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Left–Ventricular Pressure Relaxation and Diastolic Function of Isolated Working Mammalian Hearts at Hypothermia

Stefan F. J. Langer

Institute of Physiology, Charite´ University Medicine, Berlin, Germany.

Corresponding author:
Dr. Stefan F. J. Langer
Guineastr. 14, 13351 Berlin, Germany
sflanger@o2online.de

Abstract

Background

Hypothermia is well known to elevate the time constant (whatever model is used) of the isochoric left–ventricular pressure fall. Due to different critera in use, it remained unclear whether prolonged diastole in hypothermia is sufficient for complete relaxation Detecting and quantifying incomplete relaxation may become a valuable tool to prevent diastolic heart failure in hypothermia.

Methods

Left–ventricular pressure decays in isolated guinea pig and rat hearts are analysed by 4–parametric regression at different temperatures, at sinus rhythm and electrical stimulation. Residual contraction (FRC) is introduced and quantified by extrapolating the model's pressure forecast to end–systole, subtracting the asymptote, and normalising.

Results

Isochoric pressure decay fits the regression model at all temperatures and heart rates.Residual contraction is virtually absent at normothermia and remains very small (F_{RC} <3%) down to 31°C. Lower temperatures or pacing induces higher F_{RC}. Eventually, the isochoric pressure curve becomes considerably elevated and looses its concavity.

Conclusions

Despite slower pressure fall, ventricular relaxation remains fairly complete at hypothermia, and depends on considerable autoregulation of the individual heart. It is concluded (not proved) that individual emergence of negative lusitropy may indicate imminent heart failure. Asymptotic pressure is interpreted as ventricular tonus, independent from velocity of relaxation. Gradual increasing time constants may be attributed to a general slowing of bioreactions as temperature falls. Remarkable curve shape changes may be caused by aftercontractions due to elevated Ca^{2+} sensitivity at hypothermia and high Ca^{2+} load by pacing.

Keywords: ventricular pressure; myocardial relaxation, diastole, hypothermia; isolated heart

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* Corresponding author. E-mail: sflanger@o2online.de

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Introduction

Mild hypothermia is a secure and widely used method to reduce cardiac work and metabolism for surgical interventions. Experimental hypothermic heart failure only occurs during rapid or extended cooling as a sudden, rapid, and complex process. Consequently, we do not know much about prodromata and mechanisms of such failure at low temperature. As little or even positive inotropic effects of mild hypothermia has been surmised [1, 2], the diastolic function (lusitropy, relaxation) of the left ventricle became considered as a trigger of hypothermic heart failure. Due to a recent study [3], cooling renders ventricular relaxation incomplete despite its concomitant negative chronotropy. Such conclusion, however, depends critically on the soundness of modeling the isochoric (isovolumic) left–ventricular pressure fall and its extrapolation toward the onset of the subsequent systole.

Figure 1 explains the fundamental idea; as a special feature, the method only demands the high–fidelity, phasic left–ventricular pressure (LVP) curve. Ventricular relaxation is described by non–linear regression fit of the isochoric LVP decay. Any reasonable regression model, p, yields at least one time constant (τ, "velocity" of relaxation), and an asymptotic pressure (P_∞, deepest possible "extent" of relaxation). Extrapolating p into the diastolic filling phase, ending at t_{Dia}, when the following systole starts, provides us with three rational indices of diastolic function:

- $t_{\mathrm{Dia}}\tau^{-1}$ expresses the apparent duration of diastole in units of the apparent time constant, it remains unaltered if t_{Dia} and τ undergo proportional changes. (The present paper also provides the ratio $t_{\mathrm{Fill}}\tau_\infty^{-1}$.)

- $F_{\mathrm{RC}} = [p(t_{\mathrm{Dia}}) - P_\infty]\,[p(0) - P_\infty]^{-1}$ is the end–diastolic Residual Contraction, given as its fraction (hereafter: percentage) of the maximal possible pressure difference.

- $\Delta P_{\mathrm{term}} = \mathrm{LVP}(t_{\mathrm{Dia}}) - P_\infty$ is the maximal pressure augment that may occur by the actual diastolic inflow (added to the residual volume), where P_∞ serves as an estimate of the equilibrium pressure of the *fully* relaxed, hypothetically non–filling ventricle (containing its residual volume only).

The present paper introduces the latter two new indices. It should be noticed that all three indices depend on the underlying relaxation model (p) which we indicate by superscripts, if necessary. Table 1 shows respective values observed in isolated small mammalian hearts at normothermia; data from comparable sample sizes are unavailable in the literature.

The present study aims to clarify:

(a) By what means can completeness of left–ventricular relaxation be quantified from the intraventricular pressure curve?

(b) Does left–ventricular relaxation remain complete at hypothermia (by bradycardia), despite considerably increased time constants of the pressure decay?

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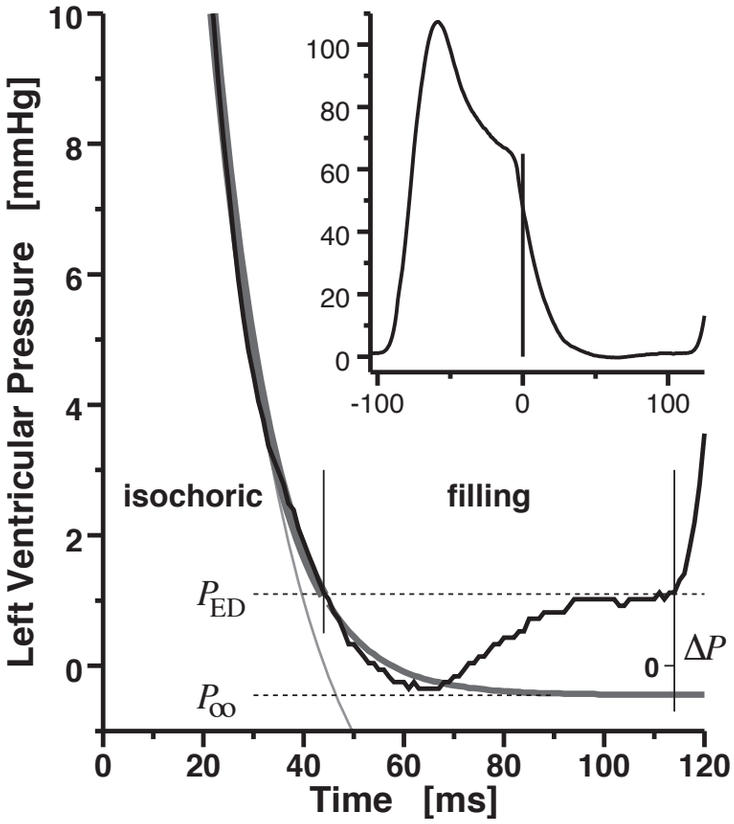

Figure 1. Indexing diastolic function from the left–ventricular pressure course (*black*). Data from an isolated SPRAGUE–DAWLEY rat heart, working at 37°C, 75 mmHg mean aortic pressure, 39 mL min^{-1} cardiac output, sinus rhythm. Time definitions (*perpendicular lines*): Zero at the steepest decay of the empirical pressure curve, –min LV$\dot{P}$; t_{Rlx}, re–encounter of the presystolic (antecedent end–diastolic) pressure; t_{Dia}, end of diastole (onset of subsequent contraction); $t_{\mathrm{Fill}} = t_{\mathrm{Dia}} - t_{\mathrm{Rlx}}$, diastolic filling time. Pressure definitions: Zero at ambient barometer pressure; P_{ED}, end–diastolic pressure; P_∞, asymptotic pressure, extrapolated from the isochoric relaxation model; $\Delta P = \Delta P_{\mathrm{term}} = P_{\mathrm{ED}} - P_\infty$. *Dark gray*: Four–parametric logistic relaxation model (Lg4, parameters fitted to isochoric pressure fall: $P_0 = 45.9$ mmHg, $P_\infty = -0.4\bar{5}$ mmHg, $\tau_0 = 20.2$ ms, $\tau_\infty = 11.0$ ms). *Light gray*: Three–parametric exponential model (Ex3, $P_0 = 45.9$ mmHg, $P_\infty = -3.5$ mmHg, $\tau = 16.7$ ms). **Interpretation**: (1.) Exponential regression is inapt to extrapolate the isochoric pressure fall if the latter deviates from exponentiality. (2.) The given pressure curve presents with complete relaxation, because the extrapolated pressure fall almost reaches its asymptote before t_{Dia} (both models). (3.) About $-P_\infty(\Delta P_{\mathrm{term}})^{-1} = 0.4/1.5 = 27\%$ of the diastolic filling work has been done by "diastolic suction" (due to model Lg4; model Ex3 estimates $3.5/4.6 = 76\%$), because $P_\infty < 0$. (4.) Ventricular filling has been almost completed within two thirds of the filling phase, because both ventricular pressure and extrapolated isochoric pressure reach their respective end–diastolic values already at $t = 95$ ms < 114 ms $= t_{\mathrm{Dia}}$ (both models).

Table 1: Lusitropy of isolated small animal hearts at standard working conditions

		G. Pig			**SD Rat**			**Ferret**		
Sample size:		182			223			20		
			C_{80}	C_{90}		C_{80}	C_{90}		C_{80}	C_{90}
t_{BI}	ms	246.5^{277}_{217}	$^{284}_{210}$		209^{237}_{180}	$^{244}_{170}$		261.5^{304}_{233}	$^{305}_{226}$	
t_{Dia}	ms	63.5^{82}_{48}	$^{86}_{41}$		72^{99}_{48}	$^{112}_{45}$		62.3^{93}_{44}	$^{93}_{43}$	
P_{ED}	mmHg	$3.19^{8.24}_{0.55}$	$^{9.24}_{-.43}$		$3.35^{8.37}_{0.84}$	$^{9.49}_{0.14}$		$2.39^{3.95}_{1.81}$	$^{3.95}_{-1.9}$	
Exponential relaxation model Ex3 (3 parameters):										
τ_{Ex3}	ms	$21.37^{29.5}_{16.3}$	$^{36.6}_{15.8}$		$17.29^{26.8}_{11.7}$	$^{30.6}_{10.8}$		$26.67^{35.4}_{17.3}$	$^{39.6}_{10.9}$	
$t_{Dia}\,\tau^{-1}_{Ex3}$	[1]	$2.88^{3.99}_{1.82}$	$^{4.55}_{1.66}$		$4.04^{6.68}_{2.33}$	$^{7.88}_{2.02}$		$1.97^{2.80}_{1.11}$	$^{3.97}_{1.11}$	
$F^{(Ex3)}_{RC}$	%	$5.60^{13.1}_{0.93}$	$^{16.1}_{0.91}$		$1.77^{5.28}_{.002}$	$^{8.67}_{.002}$		$14.00^{19.7}_{1.90}$	$^{19.7}_{0.00}$	
$P^{(Ex3)}_{\infty}$	mmHg	$-3.88^{0.84}_{-13}$	$^{2.77}_{-15}$		$-2.14^{3.26}_{-13}$	$^{4.39}_{-21}$		$-5.79^{-2.2}_{-18}$	$^{-.75}_{-18}$	
$\Delta P^{(Ex3)}_{term}$	mmHg	$6.69^{11.9}_{2.22}$	$^{16.9}_{2.22}$		$5.81^{14.9}_{1.52}$	$^{18.1}_{0.76}$		$8.37^{17.1}_{4.13}$	$^{20.5}_{4.13}$	
Logistic relaxation model Lg4 (4 parameters):										
τ_0	ms	$21.68^{29.5}_{15.8}$	$^{32.5}_{14.1}$		$18.78^{25.9}_{10.5}$	$^{29.7}_{10.5}$		$34.42^{44.4}_{17.0}$	$^{50.4}_{10.5}$	
τ_{∞}	ms	$18.37^{25.7}_{11.8}$	$^{28.4}_{10.9}$		$12.16^{15.7}_{7.62}$	$^{18.3}_{7.62}$		$15.51^{17.1}_{12.8}$	$^{18.2}_{12.2}$	
$t_{Dia}\,\tau^{-1}_{\infty}$	[1]	$3.51^{5.03}_{2.39}$	$^{5.14}_{1.85}$		$5.89^{8.23}_{3.03}$	$^{10.2}_{3.03}$		$3.68^{5.27}_{2.65}$	$^{6.00}_{2.65}$	
$t_{Fill}\,\tau^{-1}_{\infty}$	[1]	$1.26^{2.31}_{0.18}$	$^{2.97}_{.066}$		$2.72^{5.94}_{0.88}$	$^{7.50}_{0.70}$		$.779^{1.98}_{-1.0}$	$^{2.61}_{-1.0}$	
$F^{(Lg4)}_{RC}$	%	$3.45^{7.72}_{0.80}$	$^{9.01}_{0.15}$		$0.34^{1.64}_{0.00}$	$^{2.83}_{0.00}$		$3.27^{7.03}_{.001}$	$^{7.98}_{.001}$	
$P^{(Lg4)}_{\infty}$	mmHg	$-2.53^{3.01}_{-6.0}$	$^{3.50}_{-6.8}$		$.021^{5.49}_{-4.8}$	$^{6.34}_{-6.7}$		$-.144^{1.52}_{-11}$	$^{2.41}_{-11}$	
$\Delta P^{(Lg4)}_{term}$	mmHg	$5.64^{8.76}_{2.35}$	$^{10.9}_{1.97}$		$3.23^{8.09}_{0.58}$	$^{10.1}_{0.58}$		$2.68^{5.40}_{0.31}$	$^{8.05}_{0.12}$	

Entries are sample medians with the limits of the central 80%–mass (C_{80}) end central 90%–mass (C_{90}) of the sample.

Data from freshly isolated Guinea pig (G. Pig), Sprague–Dawley (SD) rat, and ferret hearts at standard condition: Protein–free perfusate, 2.5 mM Ca^{2+}, 95% O_2, 37°C; mean aortic pressure 60 mmHg (G. Pig) or 75 mmHg (rat, ferret), cardiac output 40 mL min^{-1} (G. Pig, rat; left ventricle mass below 1 g) or 60 mL min^{-1} (ferret; left ventricle mass above 2 g), spontaneous sinus rhythm.

t_{BI}, interbeat interval; t_{Dia}, diastole (isochoric and filling) interval; P_{ED}, end–diastolic pressure. Parameters of the relaxation models as described in the text.

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(*c*) What extent of incomplete relaxation can the heart deal with in steady–state?

(*d*) Are there any special characteristics in the left–ventricular pressure course related to incomplete relaxation?

2 Methods

Hearts of male Sprague–Dawley rats and of mixed sex guinea pigs were isolated and mounted to a temperature controlled artificial circulation apparatus in working left ventricle setup. Lusitropic parameters were estimated by non–linear regression with the well known 3–parametric exponential and with the more appropriate 4–parametric logistic model. Measurements were obtained at different temperature levels in steady–state and during adaptation after temperature jumps. Additional cooling experiments were performed with heart rate held constant by electrical right–atrial stimulation. Pacing was also used to increase the heart rate at selected levels of mild hypothermia, where either cardiac output or beat volume was held constant. We assess observed differences to normothermia as well as adaptation by non–parametric statistics.

2.1 Experimental setup and protocol

The isolated hearts hung loose in a temperature controlled atmosphere, fixed at the aortic stump. Protein–free buffer solution (95% O_2, 5% CO_2, pH 7.4, 2.5 mM Ca^{2+}, glucose and pyruvate) was continually pumped to the left atrium, 40 mL min^{-1}. Mean aortic pressure of 75 mmHg (rat) or 60 mmHg (guinea pig, due to poorer durability) was maintained for the duration of the experiments. Temperature of the whole circulation was changed by switching water jackets to thermostatic reservoirs of the desired temperature; perfusate temperature always became constant within five minutes. If desired, the left atrium was electrically stimulated by a wire clamp. An intracaval catheter tip manometer provides high–fidelity LVP data which is digitised at 1 kHz rate and binned in 0.075 mmHg resolution. No filtering or data smoothing is applied. Further measurands and technical details were previously described [4].

All animals recieved professional humane care according to the *Tierschutzgesetz* (German Animal Protection Act).

Test series are established as follows. All tests a specimen has been scheduled for are completed within 90 minutes after mounting the isolated heart.

A. Temperature changes with concomitant sinus bradycardia

A1. *Temperature levels.* Beginning with 37°C, the heart becomes stepwise cooled to 34°C, 31°C, and 29°C. Additional levels, 40°C or 25°C, are investigated in some rat hearts. LVP samples of 4 s length are taken at each level after ten minutes, when steady–state has been settled. Sample sizes are given in the Results section; we augment the normo– and mild hypothermia samples by adding specimens which underwent other interventions (unrelated to the present study) at the respective hypothermic level.

A2. *Adaptation.* After recording LVP at normothermia, the heart is cooled in one step down to 34°C or to 31°C. LVP samples are recorded every minute within the first five minutes after switching the thermostate, followed by eight equidistant samplings in 16 min, and two samplings during the last eight minutes. Temperature is than switched back to 37°C, and the sampling protocol repeated.

B. Pacing tests

B1. *Cooling with spontaneous versus constant heart rate.*

B1a. *Steady–state* Samples of hearts, working at mild hypothermia, are drawn from our records of previous investigations, with respect to the present standard conditions; specimens with any pretreatment or deviating setup are excluded. Hypothermia had been established either with decreasing sinus beat rate or with heart rate held constant at its intrinsic value (at 37°C) by electrical right–atrial pacing. After taking the first data recording at hypothermia, the hearts were used for investigations unrelated to the present study.
We compare the medians of samples with spontaneous *vs.* paced heart rate at 34°C (guinea pigs) or at 31°C (rats), applying U–tests.

B1b. *Adaptation* We carry out protocol A2 in new samples (8 guinea pig, 10 rat hearts). In difference, we establish electrical right–atrial stimulation at normothermia to preserve the apparent beat interval length of the heart during the temperature change.

B2. *Pacing at hypothermia.* Mild hypothermia is established in 8 guinea pig (34°C) and 8 rat hearts (34 and 31°C). Heart rate is then elevated from the apparent bradycardia in steps of 15 beats per minute up to the sinus frequency seen at normothermia. LVP is recorded in steady–state after each step. In a small number of rat hearts, the pacing series was reversed to intrinsic bradycardia while the beat volume was held constant by reducing cardiac inflow.

2.2 Data analysis

Data processing software (by the Author) disassembled each 4–second LVP recording in single beats and their subintervals, as mentioned in Fig. 1. Simple pressure data is taken as the median of the respective values in the beat intervals. Non–linear regression applies to pooled (overlayed and abscissa–adjusted) LVP data taken from all but the one most outlying isochoric decay phases within the recording [5].

We use alternative regression models to determine the lusitropic parameters from the pooled data, the conventional 3–parametric exponential,

$$p_{\mathrm{Ex3}}(t) \quad = \quad \left(P_0^{(\mathrm{Ex3})} - P_\infty^{(\mathrm{Ex3})}\right) \exp \frac{-t}{\tau} \; + \; P_\infty^{(\mathrm{Ex3})} \quad ,$$

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and its enhancement, the 4–parametric logistic model,

$$p_{\mathrm{Lg4}}(t) \quad = \quad \frac{P_0^{(\mathrm{Lg4})} - P_\infty^{(\mathrm{Lg4})}}{\gamma + (1-\gamma)\exp\frac{t}{\tau_\infty}} + P_\infty^{(\mathrm{Lg4})} \ , \qquad \tau_0 := \frac{\tau_\infty}{1-\gamma} \quad ;$$

we present the initial time constant, τ_0, instead of γ, the shape parameter [5]. If $t \to \infty$, p_{Lg4} converges to an exponential function with time constant τ_∞. τ_0 is the "local" time constant valid at the begin ($t = 0$) of the isochoric decay, whereas the logistic time constant, τ_∞, becomes asymptotically as more "valid" as t rises. Model Lg4 equals Ex3 if $\tau_0 = \tau_\infty$, and it equals the long known *3*–parametric logistic model [7] if $\tau_0 = 2\,\tau_\infty$. By that feature, Lg4 covers both models and varies continuously between them to fit the real LVP curve shape.

Samples are described by median and central 80 per cent mass C_{80}, *i.e.* the narrowest vicinity around the median that covers 80% of the sample. For drawing adaptation curves, values between adjacent recordings are linearly interpolated. Effects of interventions are assessed by Wilcoxon matched–pair tests [6] against normothermia or, respectively, intrinsic sinus frequency. U–tests [6] are used in unpaired observations. Steady–state *vs.* adaptation is assessed by Spearman's rank correlation coefficient (against t), testing the respective zero hypothesis [6]. We present (always double–sided) error probabilities, p, for refusing zero hypotheses; in adaptation tests, we note the number of significantly ($p < 0.05$) revealed non–stationarity.

3 Results

Hemodynamics settles into durable steady–state at any temperature down to 29°C; rat hearts also stand 25°C. Guinea pig hearts may work at this hypothermia level at the risk of sudden failure. Such incident is usually induced by a sudden, severe derangement (like an "aftercontraction") of the isochoric pressure fall in a single beat. Due to the constant inflow, end–diastolic pressure immediately rises, and the ventricle becomes unable to contract productively. Cardiac function can be reestablished by a transient unload, but we do not set hypothermia below 31°C to the guinea pig protocol to avoid such incidents.

Cooling always reduces coronary perfusion (concomitant rise in aortic flow) and oxygen extraction. End–diastolic pressure rises at spontaneous bradycardia. Systolic pressure rise velocity may be enhanced but is usually slightly depressed in mild and always diminished at deep hypothermia. Diastolic peak pressure fall velocity and the time constants of isochoric relaxation always indicate negative lusitropy. Relaxation model Lg4 reveals considerable changes in the shape of the isochoric LVP curve in many hearts, when temperature or heart rate varies. We further observe that τ_0 (Lg4) generally resembles the changes seen in τ (Ex3).

3.1 Steady–state hypothermia

Table 2 summarises observations from the different hypothermia levels in steady–state. In median, cooling significantly reduces LVP upstroke velocity and increases

Table 2: Lusitropic effect of temperature in steady–state, 40 mL min^{-1} cardiac output

		Guinea Pigs ($N = 11$)				**Sprague–Dawley Rats** ($N = 29$)					
Temp. [°C]:		37	34	31	29	40	37	34	31	29	25
(n):		(11)	(10)	(9)	(9)	(8)	(29)	(28)	(28)	(21)	(5)
	Unit	**abs.**	Δ%	Δ%	Δ%	Δ%	**abs.**	Δ%	Δ%	Δ%	Δ%
t_{BI}	ms	227^{246}_{215}	$\dagger 25^{32}_{19}$	$\dagger 53^{65}_{48}$	$\dagger 83^{103}_{74}$	$\dagger -12^{-6}_{-14}$	206^{229}_{171}	$\ddagger 26^{33}_{16}$	$\ddagger 53^{67}_{46}$	$\ddagger 78^{87}_{59}$	126^{162}_{120}
t_{Dia}	ms	95^{108}_{75}	$*17^{26.0}_{4.16}$	118^{168}_{106}	$\dagger 160^{204}_{141}$	$\dagger -12^{-4.7}_{-16}$	107^{135}_{84}	$\ddagger 27^{41}_{10}$	$\ddagger 49^{73}_{15}$	$\ddagger 64^{82}_{32}$	81^{132}_{69}
V_{Beat}	μL	144^{152}_{120}	$\dagger 21^{39}_{16}$	$\dagger 51^{64}_{30}$	$\dagger 58^{96}_{37}$	$\dagger -18^{-8}_{-39}$	133^{155}_{106}	$\ddagger 23^{30}_{7}$	$\ddagger 48^{64}_{24}$	$\ddagger 69^{104}_{60}$	100^{156}_{24}
F_{Cor}	$\frac{mL}{min}$	$12.7^{18.8}_{8.7}$	$\dagger -10^{-6}_{-20}$	$\dagger -13^{-2}_{-28}$	-17^{-1}_{-43}	$*-12^{0}_{-28}$	16.6^{21}_{13}	1.0^{8}_{-23}	$\ddagger -9.3^{3.1}_{-17}$	$\ddagger -11^{0}_{-24}$	-19^{-15}_{-40}
O_2 Cons	$\frac{\mu mol}{min}$	$7.3^{9.8}_{5.0}$	$\dagger -26^{-15}_{-28}$	$\dagger -44^{-27}_{-50}$	$\dagger -51^{-34}_{-66}$	—	9.4^{12}_{7}	$\ddagger -19^{-11}_{-27}$	$\ddagger -25^{-10}_{-30}$	$\ddagger -37^{-26}_{-47}$	-57^{-54}_{-66}
$\max \dot{P}$	$\frac{mmHg}{ms}$	$2.4^{2.8}_{2.1}$	$\dagger -16^{-5}_{-18}$	$\dagger -20^{-5}_{-23}$	$\dagger -26^{-10}_{-30}$	$*-11^{8}_{-21}$	$5.0^{5.7}_{4.3}$	$\ddagger -8.1^{4}_{-17}$	$\ddagger -8.3^{4}_{-15}$	$\ddagger -17^{-9}_{-23}$	-48^{-35}_{-55}
$-\min \dot{P}$	$\frac{mmHg}{ms}$	$1.9^{2.1}_{1.6}$	$\dagger -15^{-13}_{-28}$	$\dagger -29^{-19}_{-61}$	$\dagger -44^{-32}_{-59}$	10^{21}_{3}	$2.5^{2.7}_{2.0}$	$\ddagger -22^{-9}_{-37}$	$\ddagger -40^{-30}_{-49}$	$\ddagger -47^{-38}_{-55}$	-55^{-28}_{-58}
P_{ED}	mmHg	$4.5^{5.8}_{1.9}$	$\dagger 13^{26}_{-2}$	$\dagger 47^{196}_{-6}$	$*98^{445}_{-10}$	5.1^{27}_{-5}	$8.6^{20}_{3.2}$	$\ddagger 14^{33}_{-5}$	$\ddagger 14^{98}_{-2}$	$\ddagger 21^{100}_{-14}$	183^{300}_{43}
Exponential relaxation model Ex3 (3 parameters):											
τ_{Ex3}	ms	$18.4^{23.2}_{15.3}$	$\dagger 39^{62}_{20}$	$\dagger 66^{82}_{38}$	$\dagger 88^{122}_{32}$	3.2^{41}_{-24}	17.6^{29}_{12}	$\ddagger 61^{144}_{16}$	$\ddagger 122^{209}_{-3}$	$\ddagger 168^{229}_{61}$	96^{225}_{88}
$t_{Dia}\,\tau_{Ex3}^{-1}$	[1]	$4.74^{5.72}_{2.24}$	$*-12^{-1.0}_{-29}$	-16^{13}_{-34}	-11^{20}_{-24}	$-13^{4.5}_{-50}$	$6.36^{7.0}_{2.5}$	$\ddagger -26^{0.14}_{-45}$	$\ddagger -34^{-2.0}_{-52}$	$\ddagger -34^{-6.6}_{-52}$	-20^{65}_{-29}
$F_{RC}^{(Ex3)}$	%	$0.88^{1.70}_{.012}$	90^{235}_{-39}	98^{387}_{-25}	67^{137}_{-62}	$*99^{2312}_{-42}$	$.172^{2.39}_{.072}$	$\ddagger 357^{1419}_{-21}$	$\ddagger 583^{2485}_{-93}$	$\ddagger 825^{3446}_{6.4}$	154^{643}_{-81}
Logistic relaxation model Lg4 (4 parameters):											
τ_0	ms	$18.8^{23.2}_{15.7}$	$\dagger 38^{61}_{18}$	$\dagger 66^{81}_{41}$	$\dagger 93^{123}_{51}$	-0.1^{47}_{-30}	20.2^{29}_{13}	$\ddagger 59^{144}_{41}$	$\ddagger 144^{215}_{-20}$	$\ddagger 174^{264}_{60}$	71^{228}_{55}
τ_∞	ms	$17.2^{21.7}_{12.4}$	$*34^{48}_{-23}$	$*64^{122}_{-26}$	$*55^{172}_{40}$	$\dagger -56^{-46}_{-82}$	13.2^{22}_{10}	$\ddagger 30^{67}_{-19}$	$\ddagger 54^{94}_{-22}$	$\ddagger 88^{159}_{-3}$	158^{946}_{139}
$t_{Dia}\,\tau_\infty^{-1}$	[1]	$5.98^{7.38}_{4.10}$	-10^{33}_{-32}	-9.7^{26}_{-88}	17^{22}_{-14}	$\dagger 100^{234}_{32}$	$8.08^{11.7}_{5.36}$	1.78^{52}_{-26}	-4.0^{58}_{-32}	-12^{36}_{-36}	-34^{-19}_{-106}
$t_{Fill}\,\tau_\infty^{-1}$	[1]	$3.73^{5.82}_{2.37}$	$-14^{7.8}_{-49}$	-14^{10}_{-89}	-5.4^{30}_{-21}	$\dagger 180^{263}_{26}$	$5.08^{7.6}_{3.1}$	-5.2^{45}_{-33}	$*-18^{21}_{-44}$	$\ddagger -28^{2.5}_{-56}$	-66^{-28}_{-81}
$F_{RC}^{(Lg4)}$	%	$.351^{0.95}_{.045}$	100^{457}_{-60}	102^{1635}_{-84}	-37^{106}_{-93}	$\dagger -99^{-95}_{-100}$	$.052^{0.4}_{.001}$	18^{953}_{-91}	153^{1182}_{-100}	339^{1885}_{-100}	651^{9103}_{-64}
$P_\infty^{(Lg4)}$	mmHg	$-2.9^{1.0}_{-16}$	$*23^{53}_{0}$	3.8^{237}_{-97}	$*112^{275}_{-18}$	24^{57}_{-70}	4.5^{18}_{-4}	8.0^{77}_{-107}	$\ddagger 32^{114}_{-15}$	$\dagger 49^{134}_{-18}$	130^{190}_{-261}
$\Delta P_{term}^{(Lg4)}$	mmHg	$6.20^{17.3}_{4.40}$	0.56^{85}_{-40}	18^{122}_{-56}	-31^{65}_{-70}	-14^{51}_{-64}	$4.4^{8.9}_{-0.3}$	18^{85}_{-63}	-12^{73}_{-98}	-5.7^{61}_{-90}	-73^{2139}_{-102}

N, number of specimens; (n), sample size. Guinea pigs: mean aortic pressure 60 mmHg, left ventricular mass, post–experimental wet, 912±67 mg (median ± mean absolute deviation); Rats: aortic pressure 75 mmHg, LV mass 823±43 mg. Table entries are absolute values (see **Unit**) at 37°C (normothermia), and percentages of change against normothermia in the other columns; medians with upper and lower limit of the central 80% of the sample.
Error probabilities in assuming no difference against normothermia (Wilcoxon matched–pairs): * $p < 0.05$; † $p < 0.01$; ‡ $p < 0.001$; at $n \leq 10$, † becomes the highest possible significance.
O_2 Cons(umption) is tentative at hypothermia and absent at 40°C, because the Clark electrodes (Radiometer Copenhagen) are calibrated at 37°C.

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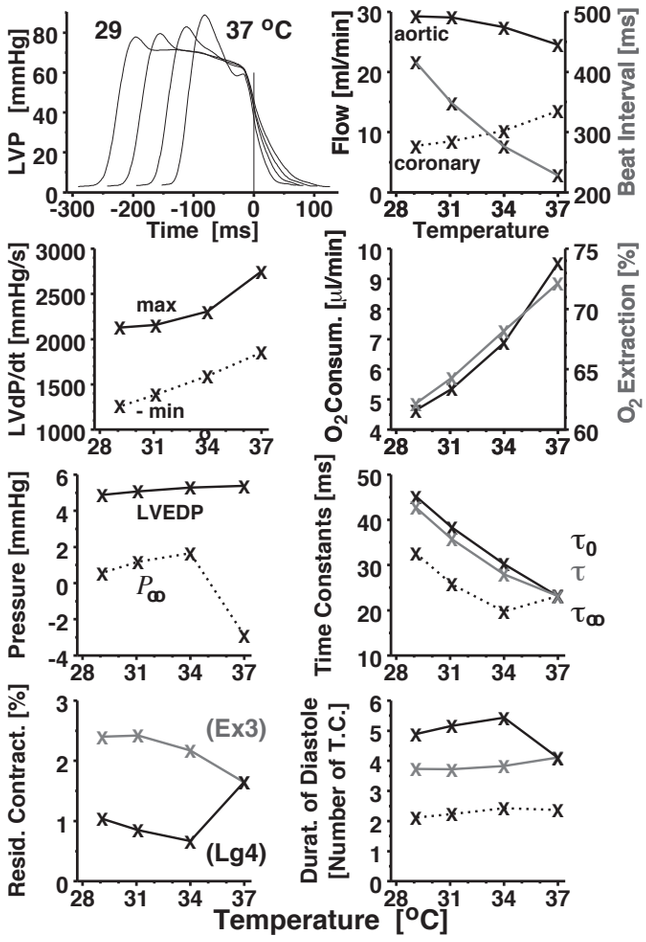

Figure 2. Isolated guinea pig heart (male, left ventricular mass 880 mg, postexperimental, wet) working in steady–state at different temperatures. Mean aortic pressure 60 mmHg, cardiac output 37 mL min^{-1}. Left–ventricular pressure (LVP) is shown from 5 s presystolic until re–encounter of end–diastolic pressure (LVEDP), adjusted to peak min LVdp/dt at t=0. O_2 Consumption is BTPS at barometer 755 mmHg. P_∞ by relaxation model Lg4, τ (*gray*) by model Ex3. *Last panel*: $t_{\mathrm{Dia}}\tau^{-1}$ (*gray*), $t_{\mathrm{Dia}}\tau_\infty^{-1}$, (*solid*), $t_{\mathrm{Fill}}\tau_\infty^{-1}$, (*dotted*).

end–diastolic pressure; however, some specimens (rat hearts, especially) stand mild hypothermia without loss of contractility. Negative lusitropic effects occur regularly in hypothermia, seen by reduced peak velocity of LVP decrease and by rising time constants. However, the asymptotic logistic τ_∞ may also decrease (at mild hypothermia) in individual specimens, while the initial τ_0 rises in almost all hearts. The relative diastolic (and filling) times, expressed in units of apparent time constants, are typically reduced in median at hypothermia, but abundant significance can be stated only in $t_{\mathrm{Dia}}\,\tau_{\mathrm{Ex3}}^{-1}$ at rat hearts. Especially, there is no systematic quantitative change with decreasing temperature level. Residual contraction remains typically below one per cent at normothermia. It does not necessarily increase, but may even multiply, at hypothermia. Becoming aware of extended relative increases in F_{RC} is a mere aftermath, no incidents to note occurred during the experiments along with expressed increases of F_{RC}. Instead, the terminal pressure difference, ΔP_{term}, seems to be noteworthy. It may increase or decrease at different temperature levels, and may almost vanish at deepest hypothermia (rat), but no heart was seen at steady work whilst $P_{\mathrm{ED}} < P_\infty$.

The mixed behaviour seen within the samples justifies a closer look on individual experiments. The guinea pig heart shown in Fig. 2 managed hypothermia with a steady but limited loss in peak pressure upstroke velocity, while end–diastolic pressure remains constant during the rising beat volume at hypothermic bradycardia. The shape of the

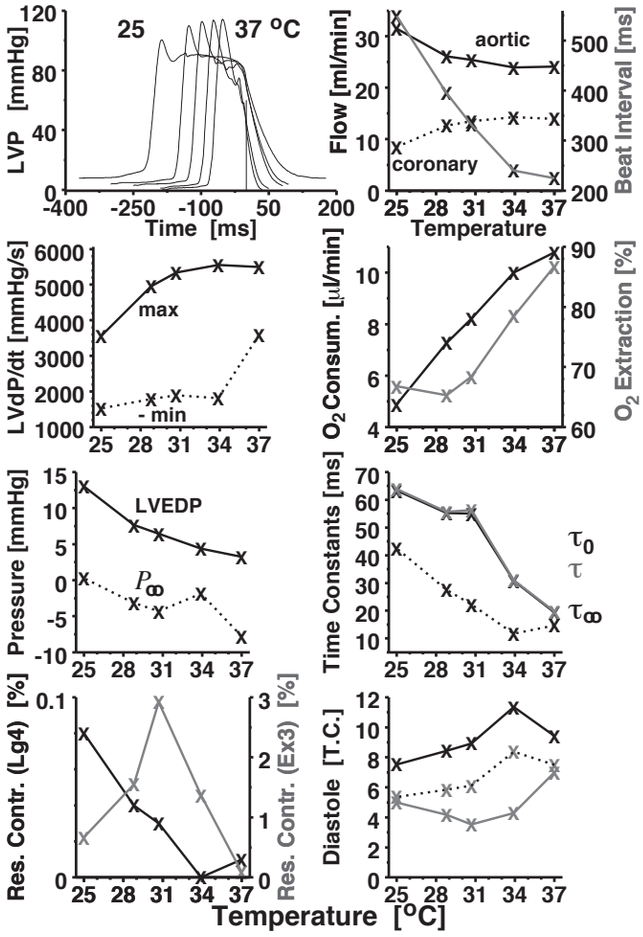

Figure 3. Isolated Sprague–Dawley rat heart (male, left ventricular mass 847 mg). Mean aortic pressure 75 mmHg, cardiac output 39 mL min^{-1}, barometer 765 mmHg. Data shown as in Fig. 2.

isochoric relaxation changes remarkably from exponential (37°C, $\tau_0 = \tau_\infty$) to logistic curves, and the negative lusitropic effect of cooling is visible from the LVP and all respective parameters. However, after an initial drop at 34°C, $\Delta P_{\text{term}}^{(\text{Lg4})}$ remains stable. Along with the mentioned shape change, models Ex3 and Lg4 purvey contrary findings about residual contraction at hypothermia, albeit both reveal "complete" relaxation, F_{RC} below 3 per cent, at all temperatures. Filling time remains stable slightly above $2 \times \tau_\infty$, while $t_{\text{Dia}}\tau_\infty^{-1}$ even increases; model Ex3 likewise indicates proportionality between t_{Dia} and τ.

The rat heart of Fig. 3 presents with end–diastolic pressure increasing as temperature is lowered, whereas peak LVP upstroke remains constant at mild hypothermia. Remarkably, peak LVP decrease velocity becomes halved yet at 34°C, but remains fairly stable against further cooling. Concomitantly, $P_\infty^{(\text{Lg3})}$ steps up at the first hypothermia level without much further increase. Again, LVP relaxation becomes non–exponential below normothermia, thus models Ex3 and Lg4 estimate grossly different residual contraction. Due to Lg4, it increases systematically with cooling, but relaxation remains complete, $F_{\text{RC}}^{(\text{Lg4})}$ less than one per mille; Ex3 yields at least tenfold higher residual contraction at hypothermia, but always less than 3 per cent. Relative diastole and filling time do not decrease by more than a fifth or a third, respectively, even at deepest temperature.

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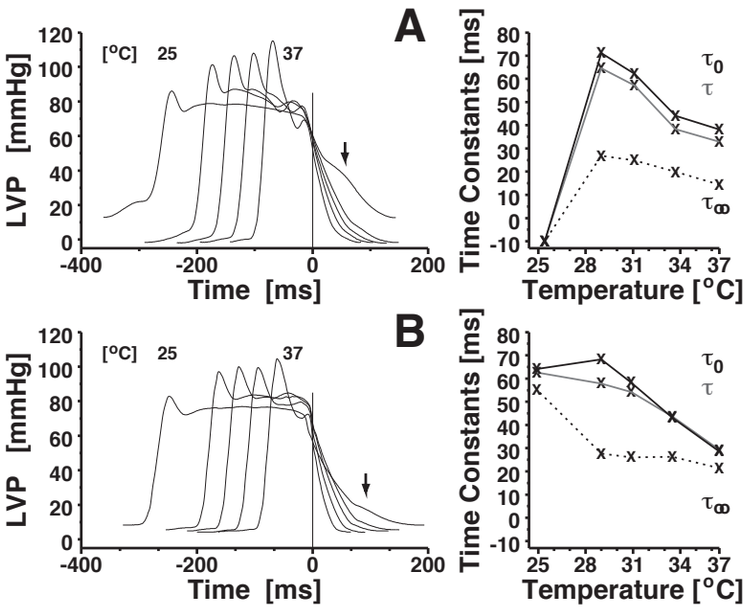

Figure 4. Emerging disturbance (↓) of left–ventricular relaxation at 25°C among two isolated Sprague–Dawley rat hearts; 75 mmHg aortic pressure, 40 mL min^{-1} cardiac output. A: Pronounced elevation of isochoric pressure decay. Violation of the relaxation models compromises the regression parameter estimates. B: More discrete disturbance. Time constants at 25°C still plausible but iffy.

Hearts from Sprague–Dawley rats resist deep hypothermia easier than those from guinea pigs. The former can present with articulated deviations from a normal isochoric left–venticular pressure fall in deep hypothermia. Such incident does not necessarily indicate imminent failure, but may be a steady–state phenomenon, seen within and beyond the LVP recordings of duration 4 s. Two examples are shown in Fig. 4. Obviously, the relaxation model assumtions (exponential or logistic shape) do not hold in such case, which is reliably detected by the goodness–of–fit index falling below 0.995.

3.2 Adaptation after jumps in temperature

Steps in temperature (protocol A2), downward as well as upward, may induce a continuous transition of the LVP curve into a new shape, but may also trigger a mixed pattern of transient changes, as arrhythmia (premature and partial recontraction, especially), or elevated diastolic pressure. However, such phenomena use to settle into steady–state within 4 to 8 minutes after three–degree steps. This also holds in rat hearts subjected to six–degree temperature steps, whereas about half of guinea pig specimens end up in failure (if not becoming deloaded or rewarmed) during six–degree jumps. We present results graphically with respect to this observation.

Cooling down guinea pig hearts by three degrees (Fig. 5) reduces LVP change velocities pertinently, with a (seemingly) slow but continuous partial recovery. End–diastolic pressure rises in all specimens (median +5%), with more extended partial recovery. Both initial and asymptotic time constant of the pressure fall rise in median at cooling. However, whereas τ_0, after an overshot for a few minutes, remains elevated during the hypothermia phase, τ_∞ presents with fluctuations which end up, in median, at the normothermic value. Concomitant variation is seen in relative diastole time and residual relaxation. Both become transiently disturbed by rapid temperature switch rather than pertinently altered. All parameter changes, including those not shown in

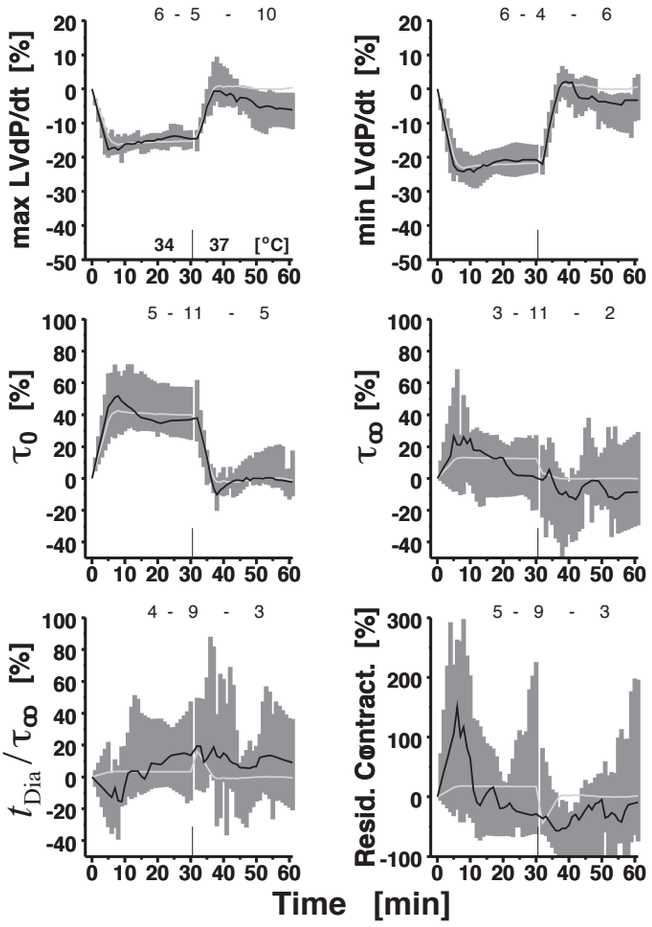

Figure 5. Half–hour adaptation after temperature steps 37°C→34°C→37°C in isolated working guinea pig hearts; N=11, left–ventricular mass 970±30 mg (median ± mean absolute deviation), 60 mmHg aortic pressure, 40 mL min^{-1} cardiac output. Median per cent differences to initial normothermia; time course (*black*) with samples' 80% range (*gray*), the *white* lines show data corrected for individually differing temperature change velocity. Statistics (*gray headlines*) are numbers of significant ($p < 0.05$) cases of non–constancy within the last 16 minutes of the respective temperature level (Spearman, *first and last* number), and of median change after rewarming (U–test, *central* number).

the Figure, are reversible by re–establishing normthermia. — Rapid cooling of guinea pig hearts by six degrees reduces the median LVP change peak velocities by fifty per cent or more. If not failing, the heart recovers partly within about ten minutes and presents with similarly altered lusitropic parameters as noted from the three–degree step samples.

Inter–individual variablity in the data is partially due to different temperature change velocity. Rescaling the time abscissa of the measurements to the median temperature–*vs.*–time course (white lines in the Figures) attenuates some overshooting but does not provide new substantial insights.

We proceed to the larger rat heart sample at temperature dropping to 31°C (Fig. 6) and stress distinctions between the Figures. First, there are remarkable transients in max $\dot{P}$ during sudden temperature changes, but only less than ten per cent (median) durable reduction at hypothermia in rat hearts. However, negative inotropy is seen from rising P_{ED} in all but two specimens, by +36% in median. Second, increasing τ_∞ is clearly revealed, with doubling in median. Slight but mostly significantly reduced $t_{\mathrm{Dia}}\tau_\infty^{-1}$ during 31°C, with small transient overshoots, is another revelation. Accordingly, F_{RC} is seemingly elevated at 31°C, but starting from tiny absolute values at 37°C, as known from Tab. 1 and protocol A1.

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Table 3: Hemodynamic effects of heart rate at mild hypothermia

		G.Pig 34°C			**Rat** 31°C		
Heart rate:		*Spt*	*Pac*		*Spt*	*Pac*	
(n):		(88)	(64)	$p <$	(168)	(80)	$p <$
t_{BI}	ms	339	260	0.001	353	226	0.001
V_{Beat}	μL	227	143	0.001	227	132	0.001
F_{Cor}	$\frac{\mathrm{mL}}{\mathrm{min}}$	9.0	9.8	0.666	14.3	13.5	0.019
O_2 Cons	$\frac{\mu\mathrm{mol}}{\mathrm{min}}$	4.7	5.1	0.268	5.9	4.7	0.001
$\max \dot{P}$	$\frac{\mathrm{mmHg}}{\mathrm{s}}$	1820	1873	0.225	4507	3504	0.001
$-\min \dot{P}$	$\frac{\mathrm{mmHg}}{\mathrm{s}}$	1366	1447	0.071	1544	1372	0.062
P_{ED}	mmHg	11.2	13.0	0.001	5.4	14.1	0.001
τ_{Ex3}	ms	32.6	26.0	0.001	47.0	51.6	0.016
τ_0	ms	32.5	27.6	0.001	55.0	55.1	0.761
τ_∞	ms	29.3	20.5	0.001	23.1	28.2	0.001
$t_{\mathrm{Dia}}\,\tau_\infty^{-1}$	[1]	4.79	4.57	0.022	8.37	3.12	0.001
$F_{\mathrm{RC}}^{(\mathrm{Lg4})}$	%	0.84	1.54	0.001	.053	4.31	0.001
$P_\infty^{(\mathrm{Lg4})}$	mmHg	4.3	8.7	0.001	–0.5	3.5	0.002
$\Delta P_{\mathrm{term}}^{(\mathrm{Lg4})}$	mmHg	6.87	3.33	0.001	$3.9\bar{5}$	12.1	0.001

Unpaired samples of size (n). Guinea pig hearts at 60 mmHg mean aortic pressure, and Sprague–Dawley rat hearts at 75 mmHg aortic pressure; cardiac output approx. 39 $\mathrm{mL\,min^{-1}}$ in all samples, steady–state. Spontaneous sinus bradycardia (*Spt*) *vs.* right–atrial electrical stimulation (*Pac*) at the intrinsic beat frequency seen in the specimen at 37°C. Sample medians and p, error probability of refusing zero hypotheses ("no median difference"; U–tests).

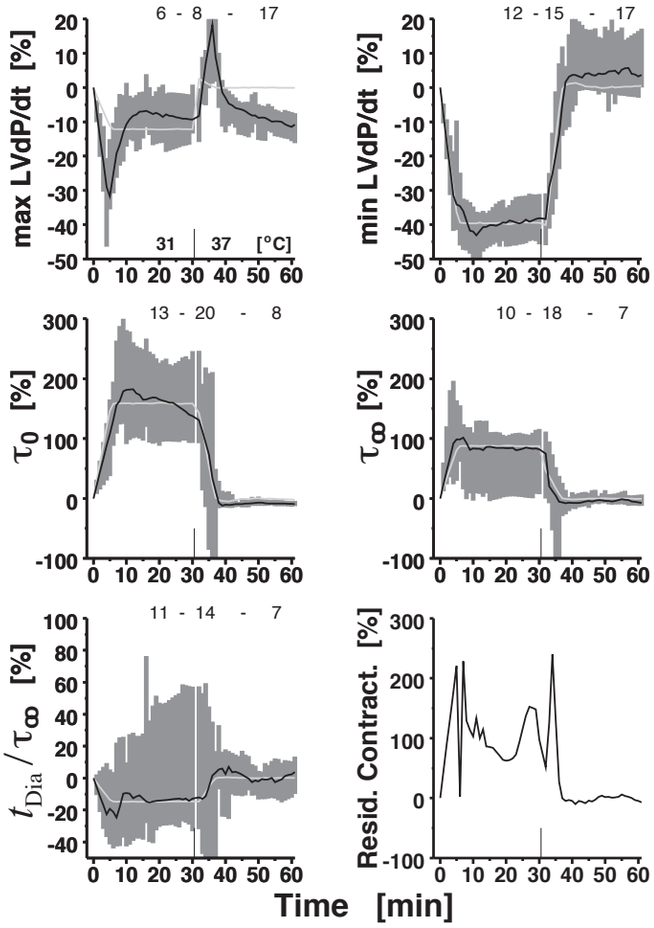

Figure 6. Half–hour adaptation after temperature steps 37°C→31°C→37°C in isolated working Sprague–Dawley rat hearts; N=21, left–ventricular mass 837±47 mg, 75 mmHg aortic pressure, 40 mL min^{-1} cardiac output. Presentment as in Fig. 5.

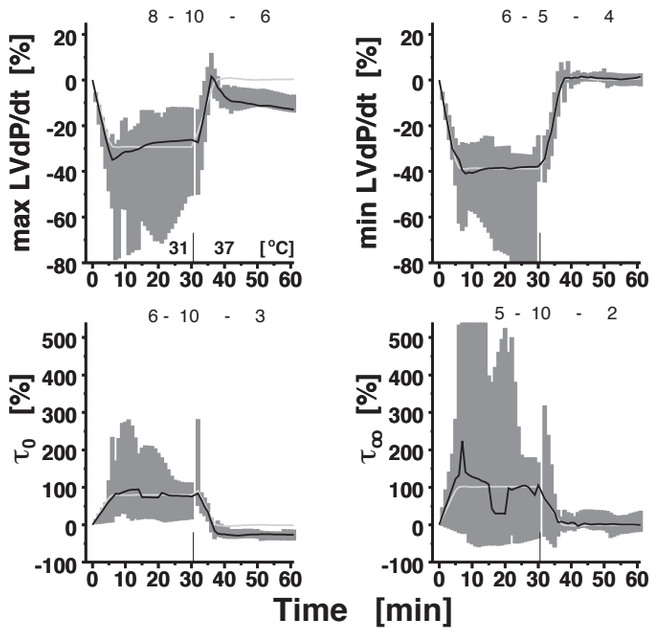

Figure 7. Adaptation after temperature steps 37°C→31°C→37°C at constant heart rate (right–atrial pacing) in Sprague–Dawley rat hearts; N=10, left–ventricular mass 840±29 mg, aortic pressure 75 mmHg, cardiac output 40 mL min^{-1}. Presentment as in Fig. 5.

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3.3 Cooling with bradycardia or electrical pacing

Table 3 compares the hemodynamics at mild hypothermia with spontaneous bradycardia and with heart rate fixed at its normothermic value.

Pacing does not affect contractility at the 34°C level in guinea pig hearts, nor does it alter the median peak pressure fall velocity or $t_{\mathrm{Dia}}\,\tau_{\infty}^{-1}$. Anyhow, residual contraction and pressure asymptote (Lg4) are significantly elevated in the pacing sample.

Pacing, compaired with spontaneous bradycardia, depresses the inotropy of rat hearts at 31°C substantially, as seen from $\max \dot{P}$ and P_{ED}. Lusitropy is also reduced; interestingly, the exponential time constant (and τ_0 of Lg4) remains unaltered, but Lg4 reveals the effect by increasing τ_{∞}. Consequently, $t_{\mathrm{Dia}}\,\tau_{\infty}^{-1}$ is halved, and a notable amount of residual contraction (absent at bradycardia) appears with pacing.

It should be mentioned that the samples do not encompass hearts at imminent failure. The experimenter had had the option to abort the cooling/pacing process in order to preserve the specimen fit for other purposes. Over nine tenth of the hearts worked properly under the said conditions, gradualness of cooling is the most pertinent requisite.

Figure 7 presents the most pertinent changes caused by constant heart rate during temperature steps in rat hearts; compare with the variable heart rate experiments (Fig. 6, take heed of ordinate scalings). As its main effect, pacing reduces, but in median only, the transient overshoots in $\max \dot{P}$; the permanent depression of $\max \dot{P}$ at 31°C (–30%) is more substantial than seen with spontaneous bradycardia (–10%). Course of depression in $-\min \dot{P}$ remains unaltered, but the broader C_{80} range (also in $\max \dot{P}$) suggests that pacing may put more hearts on the edge of failure. On the other hand, pacing halves the median τ_0 surplus, whithout changing the doubling of τ_{∞}, already seen with variable beat frequency. However, pacing impinges huge inter– and intra–individual variability and non–stationarity on the time course of τ_{∞}, and renders $t_{\mathrm{Dia}}\,\tau_{\infty}^{-1}$ and F_{RC} (not shown) quite meaningless. This phenomenon, as all other changes, vanishes after rewarming. — Exactly the same pacing effects occur in the eight guinea pig hearts (compare with Fig. 5), except $\max \dot{P}$ suffering no deeper depression as found with variable heart rate. Especially, $-\min \dot{P}$ runs almost identical to Fig. 5 (including the span of scattering), and median τ_0 increase becomes doubled by pacing, as in the rat sample. Opposit to the latter, pacing does not aggravate the scattering of τ_{∞} in the guinea pig hearts.

3.4 Heart rate stimulation at mild hypothermia

As a most unexpectant finding, the analysis of pacing series (protocol B2) frequently reveals a discontinuity of relaxation which is especially noticed in the asymptotic time constant. Overlayed LVP curves display, in about a third of the specimens, a visible "gap" between a group of fairly continuously changing curve shapes at low heart rate and the two to four curves at the high frequency end of the series, which appear also in similar shape among themselves. Even if such gap is seemingly absent in the LVP shape, a heart rate dependent sudden increase in τ_{∞} is often seen before the

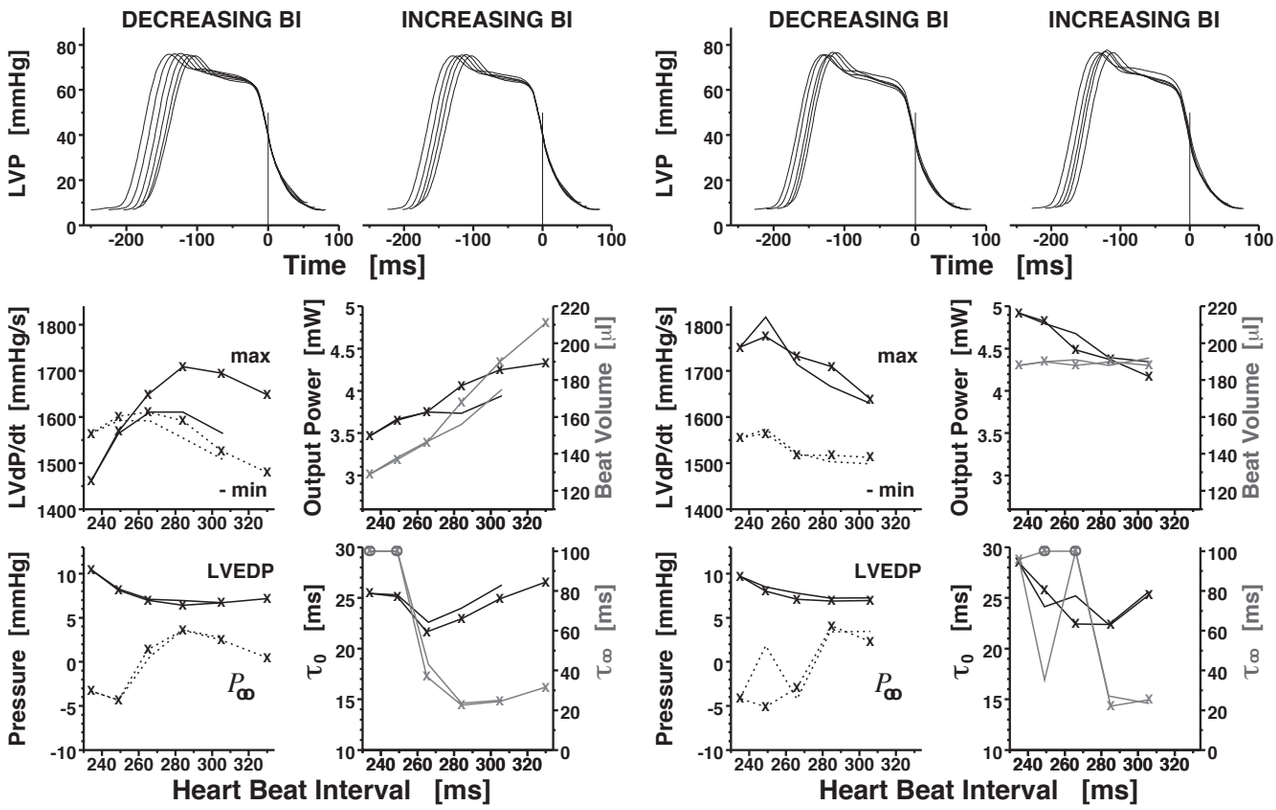

Figure 8. Right–atrial electrical stimulation of an isolated working guinea pig heart (male, left ventricular mass 950 mg) at 34°C and aortic pressure 60 mmHg, with constant cardiac output (39 mL min^{-1}, *left two colums*) or with constant beat volume (*right two columns*). Beat interval (BI) length in sinus rhythm was 242 ms at 37°C, and 331 ms at 34°C. BI DECREASING by pacing (*lines marked* with X) and reverse BI INCREASING (*lines* without plot marks). Procedure repeated with constant beat volume by reducing cardiac inflow proportionally. Left–ventricular pressure (LVP) curves adjusted as in Fig. 2. P_∞ estimated by model Lg4. $\otimes$: τ_∞ out of scale (between 142 to 153 ms); notice ordinate scale differing from τ_0.

pacing series ends. Such phenomenon, if present, is neither a transient one nor does it indicate imminent failure. Typically, the heart stands one or more additional steps in pacing rate until it fails. This holds in about two thirds of specimens (from both species), in the other hearts, the sudden τ_∞ increase coincides with functional heart failure. The said discontinuity is reversible by decreasing heart rate, and the reversion happens at the same or an adjacent rate step as in upward pacing. These incidents, as occurring inter–individually between different heart rate levels, render the median statistics scheme inappropriate. Hence we demonstrate the pertinent findings by the typical, non–outlying example shown in Fig. 8.

Accelerating the heart rate at mild hypothermia and constant cardiac output (as in Fig. 8, left columns) improves ventricular contractility. End–diastolic pressure (at its respective steady–state) does not change, despite decreasing beat volume. Lusitropy

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is also enhanced, seen by higher pressure fall velocity and decreasing time constants. Inotropy subsequently becomes as more depressed as heart rate approaches its former 37°C–value. While peak pressure fall velocity decreases gradually and by limited amounts, the relaxation time constants, τ_∞ in particular, rise remarkably at a distinct step in heart rate. The heart continues to perform steadily and even at further reduced BI lengthes. Relaxation is fairly complete at hypothermic bradycardia, $F_{RC}^{(\mathrm{Lg4})} < 1.8\%$ at the first three pacing levels (BI 330 down to 284 ms); this residual contraction rises to 6% at BI 265 ms and exceeds 20% and 23% at BI 249 and 234 ms, respectively. — All parameters but $\max \dot{P}$ (that suffers an amount of time-dependent degradation) pass through their former values if the heart rate change is reversed. LVP shape changes continuously with BI length, no "gap" becomes visible in this example.

Such "gap", however, is about to appear in the constant V_{Beat} pacing series, performed in the same specimen (Fig. 8, right columns; notice higher spontaneous heart rate as an aftermath of the preceding pacing test). More durable positive inotropic effect of pacing is observed if the heart works with constant beat volume, increasing its output and pumping power. Even so, the same leap in τ_∞ and P_∞ occurs at the same ("critical") BI length. Residual contraction is below 1.4% before the critical BI, 15% at BI 266 ms, and over 22% at shorter BI. Again, all parameter changes reverse quantitatively if pacing frequency is lowered (except one outlying τ_∞ at BI 249 ms).

4 Discussion

Incomplete ventricular relaxation as a possible mechanism of diastolic heart failure has long been discussed in concept, but still longs for a sound quantification. We hence tackle the important issues of extrapolating and of interpreting the obtained lusitropy indices first. Present findings will than find a straightforward assessment.

4.1 How to quantify completeness of ventricular relaxation

Consider the *transmural* left–ventricular pressure (LVP), *viz.* intracaval minus the pressure directly surrounding the ventricle. The latter is the intrapericardial pressure *in situ*, and is the ambient barometer pressure to the isolated heart, as in the present study. After systolic ejection, the ventricle holds the residual volume, and LVP falls along with the decreasing myocardial tension. If neither ventricular refill nor myocardial excitation occurs, LVP will eventually fall to its equilibrium pressure (residual volume, non–contracted wall) which is usually negative [8]. Concerning a distinct beat interval, ventricular relaxation is said to be complete if LVP fairly reaches this equilibrium pressure before the subsequent systole occurs. Cardiac tonus (medium–term wall tension, necessary for heterometric autoregulation of presystolic fiber length) is excluded by this definition. Complete relaxation can be detected in non–filling hearts by LVP becoming fairly constant before the onset of systole. In normally working hearts, diastolic refill compromises this criterion because end–diastolic volume no longer equals the residual volume. Completeness of relaxation in filling hearts can be estimated by

fitting a non–linear regression model to the isochoric (neither ejecting nor filling) phase of the LVP decay and extrapolating the model's LVP forecast at the end of the actual diastole.

Such extrapolation is always a scabrous issue and depends on following assumptions: (1.) The regression model covers the process in question empirically correct (absence of systematic regression error); (2.) The factors that determine the model parameters remain unaltered and effective beyond the end of the regression interval, and new regardable factors do not supervene. A recent study [3], most related to the present one, utilises two three–parametric models, exponential Ex3 and logistic Lg3. However, by reasons given before [9], four model parameters are necessary, and Lg4 covers the isochoric pressure fall properly. Present results confirm that even the individual heart changes its isochoric LVP shape during temperature or heart rate alteration. This study presents the exponential τ_{Ex3} for conventional reasons, but τ_{Ex3} carries no standalone information, because the empirical data (and some mathematical reasoning) reveals $\tau_{\mathrm{Ex3}} \approx \tau_0$. Much more important, τ_0 (and hence τ_{Ex3}) describes the initial isochoric pressure fall, whereas the late diastolic relaxation is under investigation. Consequently, the asymptotic τ_∞ is the pertinent time constant. Unacceptable in any analysis of late–diastolic relaxation, Ex3 copes with the model violations by estimating much too small (too negative) pressure asymptotes [10], as also seen in Fig. 1. The same objection holds for Lg3 (3 parameters) if the actual LVP decays exponentially [9].

Concealing the problem of inferring pressure values from inappropriate models, complete relaxation was originally declared if the time (t_{Dia}) from peak LVP fall to the onset of the next systole is not less than 3.5 times the (exponential) time constant; however, the authors also stated that shorter $t_{\mathrm{Dia}}\tau^{-1}$ "does not necessarily imply the presence of incomplete relaxation" [11]. This criterion is a mere recourse to a well known technical convention: As any exponential (with zero asymptote) falls from any value to about 3 per cent of this value within 3.5 times its time constant, the exponential process is practically "finished" within this time. Evidence for a physiological meaning of that 3.5–criterion in diastolic function has not been provided. Furthermore, the criterion was as well used with a logistic time constant [3], in spite of the fact that the enigmatic multiple is 4.2 in that model to approach asymptote by 3 per cent of the LVP at $t = 0$. Obviously, the straightforward index F_{RC} yields the desired information about completeness of diastolic relaxation, and this index can be calculated from any LVP decay model that has an asymptota. Hence, we will not consider any t_{Dia}–based surrogate further.

Another pertinent index in late diastole studies is $\Delta P_{\mathrm{term}} = \mathrm{LVP}(t_{\mathrm{Dia}}) - P_\infty$. LVP during refill was understood as the sum of active (myocardial) pressure decay and passive pressure rise due to the filling [12]; however, two issues must be made: Albeit the passive pressure–volume relation of the ventricle is linear in the range of interest [8, 12], the passive LVP rise is not, because inflow is not constant. This is well known as "fast early filling" and clearly visible in Fig. 1. Furthermore, the asymptote of the active relaxation is often erroneously assumed as zero. The first issue is accounted for by defining ΔP only at the end–point of diastole. Due to the Path–Independence Law of work, we may analytically substitute the real diastole by a 3–step

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sequence: 1., active LVP falls to its equilibrium pressure, P_∞, without any filling; 2., imagine now mitral valve opens in absence of external filling pressure, *i.e.* left–atrial transmural pressure zero; 3., finally, consider the really apparent P_{ED} and complete left ventricular filling at end–diastole (equilibrium volume). This analysis shows (Fig. 1, notice the definition of zero pressure!): ΔP_{term} represents the filling work (neglecting dynamic energy dissipation). P_∞ is the *vis a fronte*, the ventricle "sucks in" volume if $P_\infty < 0$. P_{ED} is the *vis a tergo*, moving additional volume into the ventricle. Hence $-P_\infty(\Delta P_{\mathrm{term}})^{-1}$ and $P_{\mathrm{ED}}(\Delta P_{\mathrm{term}})^{-1}$ are the fractions of "diastolic suction" and extra–ventricular filling work, respectively. No knowledge of volumes is needed, but zero pressure must be adjusted to the ambient pressure (atmospheric, in isolated hearts). Reading the barometer, checking zero calibration after the experiment has finished, and accounting for temperature changing by the protocol, have demonstrated a drift of pressure zero calibration by some mmHg. Hence we do not interprete the absolute readings. Fortunately, ΔP_{term} does not depend on the absolute pressures.

Findings from B2 (Fig. 8) require to clear away an abundant misunderstanding. It is important to understand, that *large* time constants in *linearly scaled* exponentials do not indicate "slow decay" but just indicate "poor exponentiality" of the decay curve [5]. If the asymptote is a regression parameter ("variable asymptote", now standard in cardiac relaxation studies), any straight line (at *any* slope) is perfectly fitted by an exponential with time constant $\pm\infty$. The same holds in logistic models. Hence, if the late isochoric LVP curve straightens up, the regression fit will estimate unusual high time constants together with an unreally low (negative) pressure asymptote. Such observations should be recognised as a LVP course deviating from both exponential and logistic shape at the late isochoric phase. Asymptotic parameters (τ_∞, P_∞) do not describe the future physical situation at end–diastole if such model deviation occurs.

Aforesaid model violations are exceptional. Load–clamp experiments in muscles (including cardiac) illustrated that under most different loading conditions (and temperatures) the time course of muscle relaxation *eventually* converges into exponential shape [13, 14]. This property is exactly what the logistic model describes by converging into an exponential with time constant τ_∞ as time elapses.

4.2 Interpretation of the results

Hypothermia reduces cardiac oxygen demand and became a valuable therapeutic option in heart and extra–cardial diseases, fostered by good contractility of the hypothermic ventricle. Inotropy is preserved by intramyocardial regulation [1] and is also effective in the hypothermic working ventricle [2]. Present observations, especially the up/down–regulation of contractility after abrupt temperature steps (A2), accord with these findings. Their remains a limited negative inotropic net effect of cooling in steady–state; however, long–term degradation of the isolated heart [15] plays a rôle (see max LVdP/dt curves in Figs. 5 and 6). Preserved inotropy is further seen from obviously still effective heterometric autoregulation (Frank–Starling) in the present data, especially in the constant output *vs.* constant beat volume series (B2). Temperature–dependent bradycardia in protocol A1 increases end–diastolic volume and pressure, thus augmenting

peak LVP upstroke velocity. Severe negative inotropy occurs only below 30°C.

Positive inotropy at mild hypothermia has focussed attention on concomitant diastolic dysfunction [16]. Long since known, the time constant of isochoric LVP fall increases with cooling [3, 4, 17, 18], as in present material. Its relation to diastole duration, howbeit early mentioned as an index of incomplete relaxation [11], is usually not presented in the literature, probably due to some polypragmaty of methods to calculate time constants. The 3–parametric logistic model (Lg3, *i.e.* Lg4 but with $\tau_0 = 2\tau_\infty$) was suggested to fit isochoric LVP properly, regardless of temperature in canine ventricles [18]. Present observation in small animal hearts reveal considerable LVP shape changes during cooling, properly covered only by the 4–parametric Lg4.

A recent study [3] provides pertinent data from hypothermic pig hearts in situ, using models Ex3 and Lg3. Mild hypothermia (33°C) halves the ratio $t_{\mathrm{Dia}}\tau^{-1}$ (τ taken from Ex3 as well as Lg3), but $t_{\mathrm{Dia}}\tau^{-1} > 3.5$ still holds at spontaneous bradycardia. This was originally (canine hearts, normothermia) told to exclude incompleteness of relaxation [11]. Study [3] found $t_{\mathrm{Dia}}\tau^{-1} < 3.5$ only if mild hypothermia was combined with pacing. Their authors "aimed to clarify whether this reflects increased myocardial passive stiffness or whether this is a consequence of incomplete relaxation." [3]. They assume evidence of incomplete relaxation if the end–diastolic pressure *vs.* volume curve (obtained by pacing) shifts leftward, and conclude that mild hypothermia is an example of incomplete left–ventricular relaxation [3]. Pressure asymptota (P_∞) estimates are not presented, and the said "leftward shift" not quantified to justify the term "incomplete" relaxation physiologically.

The issue is obvious: Just by definition, any asymptotic process remains incomplete at any time. We can not avoid to search for an empirically allowable or "safe" F_{RC}–limit with respect to imminent diastolic heart failure.

Shortening of $t_{\mathrm{Dia}}\tau_{\mathrm{Ex3}}^{-1}$ and of $t_{\mathrm{Dia}}\tau_\infty^{-1}$ is also seen in the present isolated hearts at hypothermia (Tab. 2). However, relaxation remains obviously complete at mild hypothermia, as evidenced straightforward by F_{RC}. The percentage of residual contraction at end–diastole is so tiny at normothermia that even the most extreme multiples, seen in A1 (Tab. 2), do not reach 3 per cent. This observation is in no conflict with the findings of the mentioned study [3]. The opposite conclusions are likely based on different definitions of a complete relaxation.

Usually, P_∞ becomes elevated at hypothermia, just indicating that the potential *extent* (as seen in the isochoric phase), but not necessarily the completeness (seen at end–diastole), of relaxation is lower in hypo– than in normothermia. The (assumed non–filling) ventricle can never encounter pressures below its equilibrium pressure (estimated by P_∞), hence incompleteness is an ambiguous term referring to elevated pressure asymptotas. With respect to cardiac tonus [19] one may ask for the smallest P_∞ ever possible at given residual volume in absence of any myocardial tonus. Cardiac tonus is not the–less–the–better but contributes to adjusting presystolic myofiber lengthes (heterometric autoregulation) [20]. Increased stiffness at hypothermia [3] requires higher end–diastolic LVP (as in Tab. 2 and Fig. 3) to stretch the pre–systolic ventricle properly. Higher P_∞ (also seen in the data) may be adequate in such situation to prevent the ventricle from becoming overstretched. If P_∞ excels a certain

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limit, it renders relaxation impossible, not incomplete. One may reasonably construe such situation also as a kind of diastolic failure, but should keep in mind that diastolic filling time is unrelated to this condition.

Incomplete relaxation can be induced by pacing at normothermia [11] and at hypothermia [3]. This is never due to the mere shortage of diastolic filling time in these steady–state observations. The time constant(s) of isochoric LVP decay increase coevally, τ_∞ of model Lg4 is the most sensitive one. Presently calculated residual contraction emerges and rises over ten per cent. Incomplete or impaired relaxation also occurs at deep hypothermia (Tab. 2, Fig. 4). All aforesaid holds in median; the eighty per cent ranges of all lusitropic parameters (Tab. 2) reveal that many specimens (as those in Fig. 2) settle to steady–state with enhanced lusitropy even at deep hypothermia. These hearts may be safe from diastolic failure, but the study's protocol keeps no track on such individual information.

We suspect re–excitation of cardiac myofibrils due to a transient intracellular Ca^{2+}–overload as the cause of incomplete relaxation. This conjecture is motivated by discrete exalterations in the isochoric LVP fall (Fig. 4) and sudden drops in the goodness–of–fit of the relaxation model Lg4, together with unusually high τ_∞ and low P_∞ estimates. Cytosolic Ca^{2+} encumbrance evokes aftercontraction of relaxing cardiac myofibers [21], hypothermia increases the Ca^{2+} sensibility [1], pacing increases myocytal Ca^{2+} load and turnover, and the present specimens work with double free Ca^{2+} concentration (no Ca^{2+} binding protein in the perfusate). Consequently, the Ca^{2+} transient occurring intracytal by unbinding Ca^{2+} from troponine may cause aftercontraction, especially if sodium or ATP dependent Ca^{2+} transporter are depressed by hypothermia.

On the other hand, preserved complete relaxation at hypothermia, as seen in many specimens, may be explained in terms of myocardial autoregulation in isolated hearts. Hypothermic bradycardia at constant cardiac flow activates positive inotropic heterometric autoregulation by larger filling volumes. Long–standing experience speaks for inotropy and lusitropy being positively correlated in the presence of regulatory pathways, as in isolated hearts. Protocol A2 confirmes such autoregulation after temperature changes.

4.3 Limitations

Pertinent lusitropic parameters are estimated by the relaxation model Lg4 which is known to leave a small systematic error, resembling an oscillation [5]. LVP always falls a little bit faster at its peak decay velocity and a little bit slower at the terminal isochoric phase (hardly seen in Fig. 1), as predicted by Lg4. We refrain from a correction, because (a.) we draw no conclusions that may depend on highly valide values; (b.) we assume that this is a mechanical vibration of the hanging heart, induced by blood momentum, that is unrelated to ventricular relaxation and does not bias the fitted regression parameters. The study is based on data from two small animal species and reveals some differences between them; other species may show deviant behavior. Impaired isochoric LVP decay is stated from inspection and obtrusive τ_∞ and P_∞ estimates; a pattern recognition algorithm should be implemented to identify and classify

typical impairments in LVP decay curves. Drift of zero calibration must be taken into account if small pressure measurements are interpreted or mutually compared with each other. The experimental protocols are designed to cover a wide range of condition within the limited operational life of the specimens; smaller steps or continuous change of experimental parameters are desired at the highly sensible conditions. Considerable inter–individual variability of the steady–state lusitropy at hypothermia demands a cohorte survey to clarify whether impaired lusitropy indicates impending failure of the individual heart. Surmised myocytal causes of impaired ventricular relaxation remain open to cell physiological investigation.

5 Conclusions

We conclude from the study in isolated working guinea pig and rat hearts:

a) There is virtually no residual contraction (above ventricular tonus) at normothermia and intrinsic spontaneous sinus rhythm of isolated working hearts, *viz.* ventricular relaxation is complete.

b) Mild hypothermia with intrinsic bradycardia signficantly decreases the left–ventricular peak pressure fall velocity and significantly increases, in median, the initial and the asymptotic time constant of the still logistic–shaped isochoric pressure fall. Relaxation remains fairly complete, with residual contraction below three per cent.

c) Deep hypothermia or pacing at mild hypothermia increases the logistic time constants of the isochoric left–ventricular pressure decay further, and induces considerable residual contraction at the onset of the subsequent systole (incomplete relaxation).

d) Severe impairment of early–diastolic (isochoric) relaxation appears as lifted concavity of the left–ventricular pressure fall and is usually a sign of imminent heart failure.

Statement of ethical publishing

The author state that he adhere to the statement of ethical publishing of the the International Cardiovascular Forum Journal [22].

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Conflict of interests

The author declares no conflicts of interest.

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