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RESEARCH ARTICLE

Is cold acclimation of benefit to hibernating rodents?

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SUMMARY

The thermal challenge associated with cold acclimation (CA) and hibernation requires effective cardio-respiratory function over a large range of temperatures. We examined the impact of acute cooling in a cold-naïve hibernator to quantify the presumed improvement in cardio-respiratory dysfunction triggered by CA, and estimate the role of the autonomic nervous system in optimising cardiac and respiratory function. Golden hamsters (*Mesocricetus auratus*) were held at a 12 h:12 h light:dark photoperiod and room temperature (21°C euthermic control) or exposed to simulated onset of winter in an environmental chamber, by progression to 1 h:23 h light:dark and 4°C over 4 weeks. *In vivo* acute cooling (core temperature T_b=25°C) in euthermic controls led to a hypotension and bradycardia, but preserved cardiac output. CA induced a hypertension at normothermia (T_b=37°C) but on cooling led to decreases in diastolic pressure below euthermic controls and a decrease in cardiac output, despite an increase in left ventricular conductance. Power spectral analysis of heart rate variability suggested a decline in vagal tone on cooling euthermic hamsters (T_b=25°C). Following CA, vagal tone was increased at T_b=37°C, but declined more quickly on cooling (T_b=25°C) to preserve vagal tone at levels similar to euthermic controls at T_b=37°C. For the isolated heart, CA led to concentric hypertrophy with decreased end-diastolic volume, but with no change in intrinsic heart rate at either 37 or 25°C. Mechanical impairment was noted at 37°C following CA, with peak developed pressure decreased by 50% and peak rate-pressure product decreased by 65%; this difference was preserved at 25°C. For euthermic hearts, coronary flow showed thermal sensitivity, decreasing by 65% on cooling (T=25°C). By contrast, CA hearts had low coronary flow compared with euthermic controls, but with a loss of thermal sensitivity. Together, these observations suggest that CA induced a functional impairment in the myocardium that limits performance of the cardiovascular system at euthermia, despite increased autonomic input to preserve cardiac function. On acute cooling this autonomic control was lost and cardiac performance declined further than for cold-naïve hamsters, suggesting that CA may compromise elements of cardiovascular function to facilitate preservation of those more critical for subsequent rewarming.

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INTRODUCTION

Thermal stress, associated with the transition towards winter, presents significant thermodynamic challenges to rodents, attributable to the high surface area to volume ratio promoting increased heat loss. Rodents adopt one of two strategies to overcome this challenge. Non-hibernators defend their thermal setpoint of core temperature by enhancing both insulation and thermogenesis. Hibernators, by contrast, adopt a new and lower thermal setpoint, thereby reducing the thermal gradient between the core and the environment, and thus energy expenditure. Effective hibernation, however, relies upon preserving sufficient fuel stores to initiate thermogenesis during the rewarming from torpor. Furthermore, mortality rates during hibernation may be high, suggesting that adopting a lower thermal setpoint requires significant physiological adaptation (Hoeck, 1987; Sherman and Morton, 1984). Critical to this transition is a functional cardiovascular system, with sufficient dynamic range to maintain cardiac output at different temperatures during the transition between torpor and normothermia to ensure adequate oxygen delivery. Although hibernation is regarded as an adaptation for improved function at low temperatures, it may be a strategy to depress biochemical reactions with cold to enable subsequent restoration of function on arousal (Van Breukelen and Martin, 2002a).

Functional adaptation to a prolonged cold challenge, cold acclimation (CA), is a transition on the spectrum towards hibernation and is likely a composite response involving multiple factors and tissues, including autonomic control of the cardiovascular and respiratory systems, and their integration. Selective alteration to skeletal muscle, including disuse atrophy in the tibialis anterior muscle in golden hamsters (*Mesocricetus auratus*), together with preserved capillary to fibre ratio, has previously been noted (Deveci and Egginton, 2002). Control of vascular tone through adrenergic and purinergic vasoconstrictors is relatively preserved in hibernators across a range of temperatures (Eliassen and Helle, 1975; Miller et al., 1986; Saito et al., 2002). However, preliminary experiments investigating the transition between normothermia and hypothermia

indicate that depressed blood pressure, heart rate (f_H) and ventilation rate (f_V) were similar when cold-acclimated (CA) and euthermic hamsters were challenged with acute cooling (Deveci and Egginton, 2007), suggesting good preservation of cardiovascular control despite hypothermic temperatures (25°C). Indeed, at a molecular level, hibernators may not demonstrate specific adaptations for low temperature, as rates of transcription show no seasonal compensation for a lower thermal setpoint (Van Breukelen and Martin, 2002b). Furthermore, the rates of oxidative phosphorylation for muscle mitochondria were unchanged following transition into hibernation for the thirteen-lined ground squirrel (*Spermophilus* [*Ictdiomys*] *tridecemlineatus*) (Muleme et al., 2006).

The left ventricle of the hibernating Richardsons' ground squirrel (*Urocitellus richardsonii*) was significantly more powerful (greater maximum developed pressure generated against a balloon) and more rapidly contracting (increased peak contractile rate, dP/dt) when acutely cooled than that of non-hibernators such as the rat (Caprette and Senturia, 1984). In part, this may result from altered cardiac excitation–contraction coupling, manifested as increased Ca^{2+} reuptake into the sarcoplasmic reticulum (Liu et al., 1997) and increased cardiac myofilament Ca^{2+} sensitivity, at low temperatures (Liu et al., 1993). Indeed, Siberian hamsters showed increased Ca^{2+} transient amplitude and increased Ca^{2+} flux in cardiomyocytes following exposure to short photoperiod (Dibb et al., 2005), consistent with preserved contractile performance during seasonal hypothermia. Furthermore, CA in the ground squirrel resulted in higher threshold potentials from L-type Ca^{2+} channels and higher rates of depolarisation for isolated cardiomyocytes, indicative of preserved Ca^{2+} homeostasis at low temperatures. Interestingly, CA did not prevent the formation of 'j' waves within the echocardiogram (ECG) or atrio-ventricular block for CA hamsters subjected to extreme cooling, yet both were absent during actual hibernation to similar core temperatures (Miyazawa et al., 2008), implying that for the heart, CA and hibernation are physiologically distinct. However, CA will precede hibernation and may be viewed as essential preparation for hibernation.

We have recently demonstrated that for non-hibernators (*Rattus norvegicus*), CA represents a partial compensation characterized by altered sympathovagal balance and poor left ventricle function (Hauton et al., 2011). Yet no such study has been undertaken in hibernators. We therefore investigated which of the physiological adaptations to cold exposure are critical to this strategy. We exploited the anaesthetized, instrumented golden hamster (*Mesocricetus auratus*) and Langendorff-perfused heart preparation to examine the integrative (extrinsic) and phenotypic (intrinsic) responses, respectively, comparing the degree of cardiovascular compensation in euthermic control (cold-naïve) and CA hamsters at normothermia (T_b=37°C) and when exposed to acute cooling (hypothermia; T_b=25°C).

MATERIALS AND METHODS

Animals

Male golden hamsters (*Mesocricetus auratus* Waterhouse 1839) (Charles River Laboratories, Margate, UK) of ~100 g (*in vivo* studies) or ~120 g body mass (*ex vivo* studies) at experimentation were randomly assigned to control and CA groups. All reagents were supplied by BDH (Poole, UK) or Sigma-Aldrich (Poole, UK) unless otherwise stated. Ventricular balloons were constructed 'in house' using Saran Wrap® polythene film.

Animals were maintained in accordance with the UK Home Office, Animal Scientific Procedures Act (1986) and experiments were approved by the University of Birmingham Ethical Review Committee. Animals were housed at 22°C under a 12 h:12 h light:dark photoperiod with *ad libitum* access to food and water. Animals were acclimated to cold as previously outlined (Deveci and Egginton, 2007; Hauton et al., 2009). Briefly, hamsters (~80 g body mass) were housed singly in an environmental chamber and had *ad libitum* access to both food and water. The chamber was cooled from 21 to 4°C over a period of 4 weeks, with day length reduced from 12 h:12 h to 1 h:23 h light:dark over the same period. These conditions were maintained for 4–6 days, and then animals were entered into experiments.

Surgical preparation

Animals were initially anaesthetised with isofluorane (Fluothane, ICI; 3–4% in oxygen) while the left jugular vein was cannulated, patency being maintained with heparinised saline (20 U ml^{-1}), then replaced with an infusion of α-chloralose and urethane (165 mg and 2.5 g in 10 ml saline i.v.) (Sabharwal et al., 2004a). A tracheal cannula was inserted to maintain a patent airway and to attach a spirometer. The left femoral vein and artery were cannulated and 3-lead ECG electrodes were inserted into the chest wall and paws. After surgery, animals were transferred to a thermostatically controlled stage (Sabharwal et al., 2004b) and physiological saline solution (0.9% NaCl) was given by continuous infusion (1.25 ml h^{-1}). A J-type thermistor was inserted deep into the oesophagus to measure body temperature (T_b; °C) throughout the experimental protocol.

In vivo cardiac performance

In one group of animals (Table 1) were allowed to stabilise for 90 min, and then recordings were taken for 5 min at T_b=~37°C and 25°C. After cooling to the low T_b (at a rate of ~1°C every 10 min) the animals were allowed to stabilise for 30 min and further readings taken, before the animal was rewarmed to T_b=37°C. At T_b=~37°C and 25°C some animals received radiolabelled microspheres (^{113}Sn, ^{46}Sc; DuPont NEN, Mechelen, Belgium) delivered into the left ventricle *via* a right carotid arterial cannula to estimate myocardial blood flow. Reference flow was obtained by sampling from one brachial artery using a precision withdrawal pump (Hauton et al., 2011).

In other animals, the baroreflex control of heart rate was assessed by pharmacological manipulation of mean arterial blood pressure (MABP), again at T_b=~37°C and 25°C. MABP was increased by giving a bolus injection *via* the femoral vein of ~10 μg phenylephrine hydrochloride, and decreased using ~10 μg sodium nitroprusside, at a dose of 1 mg kg^{-1}, given in random order. ECG signals and MABP were recorded during and after the bolus injections; all variables were stable for 5 min prior to each bolus. The influence of vagal tonus on cardiac cycle duration was examined using high-resolution ECG recordings in animals with intact vagi. ECG signals were amplified and recorded using a bioamplifier and Chart software (ADInstruments, Oxford, UK). Optimal settings for well-defined R waves at 37°C were: range 1 mV, high pass 0.3 Hz, low pass 50 Hz, sampling rate 1 kHz. The trace was used to calculate f_H (beats min^{-1}) and R–R intervals (ms), as well as the relative duration of the cardiac cycle components (the latter sampled at 10 kHz). Cardiovascular and respiratory measurements were collected as previously detailed (Hauton et al., 2011).

Ex vivo cardiac performance

Hamsters were prepared surgically, as outlined previously for rats (Hauton et al., 2001). Briefly, anaesthesia was induced with isofluorane (4% v/v in oxygen) and following thoracotomy, hearts,

Table 1. The impact of acute cold (hypothermia) or cold acclimation on *in vivo* physiological parameters in the anaesthetised golden hamster (*Mesocricetus auratus*)

	Normothermia		Hypothermia	
	Euthermic (*N*=12)	Cold acclimated (*N*=11)	Euthermic (*N*=9)	Cold acclimated (*N*=7)
Body mass (g)	136±1	130±3	146±5	136±4
MABP (mmHg)	107±3	116±3^{+}	89±3*	68±3$^{*,+}$
Systolic pressure (mmHg)	138±3	150±5^{+}	128±5	113±7*
Diastolic pressure (mmHg)	91±3	99±3	70±3*	45±2$^{*,+}$
Pulse pressure (mmHg)	48±3	51±3	58±4*	67±6*
Cardiac output (ml min^{-1})	60±6	65±10	63±8	35±7$^{*,+}$
LV blood flow (ml min^{-1} 100 g^{-1})	301±37	227±27	177±16*	237±54
LV conductance (ml min^{-1} 100 g^{-1} mmHg^{-1})	2.88±0.32	1.99±0.28^{+}	1.97±0.14*	3.49±0.75$^{*,+}$
Heart rate (beats min^{-1})	369±7	406±4^{+++}	213±9*	168±13$^{*,+}$
Stroke volume (ml)	0.16±0.01	0.16±0.02	0.29±0.03*	0.22±0.04
Cardiac index (ml min^{-1} 100 g^{-1})	89±6	77±8	109±8	86±3$^{*,+}$
Cardiac minute work (J kg^{-1})	527±74	578±57	655±86	305±63$^{*,+}$
Stroke work (J kg^{-1})	1.43±0.23	1.42±0.14	3.01±0.32*	1.90±0.36^{+}
TPR (mmHg min^{-1} ml^{-1})	2.24±0.36	2.03±0.32	1.60±0.17	2.34±0.44

MABP, mean arterial blood pressure; LV, left ventricle; TPR, total peripheral resistance.
Data represent means ± s.e.m. Statistical significance indicated as: effect of acute cooling, $^{*}P$<0.05; effect of cold acclimation, ^{+}P<0.05, ^{+++}P<0.001.

excised with lungs and thymus *in situ*, were immersed in ice-cold Krebs–Hensleit medium, which contained the following (in mmol l^{-1}): NaCl (120), KCl (4.8), $NaHCO_3$ (25), $MgSO_4 \cdot 7H_2O$ (1.2), KH_2PO_4 (1.2), glucose (10) and $CaCl_2$ (1.3), gassed with oxygen/CO_2 (95:5). The aorta was cannulated (16 G cannula), and then perfused in retrograde fashion by the method of Langendorff, with modifications (Hauton and Caldwell, 2012). Hearts were maintained at either 25 or 37°C and perfused at a constant pressure (100 cm H_2O). Starting temperature was assigned at random to minimise any impact of 'programming' changes to cardiac performance with temperature. Ventricle performance was measured with a fluid-filled balloon, attached to a fine plastic catheter, connected to a pressure transducer and a graduated syringe (0–1000 µl, Hamilton, NV, USA) inserted into the left ventricle.

Ventricular performance

Ventricular performance was estimated as outlined previously (Hauton and Ousley, 2009). All hearts were unpaced, with developed pressure (mmHg) measured following isovolumic contraction and recorded to a computer using a digital interface (ADInstruments). The initial balloon volume was adjusted until measured end-diastolic pressure was 0 mmHg and the systolic pressure was recorded. Balloon volume was increased in incremental steps (25 µl), and developed pressures were recorded continuously until end-diastolic pressure was constant before further subsequent increases in balloon volume. Increases in balloon volume continued until the systolic pressure exceeded 200 mmHg. Coronary flow was estimated from timed collections of a known volume of perfusate freely draining from the apex of the heart and expressed as volume/unit time/unit mass of cardiac tissue (typical volume=2.0 ml). Ventricular performance was calculated off-line, as detailed previously (Hauton and Ousley, 2009).

Capillary density and fibre size

Tissues were blotted and weighed. The lower portion (apex) of the heart was taken and the left ventricle (LV) free wall was dissected and mounted onto cork disks, as detailed previously (Hauton and Ousley, 2009). Cryostat sections (10 µm) were fixed onto glass slides and capillaries were visualized using an FITC-labelled lectin method (Williams et al., 2006). Capillary density was quantified from digital images (magnification ×200) by estimating capillary number in transverse-sectioned regions of the LV free wall of known area (Hauton and Ousley, 2009). Both capillary density and muscle fibre number were estimated using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Data analysis

Heart rate variability was analysed with the HRV extension software (ADInstruments) using 512 consecutive beats (R waves), a Hann window to remove noise interference to the power spectrum, and a 2/3 overlap to minimise spectral leakage due to baseline instability. The component bandwidths were set as very low frequency (VLF)=0–0.04 Hz, low frequency (LF)=0.04–1 Hz and high frequency (HF)=1–3 Hz (Kuwahara et al., 1994; Sabharwal et al., 2004a). A fast Fourier transform algorithm was used to calculate the power spectrum, and the boundaries for ectopic beats and artefacts were defined using Poincare plots.

ECG and blood pressure traces, obtained during the pharmacological manipulation of MABP, were used to analyse the baroreflex control of f_H. Systolic and diastolic readings were used to calculate MABP, the timing of systolic pressure was used to confirm R waves in the ECG trace, and the average of the next three consecutive R–R intervals was used to determine instantaneous f_H for each temperature. f_H was normalised by converting values into a change from baseline (%), which was then plotted against MABP (DeltaGraph, Rock Software, Salt Lake City, UT, USA). A sigmoid logistic function equation (Kent et al., 1972) was used to fit a baroreflex curve to the plotted data at T_b=37°C and 25°C for each animal:

$$f_H = A / \{1 + \exp [B (\text{MABP} - C)]\} + D, \quad (1)$$

where A is the response range for f_H, B is the gain, C is the midpoint pressure at the midrange of the curve and D is the minimum response for f_H. From these parameters, other parameters such as the maximum response, saturation pressure for MABP (P_{sat}), threshold pressure for MABP (P_{thr}), maximal gain and operating range can be calculated (Miki et al., 2003; Sabharwal et al., 2004a).

All data are expressed as means ± s.e.m., unless otherwise stated. Statistical evaluation was performed using factorial and repeated-

measures ANOVA, as appropriate, with Fisher's protected least significant difference test to estimate the *post hoc* significance (StatView, SAS Institute, Cary, NC, USA). Statistical significance was accepted at $P<0.05$.

RESULTS

Throughout the cooling cycle all animals remained active and healthy, with no hamsters commencing hibernation. No differences in body mass were noted at the end of 4 weeks CA (n.s.; Table 1). However, cardiac mass relative to body mass was significantly increased following CA (euthermic=0.465±0.026% *versus* CA=0.602±0.48; $P<0.05$).

Effect of cold acclimation at normothermia

CA hamsters were moderately hypertensive ($P<0.05$; Table 1) with preserved diastolic pressures and elevated peak systolic pressure ($P<0.05$; Table 1). Both cardiac output and LV blood flow were maintained (n.s.; Table 1), but LV conductance was decreased following CA ($P<0.05$; Table 1). CA also induced a tachycardia (10%, $P<0.05$; Fig. 1), but stroke volume of the heart was unchanged following CA (n.s.; Table 1). Cardiac index, minute work and stroke work were all unchanged following CA (n.s. for all; Table 1), and CA had no effect on total peripheral resistance (n.s.; Table 1).

Effects of acute hypothermia on the cardiovascular system

Hypothermia decreased mean arterial blood pressure for both euthermic and CA hamsters ($P<0.05$ for both; Fig. 1), but for CA this was decreased further than for euthermic hamsters ($P<0.05$; Fig. 1). As systolic pressure was preserved for euthermic hamsters on cooling, this was a direct consequence of decreased diastolic pressures ($P<0.05$; Table 1). For CA hamsters, both systolic and diastolic pressures significantly decreased ($P<0.05$ for both; Table 1), yielding a significant reduction in MABP on cooling ($P<0.05$ compared with euthermic controls; Fig. 1), although in compensation pulse pressure was increased in both euthermic and CA hamsters ($P<0.05$ for both; Table 1). Interestingly, for both euthermic and CA hamsters, total peripheral resistance was preserved on acute cooling (n.s. for both; Table 1).

For euthermic hamsters, cardiac output was maintained following hypothermia, and this was sustained through an increase in stroke volume ($P<0.05$; Table 1) to compensate for decreased f_H. For CA hamsters, cardiac output fell by 45% ($P<0.05$; Table 1), although in this case bradycardia was not compensated by increased stroke volume. Acute cooling decreased LV blood flow for euthermic hamsters ($P<0.05$; Table 1), but this was maintained in CA hamsters (n.s.; Table 1). Conductance of the LV was also decreased for euthermic hamsters ($P<0.05$; Table 1), but was increased 75% beyond normothermic levels for CA

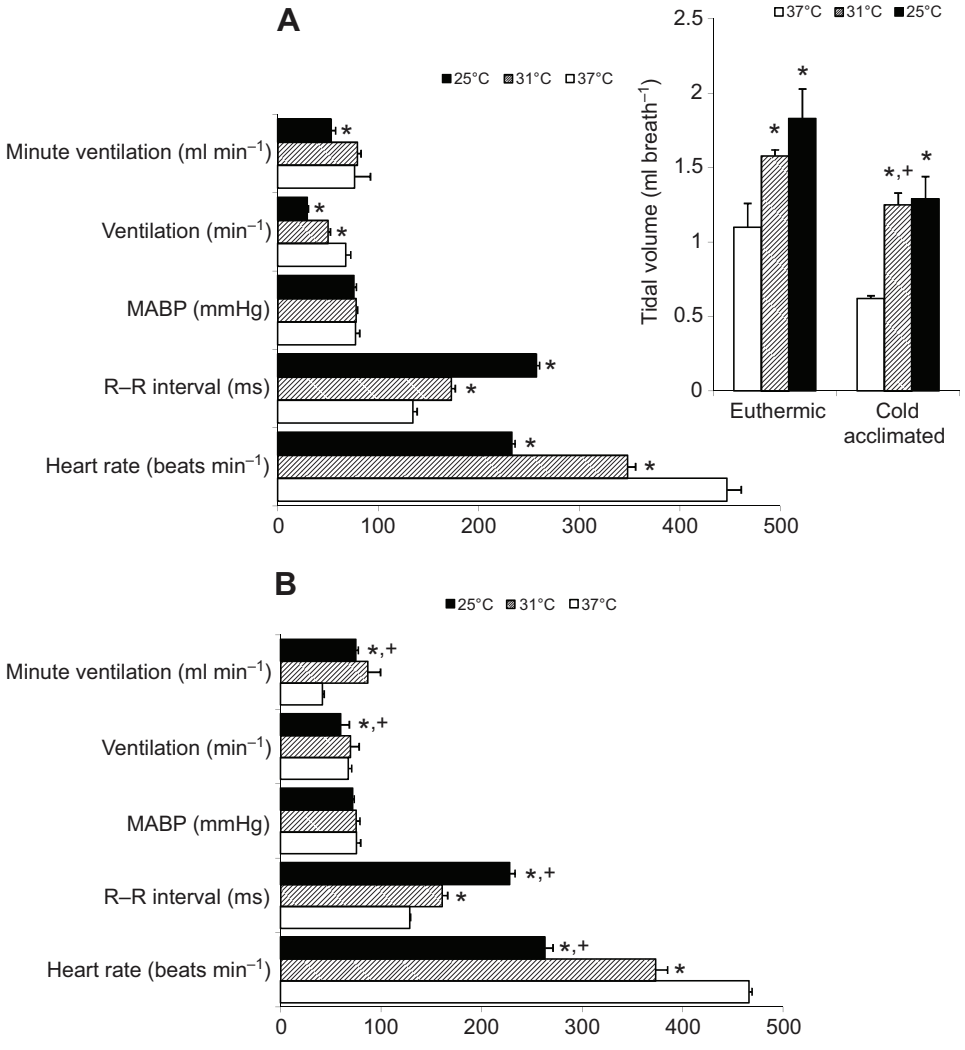

Fig. 1. Cardio-respiratory parameters measured in (A) euthermic and (B) cold-acclimated (CA) hamsters. Anesthetised animals were instrumented to measure blood pressure, ventilation and echocardiogram (ECG) parameters during acute cooling from T_b=37°C to T_b=25°C. Inset shows increase in tidal volume with acute cooling. Data represent means ± s.e.m. for N=4 euthermic and CA hamsters. Statistical significance indicated as: effects of acute cooling, $^*P<0.05$; effects of cold acclimation, $^+P<0.05$.

Table 2. The impact of acute cold (hypothermia) or cold acclimation on heart rate variability in the anaesthetised golden hamster (*Mesocricetus auratus*)

		Core temperature		
		37°C	31°C	25°C
High frequency peak (Hz)	Euthermic	1.066±0.75	0.818±0.029*	0.389±0.098*
	Cold acclimated	1.797±0.349^{+}	1.132±0.160^{+}	1.026±0.144^{+}
Low frequency power (ms^2)	Euthermic	0.060±0.019	0.243±0.228	0.403±0.220
	Cold acclimated	0.137±0.070	0.071±0.029	0.269±0.053
High frequency power (ms^2)	Euthermic	0.843±0.232	1.598±0.262	2.599±0.545*
	Cold acclimated	0.225±0.079	0.442±0.170^{+}	1.922±0.893
Very low frequency power (ms^2)	Euthermic	0.102±0.042	0.300±0.096	0.496±0.301
	Cold acclimated	0.090±0.027	0.033±0.003	0.089±0.067
Low frequency/High frequency ratio	Euthermic	0.134±0.0.61	0.187±0.176	0.197±0.118
	Cold acclimated	0.587±0.135^{+}	0.154±0.014*	0.178±0.049*
s.d. of the R–R interval	Euthermic	1.128±0.067	1.646±0.127*	2.662±0.387*
	Cold acclimated	0.778±0.110	1.065±0.232	1.770±0.247*

Data represent means ± s.e.m. (euthermic hamsters N=5; cold-acclimated hamsters N=5). Statistical significance indicated as: effect of acute cooling, *P<0.05; effect of cold acclimation, ^{+}P<0.05.

hamsters (P<0.05; Table 1). Acute cooling led to bradycardia in all animals (P<0.05; Figs 1, 2) with a corresponding increase in R–R interval (P<0.05; Fig. 2), but CA hamsters showed a further 20% decrease (P<0.05; Fig. 1). Cardiac index and minute work were unaltered by acute cooling in euthermic hamsters (n.s.; Table 1), while both were decreased in CA hamsters (P<0.05 for both; Table 1). Cardiac stroke work doubled on cooling for euthermic hamsters (P<0.05; Table 1), but was maintained for CA hamsters (n.s.; Table 1).

Effects of acute hypothermia on ventilation

For euthermic hamsters, f_V was thermally sensitive, showing a decline on cooling (P<0.05; Fig. 1). However, tidal volume showed a temperature-dependent response to acute cooling, being increased at 31°C (P<0.05; Fig. 1, inset) and further increased at 25°C (P<0.05; Fig. 1, inset). For CA hamsters this decline in f_V was less pronounced, being preserved at 31°C and decreased by only 15% on further cooling to 25°C (P<0.05; Fig. 1). For euthermic hamsters, minute ventilation was well maintained on cooling despite decreased f_V, through greater respiratory volume, increasing at 31°C (P<0.05, Fig. 1) with further increases at 25°C (P<0.05; Fig. 1). For CA hamsters, minute ventilation doubled on cooling (P<0.05; Fig. 1), and this was sustained through increased depth of ventilation (P<0.05 for both 31 and 25°C; Fig. 1, inset) and by maintaining f_V.

Heart rate variability

Upon cooling, peak frequencies measured by power spectral analysis declined, as evident from the pronounced bradycardia seen in euthermic hamsters (P<0.05 at 31°C, P<0.05 at 25°C; Table 2). The standard deviation of the R–R interval was thermally sensitive, showing increases with decreasing temperature, suggesting increased beat-to-beat variability (P<0.05 at 25°C; Table 2). For CA hamsters, peak frequencies were higher than for euthermic hamsters (P<0.05; Table 2) and were preserved despite acute cooling, remaining elevated above similarly cooled euthermic hamsters (P<0.05; Table 2). Both low frequency and very low frequency power were unchanged by either cold acclimation or acute cooling (n.s.; Table 2). CA hamsters had a greater LF/HF ratio (an index of sympathovagal balance) at 37°C (P<0.05; Table 2); however, on acute cooling this declined to levels similar to those for euthermic hamsters (n.s.; Table 2).

Effects of rewarming from 25°C

For euthermic hamsters, acute cooling to 25°C did not alter minute ventilation but resulted in a significant decrease in f_V (P<0.05; Fig. 2) coupled with an increase in tidal volume (P<0.05; Fig. 2), which was preserved on rewarming to 37°C. MABP was unchanged by cooling or rewarming; however, cooling led to a 50% decrease in f_H (P<0.05) that was only partially restored on rewarming to 37°C (Fig. 1).

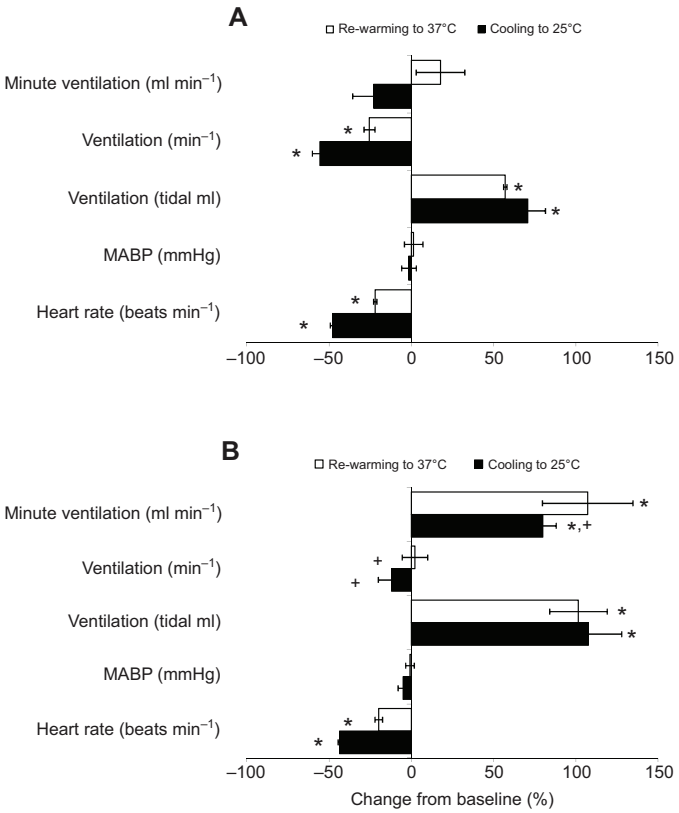

Fig. 2. Change in cardio-respiratory parameters on acute cooling to T_b=25°C and subsequent rewarming to T_b=37°C for (A) euthermic and (B) CA hamsters. Data represent means ± s.e.m. for N=4 euthermic and CA hamsters. Statistical significance indicated as: effects of acute cooling and rewarming, *P<0.05; effects of cold acclimation, ^{+}P<0.05.

For CA hamsters, minute ventilation was doubled on cooling to 25°C (P<0.05; Fig. 2) and this was preserved despite rewarming (P<0.05; Fig. 2B). Furthermore, unlike euthermic controls, this increased ventilation was accounted for entirely by increased tidal volume on acute cooling (P<0.05; Fig. 2) and with subsequent rewarming (P<0.05; Fig. 2B), with rate of ventilation unaffected (P<0.05 *versus* euthermic for both cooling and rewarming). For CA hamsters, f_H declined on acute cooling (P<0.05; Fig. 2) and was only partially restored on rewarming (P<0.05; Fig. 2), being unchanged from similarly treated euthermic hamsters (n.s. for both; Fig. 2).

Intrinsic myocardial performance

The intrinsic (unpaced) rate of perfused hamster hearts was significantly decreased in CA hamsters (P<0.05; Fig. 3A), yet both euthermic and CA hamster hearts showed thermal sensitivity, declining with decreasing temperature (P<0.05 for both; Fig. 3A). Arrhenius transformation of f_H indicated that the degree of thermal sensitivity was unchanged following CA (n.s.; Fig. 3, inset). For euthermic hearts acute cooling halved the peak rate of pressure development (dP/dt) (P<0.05; Fig. 3B). However, estimated peak dP/dt was significantly decreased following CA (P<0.05; Fig. 3B), but thermal sensitivity was lost (n.s.; Fig. 3B).

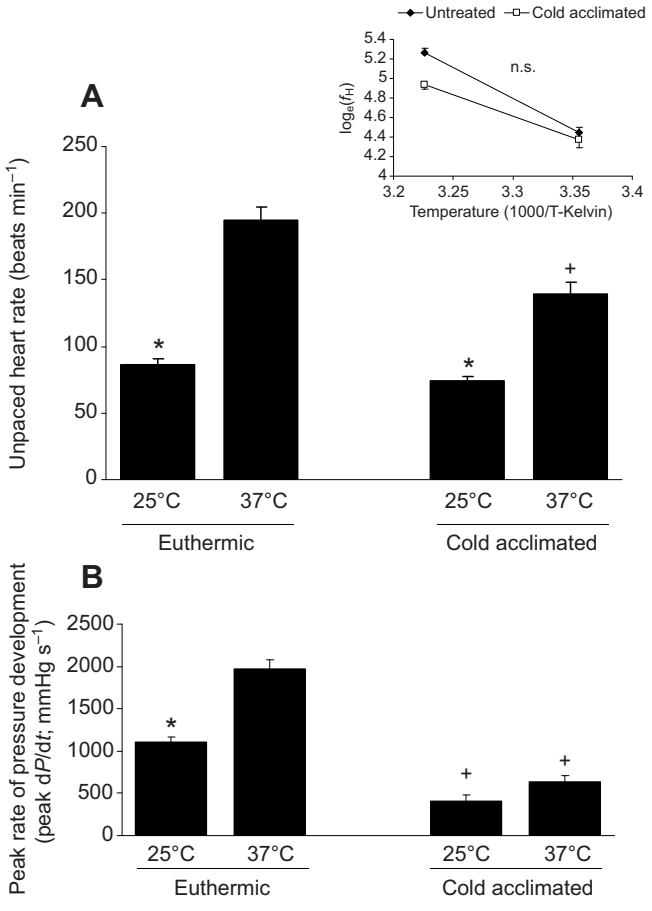

Fig. 3. Effects of acute cooling on (A) unpaced heart rate (f_H) and (B) rate of pressure development (+dP/dt) for the isolated Langendorff-perfused hamster heart, isolated from euthermic and CA hamsters. Hearts were acutely cooled from 37 to 25°C. Inset: Arrhenius transformation of f_H to indicate preservation of thermal sensitivity following CA. Data represent means ± s.e.m. for N=6 euthermic and CA hamsters. Statistical significance indicated as: effects of acute cooling, *P<0.05; effects of cold acclimation, ^+P<0.05.

For both euthermic and CA hamsters, diastolic performance was unchanged by acute cooling (n.s.; Fig. 4), with diastolic stiffness (Δdiastolic pressure/unit volume change) in euthermic (Fig. 4A) and CA hamsters (Fig. 4B) unchanged by acute cooling. Estimates of LV end-diastolic volume (EDV) decreased in CA hamsters (euthermic=156±5 µl *versus* CA=131±7 µl; P<0.05), suggesting a concentric hypertrophy of the heart on cold acclimation. For euthermic hamster hearts, acute cooling had no effect on peak developed pressure (n.s.; Fig. 5A), while CA decreased peak developed pressure at both 37 and 25°C (P<0.05 for both; Fig. 3B). Interestingly, acute cooling altered ventricular dynamics, inducing a right shift in the performance characteristics (greatest pressure at increased balloon volume; Fig. 5A) in euthermic hearts. By contrast, for CA hearts, acute cooling induced a left shift in the performance characteristics (greatest pressure at lower volume; Fig. 5B). This was similarly shown in calculated rate-pressure products (RPP). For euthermic hearts, acute cooling decreased RPP (P<0.05; Fig. 5C) and displaced the peak RPP towards a larger balloon volume. CA significantly decreased RPP in hearts (P<0.05 for both 25 and 37°C; Fig. 5D), yet acute cooling induced a left shift in the relationship with peak RPP occurring at lower balloon volumes (Fig. 5D).

Estimates of coronary flow for euthermic hearts indicated a decrease with increasing balloon volume, varying with increasing ventricular wall stress (Fig. 6A). However, on acute cooling, coronary flow was significantly decreased at all balloon volumes, with differences declining with increasing balloon volume (P<0.05 for balloon volumes up to 175 µl). For CA hearts perfused at 37°C, coronary flow was significantly decreased at all balloon volumes, compared with euthermic hearts (P<0.05 for all; Fig. 6B). Yet for CA hearts thermal sensitivity was lost, coronary flow being preserved on acute cooling to 25°C (n.s.; Fig. 6B).

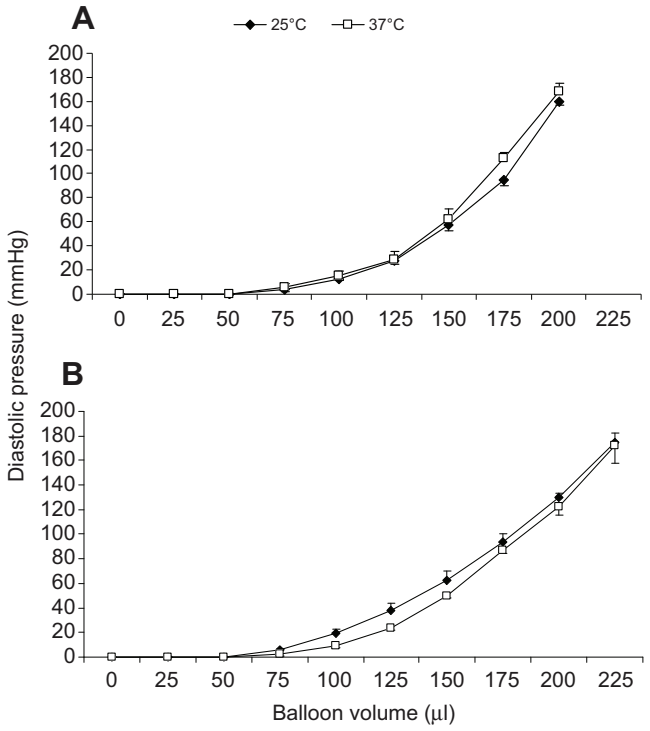

Fig. 4. Diastolic performance of isolated Langendorff-perfused hamster hearts, isolated from (A) euthermic and (B) CA hamsters, acutely cooled from 37 to 25°C. Data represent means ± s.e.m. for N=6 euthermic and CA hamsters.

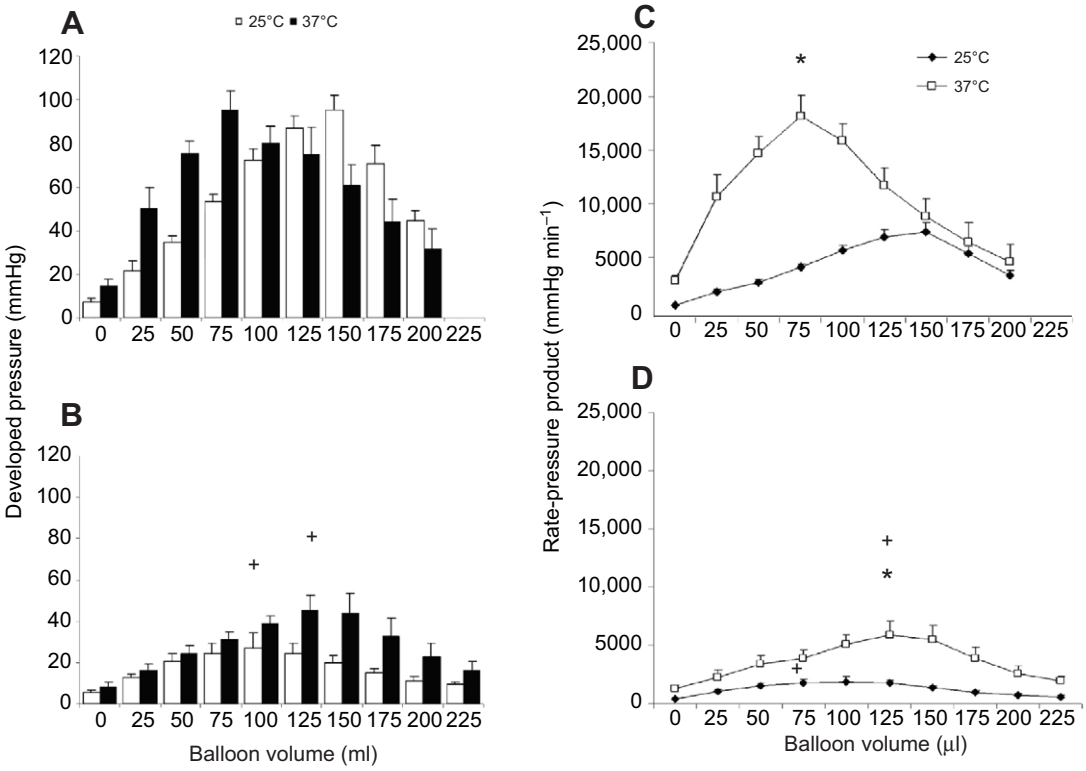

Fig. 5. Effect of acute cooling on developed pressure of isolated Langendorff-perfused hamster hearts, isolated from (A) euthermic and (B) CA hamsters, and rate-pressure product for (C) euthermic and (D) CA hamsters. Hearts were acutely cooled from 37 to 25°C. Data represent means ± s.e.m. for N=6 euthermic and CA hamsters. Statistical significance indicated as: effects of acute cooling, *P<0.05; effects of cold acclimation, ^{+}P<0.05.

Cardiac morphology

An increase in capillary density for papillary muscle (79%), endocardium (51%) and epicardium (40%) was seen following 4 weeks CA (P<0.05 for all; Fig. 7). When CA was extended to 8 weeks this increased capillarity was maintained compared with euthermic hearts, but to a lesser extent (papillary muscle 22%, endocardium 30%, epicardium 39%, P<0.05 for all; Fig. 7), presumably as a result of cardiac hypertrophy.

DISCUSSION

We demonstrate that for the hamster, CA increased sympathetic drive in an attempt to augment cardiac output. However, the physiological response of the myocardium to the challenge of CA, i.e. hypertrophy, led to marked reduction in cardiac performance at euthermia, and as a consequence mechanical performance was compromised to a far greater extent on cooling. The additional sympathetic drive alone was not sufficient to sustain cardiac performance, partly because this drive was not maintained on cooling. Paradoxically, therefore, the hamster may be particularly sensitive to severe reductions in environmental temperature during preparations for over-winter survival.

Physiological response to acute cooling

Acute hypothermia-induced hypotension for euthermic hamsters was a consequence of cooling-induced bradycardia and decreased diastolic pressure. However, systolic function was better maintained in cold-naïve hamsters on cooling than in CA hamsters, improving relative maintenance of MABP. Indeed, diastolic function in CA hamsters was relatively poor at hypothermia, suggesting that the primary limitation in their cardiovascular performance was cardiac in origin, rather than the integration between organ systems.

The increase in stroke volume observed for euthermic hamsters on acute cooling preserved cardiac output despite decreased f_H, and demonstrates a Frank–Starling-mediated partial compensation in a manner similar to that found in non-hibernators (*R. norvegicus*) noted previously (Hauton et al., 2011). Preservation of total peripheral resistance also suggested that vascular tone and hence afterload were maintained. Consequently, for cold-naïve hamsters, poor cardiovascular performance likely resulted from thermodynamic considerations. Decreased LV blood flow may also be a contributor, yet rates of oxygen delivery were coincidentally improved as f_H declined, as increasing the diastolic period prolongs diffusive exchange and reduces cardiac oxygen consumption. The CA hamsters showed an increase in LV conductance during hypothermia, which, coupled with increased capillary densities, indicates an 'active' increase in flow that may be demand driven (cf. functional hyperaemia in skeletal muscle).

The hypertension noted at normothermia in CA hamsters was partly a result of increased systolic pressure, associated with 'volume overload' of the extracellular fluid compartment (Sun et al., 1999), coupled with tachycardia following the augmented secretion of catecholamines (Cassis et al., 1998; Sun et al., 2003). Despite the vasoconstrictor properties of catecholamines, a preserved baroreceptor reflex (supplementary material Fig. S1) suggests that a decrease in total peripheral resistance was anticipated. However, this may reflect a tissue-specific or regional desensitisation to the effects of chronic catecholamine release, preferentially targeting the vasculature (Chang et al., 1982). However, CA hamsters were adversely affected on acute cooling, displaying hypotension due to

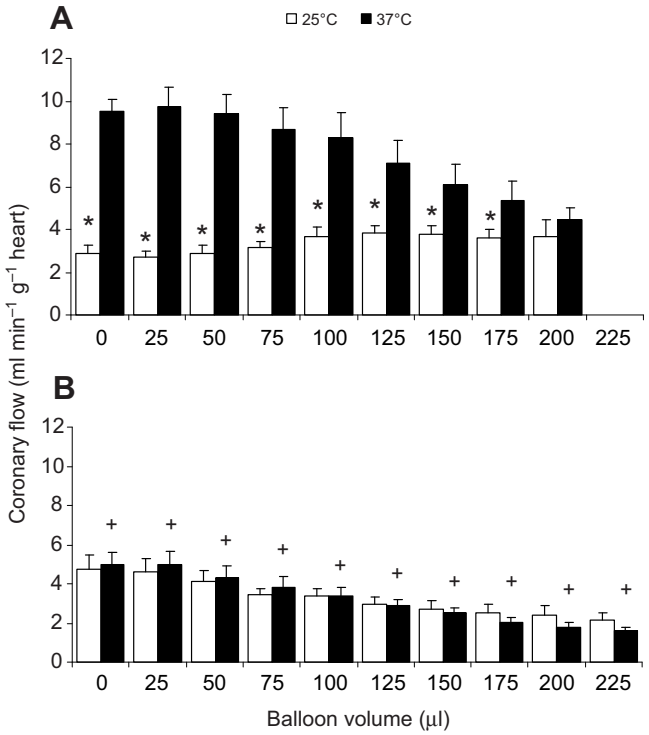

Fig. 6. Effect of acute cooling on coronary flow for the isolated Langendorff-perfused hamster heart, isolated from (A) euthermic and (B) CA hamsters, estimated as timed recovery of known volume of effluent collected from apex of heart. Data represent means ± s.e.m. for N=6 euthermic and CA hamsters. Statistical significance indicated as: effects of acute cooling, *P<0.05; effects of cold acclimation, ^{+}P<0.05.

decreased systolic and diastolic pressures, coupled with reduced f_H, leading to a lower cardiac output. As LV blood flow was preserved, implying that oxygen delivery to the myocardium was potentially better for the CA hamster than euthermic hamster on acute cooling, functional impairment was likely not perfusion-mediated (Zhang et al., 2012). One possible outcome of CA may also be the chronic β-adrenergic desensitisation of cardiac tissue, previously noted for CA rat hearts (Cheng and Hauton, 2008). Recent temporal changes noted in the transcriptome of the thirteen-lined ground squirrel suggested that hibernation triggers changes in Ca^{2+} handling proteins within the cell to increase the amplitude of Ca^{2+} release during torpor (Hauton et al., 2011). Interestingly, no changes were noted in the preceding period, which we suggest may correspond to 'cold acclimation' of the present study. Taken together with our data, these observations indicate that the transition into torpor may increase the vulnerability to changes in core temperature (as exemplified by our data), but that during torpor, cardiac-specific changes to increase Ca^{2+} transient amplitude may improve cardiovascular performance at lower core temperatures (Dibb et al., 2005; Liu et al., 1997).

Heart rate variability

Bradycardia on cooling increased heart rate variability, but this was better preserved following CA, possibly reflecting better maintenance of sympathetic control. Vagal tone, evident from the HF peak, was poorly maintained on acute cooling for euthermic hamsters, also noted for the ground squirrel (Milsom et al., 1993). Yet, given the low intrinsic f_H of the *ex vivo* perfused heart at low temperature, a decline in vagal tone may have limited effect. This infers a greater contribution from sympathetic tone in hamsters at normothermia, as noted for other rodents, including the mouse (Ishii et al., 1996) and the rat (Hashimoto et al., 1999; Hauton et al., 2011). Interestingly, vagal tone was higher following CA and remained at levels corresponding to those of euthermic hamsters at normothermia despite falling on acute cooling to 25°C, indicating a preserved baseline vagal activity. This may be of functional significance given the low minute ventilation for CA hamsters at normothermia, as vagal tone is associated with bronchoconstriction (Fisher et al., 2004), minimising heat loss during ventilation but at a cost of disproportionate ventilation of the dead space. The increased sympathetic tone, coupled with decreased vagal tone following CA, may be critical for controlling regional blood flow, with high peripheral adrenergic tone in the hamster hindfoot facilitating rapid rewarming from hibernation with limited energy resources (Osborne et al., 2005).

Intrinsic cardiac performance

Of surprise is the low intrinsic f_H for all isolated perfused hearts, suggesting a high sympathetic drive *in vivo*, with similar observations previously noted for both untreated and CA hamster hearts over a greater temperature range (Merrill et al., 1981). The increase in cardiac mass coupled with decreases in estimated EDV suggested concentric hypertrophy following CA (Deveci and Egginton, 2007), analogous to pathological hypertrophy. Coupled with poor contractility, this decrease in EDV following CA may limit cardiac output in response to stresses such as exercise. This is in contrast to CA in rats, which increased both cardiac mass and EDV associated with LV remodelling (Cheng and Hauton, 2008). Also unlike the rat, CA in hamsters increased LV capillary density, which implies the stimulation of angiogenesis to facilitate improved

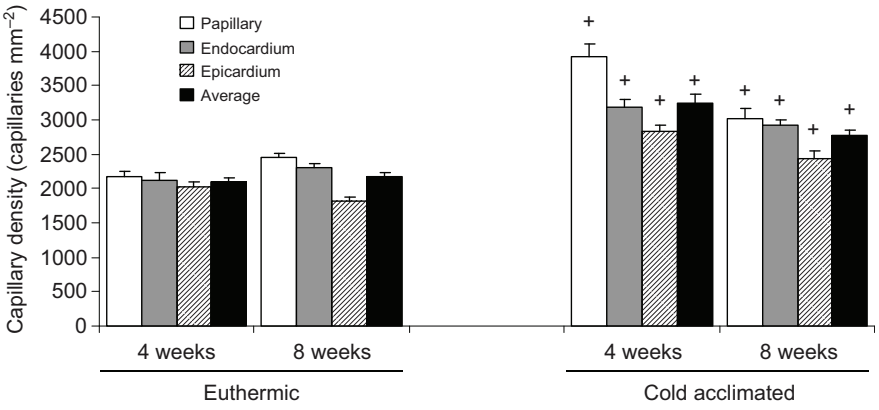

Fig. 7. Effect of cold acclimation for 4 or 8 weeks on cardiac capillary density in the epicardium, endocardium and papillary muscle of the heart. Average capillary density represents the arithmetic mean of the data collected from each region of the heart. Data represent means ± s.e.m. for N=6 euthermic and CA hamsters. Statistical significance indicated as: effects of cold acclimation, ^{+}P<0.05.

oxygen delivery in response to increased cardiac work. Hence, a reduction in the maximum oxygen diffusion distance for myocardium potentially increased the delivery of oxygen following CA.

Cardiac fibrosis, noted following prolonged exposure to cold (Zhang et al., 2012), which would increase ventricle stiffness, was not observed. However, the changes to developed pressure and RPP following cooling directly support the thermal sensitivity of the LV. The tension developed in the LV results from a combination of the numbers of cross-bridges formed and the rate of myosin-ATPase activity, hence for the higher perfusion temperature (37°C) peak tension is achieved at lower balloon volumes than at 25°C. Teleologically this may be beneficial as ventricular filling may be greater at lower f_H, and therefore changes to the tension-length characteristics of sarcomeres will preserve cardiac output despite the low f_H. For CA hamster hearts, the peak in developed pressure and RPP occur at different balloon volumes to those of euthermic hamsters, possibly as a consequence of hypertrophy and altered acto-myosin interactions changing the position of peak tension development for individual sarcomeres.

Coronary flow

The thermal sensitivity of coronary flow for euthermic hamsters suggested that local control mechanisms (e.g. nitric oxide synthase, cyclo-oxygenases and K^+ flux) were important for the hamster under normal conditions, but that these were thermodynamically unfavourable at lower perfusion temperatures. LV blood flow *in vivo* was also decreased, suggesting that centrally mediated mechanisms are not sufficient to preserve ventricle perfusion. Indeed, previous experiments document a loss of coronary autoregulation *in vivo*, in response to exogenous adenosine or ischaemia-induced hyperaemia for euthermic or CA hamsters perfused at low temperature (9°C) (Merrill et al., 1981).

Similarly, CA hamsters also showed poorly maintained coronary flow irrespective of perfusion temperature, implying that centrally mediated factors may be critical for preserving the LV blood flow noted *in vivo*. We cannot ignore a shortcoming of the perfusion technique, where both untreated and CA hearts were perfused at the same coronary perfusion pressure, while in intact animals elevated systemic blood pressure may support increased blood flow in the CA heart, evident from the *in vivo* LV blood flow estimates. Immunolocalisation revealed a transfer of endothelial nitric oxide synthase towards an intracellular compartment for the CA and hibernating hamsters (Saitongdee et al., 1999), suggesting a reduced endothelial contribution to maintenance of vascular tone. Furthermore, isolated carotid arteries taken from CA hamsters showed a loss in sensitivity to endothelium-dependent and -independent vasodilators at 10°C, suggesting a possible high thermal sensitivity in terminal arterioles from CA hamsters (Saito et al., 2002). Taken together, these results suggest that the decline in coronary flow for the perfused heart results from attenuation of locally mediated vasodilators, despite increased capillary density, and indicate a perfusion limitation of the CA myocardium that is overcome *in vivo* by increased autonomic inputs to improve cardiac contractility by partially restoring coronary flow.

Context of the experiment

CA may be only a transition phase towards hibernation, and hence represent an incomplete phenotype, consistent with the large coefficient of variation noted in our measurements. Anaesthesia may also have blunted sympathetic nerve activity, with early experiments demonstrating an α-chloralose-induced bradycardia (Korner et al., 1968), although in the cat α-chloralose increased baroreceptor reflex sensitivity (Matsukawa and Ninomiya, 1989). While more recent experiments demonstrated no change in response to baroreceptor unloading in the rat induced by chloralose-urethane (Usselman et al., 2011), few studies have examined the effect of anaesthesia in hibernators, with chloralose-urethane yielding only modest decreases in MABP and f_H for the marmot (*Marmota flaviventris*) (Zatzman and Thornhill, 1988).

Chloralose is a chlorinated glucose derivative, which at the highest dose given in these experiments may represent 5% (by calculation) of the total glucose present in the extracellular compartment. However, limited effects on glucose oxidation rates have been noted using α-chloralose anaesthesia (Ueki et al., 1992; Yang and Shen, 2006), suggesting that glucose metabolism may not be limited during our experiments.

Concluding remarks

We demonstrate that CA led to changes in the cardiovascular function of hamsters that were less favourable when subjected to acute cooling. Inefficiencies were manifest in the myocardium, and represented reductions in coronary perfusion, despite apparent angiogenesis to minimise intracardiac oxygen diffusion distances. This implies that CA in the hamster is a composite response to address the physiological requirements of cold exposure, and our data suggest that increased sympathetic tone may be important for maintaining cardio-respiratory function but that it is poorly preserved at lower core temperatures. Given the high consumption of oxygen required for thermogenesis (Chi and Wang, 2011), this may account for the progressive, regional rewarming noted for the hamster (Osborne et al., 2005). Furthermore, this may in part explain the 'social thermoregulation' previously noted for the Siberian hamster (Jefimow et al., 2011), as grouped animals show narrower ranges of core temperatures during torpor, which may prevent a catastrophic fall in core temperature beyond which cardiovascular control is inadequate. CA may represent an indefinite period of preservation prior to committing to a wholesale transcriptome remodelling during hibernation. Nevertheless, the high mortality and lack of molecular adaptations associated with hibernation (Van Breukelen and Martin, 2002a) are consistent with the current data showing a lack of enhanced function. However viewed, an animals' response to cold exposure represents a gamble for survival.

LIST OF SYMBOLS AND ABBREVIATIONS

CA	cold acclimation/cold acclimated
ECG	echocardiograph
EDV	end-diastolic volume
f_H	heart rate
f_V	ventilation rate
HF	high frequency
LF	low frequency
LV	left ventricle
MABP	mean arterial blood pressure
RPP	rate-pressure product
VLF	very low frequency

AUTHOR CONTRIBUTIONS

S.E. and D.H. devised and designed the experiments; S.E., S.M., D.D. and D.H. conducted the experiments; S.E., S.M., D.D. and D.H. analysed the data; S.E. and D.H. interpreted the data and wrote the manuscript.

COMPETING INTERESTS

No competing interests declared.

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