (mmHg)	89.2 ± 16.9	$78.2 \pm 17.1^*$	$69.4 \pm 20.6^{**}$
Phase IV			
P_{4A} (mmHg)	43.7 ± 17.2	35.2 ± 9.2	$32.2 \pm 11.8^*$
P_{40} (mmHg)	49.0 ± 18.9	$38.4 \pm 10.0^*$	$35.1 \pm 12.6^{**}$
P_{4B} (mmHg)	-3.3 ± 6.3	-2.9 ± 7.6	-2.3 ± 7.7
$P_{4\infty}$ (mmHg)	-7.2 ± 5.4	-4.6 ± 7.9	$-3.9 \pm 8.1^*$

Values indicate the means $\pm$ SD in eight excised, cross-circulated canine hearts. P_{1A} , P_{2A} , P_{3A} , and P_{4A} are the h-L amplitude constants for Phases I, II, III, and IV, respectively. P_{10} , P_{20} , P_{30} , and P_{40} are the m-E amplitude constants for Phases I, II, III, and IV, respectively. P_{1B} , P_{2B} , P_{3B} , and P_{4B} are the h-L nonzero asymptotes for Phases I, II, III, and IV, respectively. $P_{1\infty}$, $P_{2\infty}$, $P_{3\infty}$, and $P_{4\infty}$ are the m-E nonzero asymptotes for Phases I, II, III, and IV, respectively.

* $p < 0.05$ vs. 33°C.

** $p < 0.001$ vs. 33°C.

$p < 0.05$ vs. 36°C.

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Table 3. Goodness of half-logistic (h-L) and mono-exponential (m-E) fits for Phases I–IV in the isovolumic left ventricular (LV) pressure–time curves (PTCs) at 33°C, 36°C, and 38°C

	Temperature (°C)		
	33	36	38
Phase I			
h-L r [Z]	0.9995 [4.1 ± 0.6**]	0.9996 [4.2 ± 0.6**]	0.9995 [4.1 ± 0.5**]
m-E r [Z]	0.9976 [3.4 ± 0.3]	0.9973 [3.3 ± 0.3]	0.9973 [3.3 ± 0.3]
h-L RMS (mmHg) ²	0.231 ± 0.382#	0.172 ± 0.279#	0.103 ± 0.106#
m-E RMS (mmHg) ²	0.754 ± 1.027	0.638 ± 0.763	0.427 ± 0.339
Phase II			
h-L r [Z]	0.9997 [4.3 ± 0.3*]	0.9997 [4.4 ± 0.4*]	0.9997 [4.4 ± 0.3*]
m-E r [Z]	0.9986 [3.6 ± 0.2]	0.9988 [3.7 ± 0.2]	0.9989 [3.7 ± 0.3]
h-L RMS (mmHg) ²	0.169 ± 0.074#	0.118 ± 0.071#	0.095 ± 0.100#
m-E RMS (mmHg) ²	0.704 ± 0.289	0.478 ± 0.300	0.409 ± 0.418
Phase III			
h-L r [Z]	0.9995 [4.1 ± 0.6**]	0.9996 [4.2 ± 0.5**]	0.9995 [4.2 ± 0.4**]
m-E r [Z]	0.9983 [3.5 ± 0.5]	0.9984 [3.6 ± 0.3]	0.9983 [3.5 ± 0.2]
h-L RMS (mmHg) ²	0.286 ± 0.500#	0.151 ± 0.163#	0.129 ± 0.148#
m-E RMS (mmHg) ²	0.656 ± 0.664	0.456 ± 0.242	0.514 ± 0.330
Phase IV			
h-L r [Z]	0.9997 [4.5 ± 0.7**]	0.9997 [4.5 ± 0.9*]	0.9996 [4.3 ± 0.5**]
m-E r [Z]	0.9983 [3.5 ± 0.3]	0.9976 [3.4 ± 0.3]	0.9975 [3.3 ± 0.2]
h-L RMS (mmHg) ²	0.241 ± 0.380#	0.158 ± 0.186#	0.102 ± 0.109#
m-E RMS (mmHg) ²	0.894 ± 1.289	0.625 ± 0.419	0.530 ± 0.416

Values indicate the means ± SD in eight excised, cross-circulated canine hearts. r is the correlation coefficient. The r value indicates the mean following Z-transformation (Z). RMS is the residual mean square.

* $p < 0.05$.

** $p < 0.001$ vs. m-E Z.

$p < 0.05$ vs. m-E RMS.

Table 4. Temperature coefficients (Q_{10}) of the reciprocal values of the half-logistic (h-L) and mono-exponential (m-E) time constants obtained by fitting using h-L and m-E functions for Phases I–IV in the isovolumic left ventricular (LV) pressure–time curve (PTC)

Q_{10}	
Phase I	
$1/P\tau_{1L}$	1.3 ± 0.4
$1/P\tau_{1E}$	1.4 ± 0.4
Phase II	
$1/P\tau_{2L}$	1.7 ± 0.6
$1/P\tau_{2E}$	1.8 ± 0.9
Phase III	
$1/P\tau_{3L}$	2.2 ± 0.7
$1/P\tau_{3E}$	2.2 ± 0.9
Phase IV	
$1/P\tau_{4L}$	2.4 ± 0.5
$1/P\tau_{4E}$	2.9 ± 0.7

Values indicate the means $\pm$ SD in eight excised, cross-circulated canine hearts. $P\tau_{1L}$, $P\tau_{2L}$, $P\tau_{3L}$, and $P\tau_{4L}$ are the h-L time constants for Phases I, II, III, and IV, respectively. $P\tau_{1E}$, $P\tau_{2E}$, $P\tau_{3E}$, and $P\tau_{4E}$ are the m-E time constants for Phases I, II, III, and IV, respectively.