Cardiac activation heat remains inversely dependent on temperature over the range 27 °C – 37 °C

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Abstract

The relation between heat output and stress production (force per cross-sectional area) of isolated cardiac tissue is a key metric that provides insight into muscle energetic performance. The heat-intercept of the relation, termed ‘activation heat’, reflects the metabolic cost of restoring transmembrane gradients of Na^+ and K^+ following electrical excitation, and myoplasmic Ca^{2+} concentration following its release from the sarcoplasmic reticulum. At sub-physiological temperatures, activation heat is inversely dependent on temperature. Thus, one may presume that activation heat would decrease even further at body temperature. However, this assumption is *prima facie* inconsistent with a study, using intact hearts, which revealed no apparent change in the combination of activation and basal metabolism between 27 °C and 37 °C. It is thus desired to directly determine the change in activation heat between 27 °C and 37 °C. In this study, we use our recently constructed high-thermal resolution muscle calorimeter to determine the first heat-stress relation of isolated cardiac muscle at 37 °C. We compare-the relation at 37 °C to that at 27 °C to examine whether the inverse temperature-dependence of activation heat, observed under hypothermic conditions, prevails at body temperature. Our results show that activation heat was reduced (from 3.5 $kJ \cdot m^{-3}$ ± 0.3 $kJ \cdot m^{-3}$ to 2.3 $kJ \cdot m^{-3}$ ± 0.3 $kJ \cdot m^{-3}$) at the higher temperature. This leads us to conclude that activation metabolism continues to decline as temperature is increased from hypothermia to normothermia, and allows us to comment on results obtained from the intact heart by previous investigators.

New and noteworthy

Using isolated cardiac trabeculae, we provide the first measurement of cardiac activation heat at body temperature. This measurement eliminates a concern regarding the temperature

dependence of activation heat between hypothermic and normothermic temperatures and allows us to comment on results obtained from intact hearts.

Keywords: muscle energetics, calorimetry, heat-stress relation, cardiac trabeculae, heat

Introduction

The heat generated during the contraction of cardiac muscle arises from two independent sources: ‘activation’ and ‘contraction’. These two conceptually distinct thermal components can be partitioned, without the need for pharmacological intervention, from the relationship defining heat as a function of stress (force per cross-sectional area). The heat-intercept of the heat versus stress relationship of cardiac muscles gives the ‘activation heat’, defined as the energy required for activation of the contractile unit, attributed to the energetic cost of the sarcoplasmic reticular Ca^{2+}-ATPase to sequester calcium from the cytosol, and the energy expended by the Na^{+}-K^{+}-ATPase to restore ionic homeostasis disturbed by both the action potential and calcium removal via the Na^{+}-Ca^{2+} exchanger (7, 10, 11, 17, 27, 44, 51). The supra-activation heat is the contraction-dependent heat resulting from the Ca^{2+}-triggered, actin-activated, myosin ATPase and thus is dependent on the stress developed. Hence, the inverse of the slope of the heat versus stress relationship quantifies the stress development per unit energetic cost of the contractile apparatus (herein referred to as ‘crossbridge economy’).

Given that the relationship between supra-basal heat and stress can be readily obtained by varying the length of isolated cardiac tissue preparations, and is independent of stimulus frequency (6, 14, 22), it has become one of the key metrics for examining the energetic performance of cardiac muscle. Much has been learned from previous studies that have adopted this protocol. For example, activation heat has been shown to be dependent on age (35), species (38), ventricle (23), and extracellular Ca^{2+} concentration (17, 22), but is not affected by systemic (24) or pulmonary (34) hypertension-induced hypertrophy, a high-fat diet (20), or diabetes (25). Despite extensive quantification of the cardiac heat-stress relation, it has not yet been determined at body temperature.

An inverse dependence of temperature of cardiac activation heat has been reported at sub-physiological temperatures. This result was obtained by Gibbs and Vaughan (18) at 20°C and 32°C, Gibbs and Gibson (14) at nominally 21°C and 31°C, Barclay *et al.* (6) at 19°C and 29°C and Loiselle (41) at 20°C and 27°C. It is thus plausible to assume that the inverse temperature-dependence of activation heat continues up to body temperature. However, this assumption is *prima facie*, inconsistent with the results of Suga *et al.* (53) who reported no apparent effect of temperature (between 27 °C and body temperature) on the VO_2-intercept of the relationship between oxygen consumption (VO_2) and pressure-volume area (PVA). From these results of Suga *et al*., it may be hypothesised that the temperature-dependence of cardiac activation metabolism has reached its minimum at 27°C. On the other hand, the measurements by the Suga group were made on whole hearts rather than isolated tissues, were based on PVA rather than stress, and were confounded by inclusion of basal metabolism in the VO_2-intercept, all of these rendering this hypothesis uncertain. This unsatisfying state of affairs has been the primary motivation to undertake this study. We thus address the question whether cardiac activation heat decreases between 27°C and body temperature. In addressing this question, we provide the first measurement of the heat-stress relation of isolated cardiac tissue at body temperature.

We use a recently constructed muscle calorimeter, with greatly increased thermal resolution, to measure the low rate of heat output of rat ventricular trabeculae undergoing isometric contractions at body temperature (31). The use of cardiac trabeculae greatly reduces the concern regarding insufficient diffusion of oxygen supply into muscle core. Mathematical modelling studies suggest that, even when the experimental solution is supplied with 100 %

oxygen, muscle radius should not exceed 150 μm to 200 μm to preclude the possibility of an anoxic core due to insufficient diffusion of oxygen throughout the sample (5, 21). Isolated cardiac trabeculae, with a typical radius of less than 150 μm, are thus suitable preparations in this regard.

Methods

Animal handling and experiments were conducted according to protocols approved by the University of Auckland Animal Ethics Committee.

Muscle preparation Male Wistar rats (3 months old, 380 g to 500 g) were anaesthetized using isoflurane, followed by cervical dislocation, thoracotomy, and cardiectomy. The excised hearts were immediately plunged into chilled saline to induce arrest prior to being Langendorff-perfused with dissection solution at room temperature. The dissection solution was a Tyrode solution comprising: 130 $mmol \cdot L^{-1}$ NaCl, 6 $mmol \cdot L^{-1}$ KCl, 1 $mmol \cdot L^{-1}$ $MgCl_2$, 0.5 $mmol \cdot L^{-1}$ NaH_2PO_4, 0.3 $mmol \cdot L^{-1}$ $CaCl_2$, 10 $mmol \cdot L^{-1}$ Hepes, 10 $mmol \cdot L^{-1}$ glucose and 20 $mmol \cdot L^{-1}$ 2,3-butanedione monoxime (BDM). The dissection solution was aerated with 100 % O_2 and had a pH of 7.4 (adjusted using Tris).

A right ventricular trabecula was dissected then transferred to, and mounted in, the calorimeter. Once in the measurement chamber, it was superfused with experimental solution which was the same as the dissection solution except for elevation of Ca^{2+} concentration to 1.5 mmol/L, and the absence of BDM. Each trabecula was required to undergo experiments at both 27 °C and 37 °C. The latter temperature necessitated the experimental solution in the reservoir upstream to be maintained at 41 °C during aeration to prevent bubble formation downstream in the measurement chamber. The superfusate flow rate through the chamber was 0.5 µL/s. This flow rate represents a careful reconciliation between the need to ensure adequate oxygenation (5, 21) and the desirability of maximizing the thermal signal-to-noise ratio (31).

Flow-through muscle calorimeter The new muscle calorimeter used for this study has been described previously (31). Briefly, it consists of a flow-through microcalorimeter, a silicon-beam force transducer, and a linear motor. In the microcalorimeter chamber, the muscle is held between two platinum hooks attached to the ends of a pair of borosilicate capillary tubes which, in turn, are attached to either the force transducer or the motor. Superfusate (experimental Tyrode solution) flows over the length of the muscle. The change of temperature of the superfusate is measured using thermoelectric modules (abutting the outside surface of the microcalorimeter chamber). The rate of heat production of the muscle is thus inferred from the flow rate of the superfusate and the difference of temperature between the upstream and downstream thermoelectric modules.

Experimental protocols Muscles were field-stimulated at 3 Hz during a period of recovery following dissection and mounting. Completion of recovery (typically requiring at least thirty minutes) was achieved when the stress production had reached a steady state and lowering the stimulus voltage or stimulus duration caused a negligible change in developed stress. The muscle was then gradually stretched to optimal length (L_o), i.e. the length that produces maximal developed (active) stress.

Measurement of activation heat requires extrapolating the heat-stress relation to the point of zero stress. Populating this relation to minimize the extent of extrapolation can be achieved by two protocols: reducing muscle length and changing stimulus frequency. The relationship between heat and stress was determined through use of both protocols comprising a total of

15 interventions. Force and rate of heat production were analyzed when the signals had reached steady state during each intervention. The muscle was quiescent between interventions, thereby providing references for zero force and zero heat baselines.

First, a 'frequency-change' protocol was performed at optimal length. Five stimulus frequencies were presented in random orders. The temperature-dependence of twitch duration and muscle stress production meant that at 27 °C, compared with that at 37 °C, muscles were unable to follow higher stimulus frequencies due to incomplete relaxation of the twitch (i.e., a severe increase of diastolic stress ensued). Hence, the frequencies examined were necessarily different between the two temperatures: 0.5 Hz, 1 Hz, 2 Hz, 3 Hz, and 4 Hz at 27 °C, and 1 Hz, 3 Hz, 5 Hz, 6 Hz, and 8 Hz at 37 °C. The initial frequency was repeated at the end of the protocol to examine the extent of stress diminution (i.e., irreversible decrease of developed stress with time). A muscle was discarded if its stress fell more than 20 % below its initial value.

Once the 'frequency-change' protocol had been completed, a 'pre-shortening' protocol was undertaken. This protocol involved progressively reducing muscle length (5 steps of 4 % decrements from L_o to 80 % of L_o), thereby decreasing active stress production. Each muscle was stimulated at two frequencies (1 Hz and 3 Hz at 27 °C, or 3 Hz and 5 Hz at 37 °C. Heat and stress were recorded at muscle lengths of 80 %, 84 %, 88 %, 92 %, 96 %, and 100 % of L_o.

The heat artifact resulting from electrical stimulation was quantified at the end of each experiment in the absence of a trabecula. Stimulus heat was quantified by recording the output that resulted from stimulating at each combination of temperature and frequency. The resulting values retrospectively corrected the rate of heat production of each trabecula.

Trabeculae dimensions The cross-sectional area and volume of each trabecula at optimal muscle length were calculated from estimates of muscle diameters in two orthogonal planes. It was assumed that each trabecula had an elliptical cross-section and that we were measuring the diameters of the principal axes. In total, six trabeculae from five hearts were studied. They had mean major and minor diameters of 0.28 mm ± 0.04 mm and 0.23 mm ± 0.02 mm, respectively, resulting in a mean cross-sectional area of 0.051 mm^2 ± 0.0085 mm^2. Their mean length was 2.65 mm ± 0.39 mm.

Definitions and data normalization Stress was calculated as force divided by muscle cross-sectional area. Heat rate was normalized to muscle volume. The heat per twitch was quantified by dividing heat rate by stimulus frequency.

Three different stress components were analyzed – passive stress, diastolic stress, and active stress – which can be distinguished in Fig. 1a. Passive stress was defined as the stress (relative to that at 80 % L_o) when the muscle was unstimulated. Active stress was defined as the difference between the peak developed stress and the baseline of developed stress during a twitch. Diastolic stress was defined as the extent of baseline stress above passive stress

during a twitch. Twitch duration was defined as the time the stress is above 5 % of peak stress.

Statistical analyses Variables of interest (heat rate, heat per twitch, stresses and twitch duration) were expressed as mean ± SEM, and plotted as functions of frequency and of muscle length.

F-tests were conducted to assess whether the heat versus stress relation should be fitted with a linear or quadratic regression line. The F-statistic was found for each relation and compared with the critical value of the F-distribution for that relation. F-tests revealed that a quadratic fitting did not significantly improve the fit; i.e. the residual variance was not significantly reduced. Hence, the heat-stress data were fitted using linear regression.

To test for the effect of temperature, the 'random coefficient' model within Proc Mixed of the SAS software package was used. Using this model, the linear regression lines of the six heat versus stress relations (each obtained from a single trabecula) at a given temperature were averaged using the method of Feldman (13) in which regression coefficients are assumed to arise from a random sample of a multivariate normal population (37). The distribution of residuals was confirmed to be random, in accord with an assumption of the model. Statistical significance was tested for the difference between the average intercepts (activation heat) and the average slopes (inverse of the 'crossbridge economy') at the two temperatures. In all cases, $P < 0.05$ was considered as indicating statistical significance.

Results

In order to construct the heat-stress relations, isometric twitch force and rate of heat production were simultaneously measured while the trabeculae were stimulated at a range of frequencies at various muscle lengths and at two temperatures. Fig. 1 displays a subset of typical records of stress production and rate of heat production at different stimulus frequencies, muscle lengths and temperatures. The active stress production decreased while the rate of heat production increased with increasing stimulus frequency. At higher stimulus frequencies, the trabeculae were unable to relax completely between subsequent twitches, leading to a frequency-dependent increase of diastolic stress. Both active stress production and rate of heat production decreased with decreasing muscle length. Decreasing muscle length also reduced passive stress.

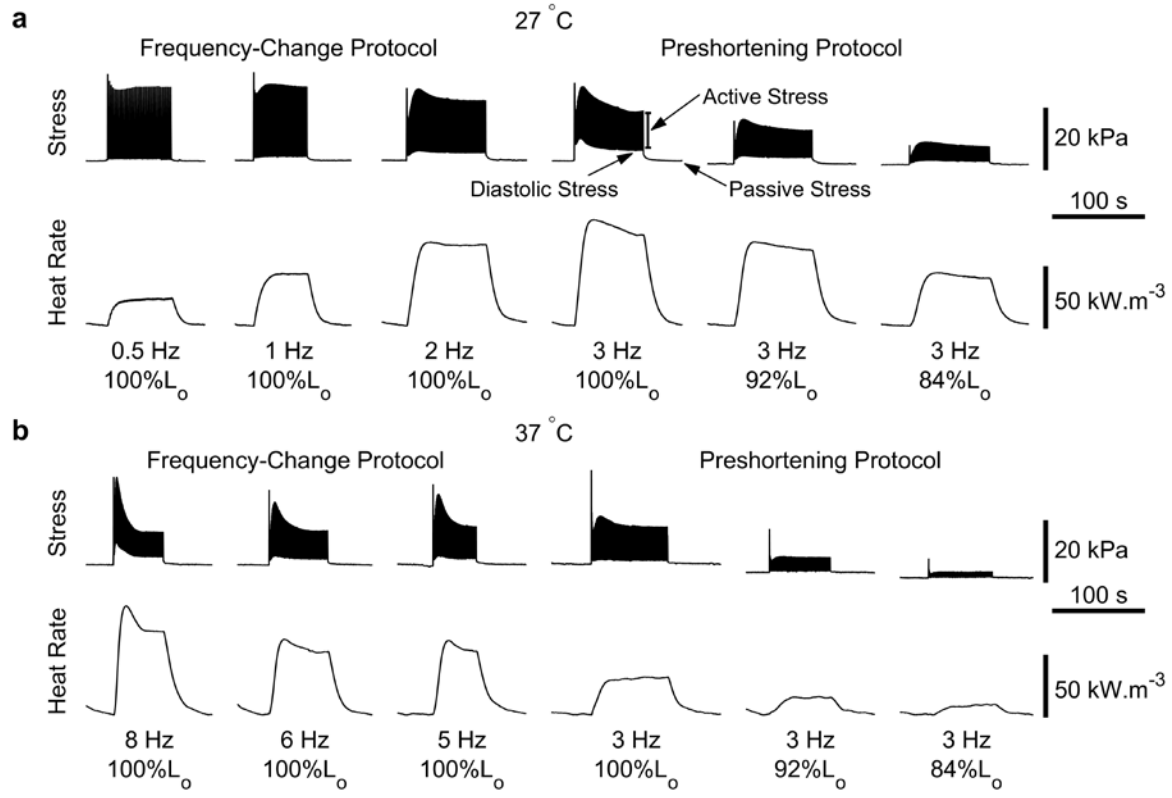

Fig. 1 A subset of typical experimental records of stress production (top) and rate of heat production (bottom) of a trabecula at 27 °C (a) and 37 °C (b) in response to various stimulus frequencies and muscle lengths.

Effect of stimulus frequency on heat and stress production

Heat rate, heat per twitch (i.e., heat rate divided by stimulus frequency), active stress, diastolic stress, and twitch duration, as functions of stimulus frequency at both temperatures (27 °C and 37 °C), are shown in Fig. 2. These variables, except for the heat rate ($p = 0.075$), were lower at 37 °C compared with those at 27 °C.

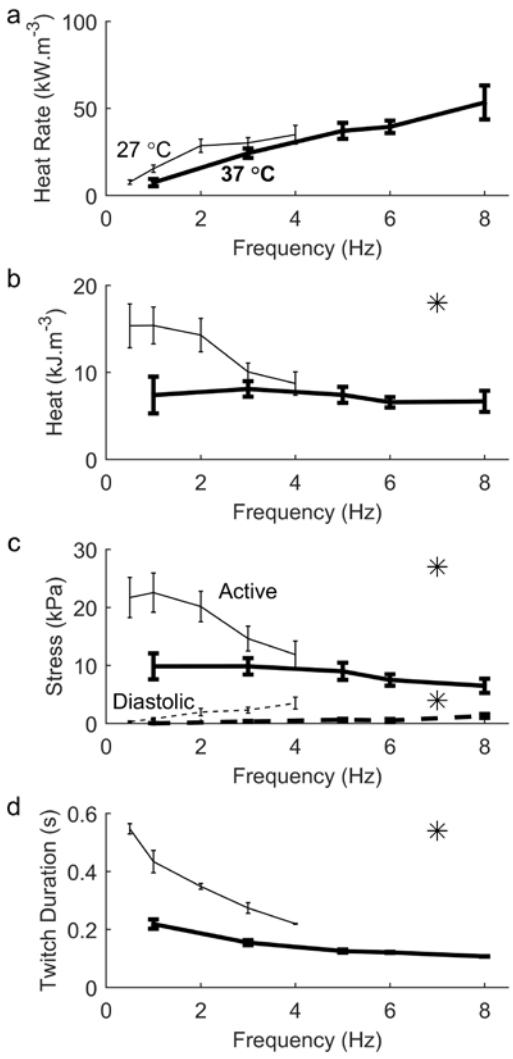

Fig. 2 Steady-state heat rate (a), heat per twitch (b), active and diastolic stresses (c), and twitch duration (d) at optimal muscle length, as functions of stimulus frequency. The asterisk indicates statistically significant effect of temperature at the two stimulus frequencies (1 Hz and 3 Hz) common to the two temperatures (27 °C and 37 °C).

Effect of muscle length on heat and stress production

Heat rate, heat per twitch, active stress, passive stress, and twitch duration were expressed as functions of muscle length at both temperatures (27 °C and 37 °C), as shown in Fig. 3. All metrics, with the exception of passive stress, were temperature-dependent with significant reductions at the higher temperature (37 °C).

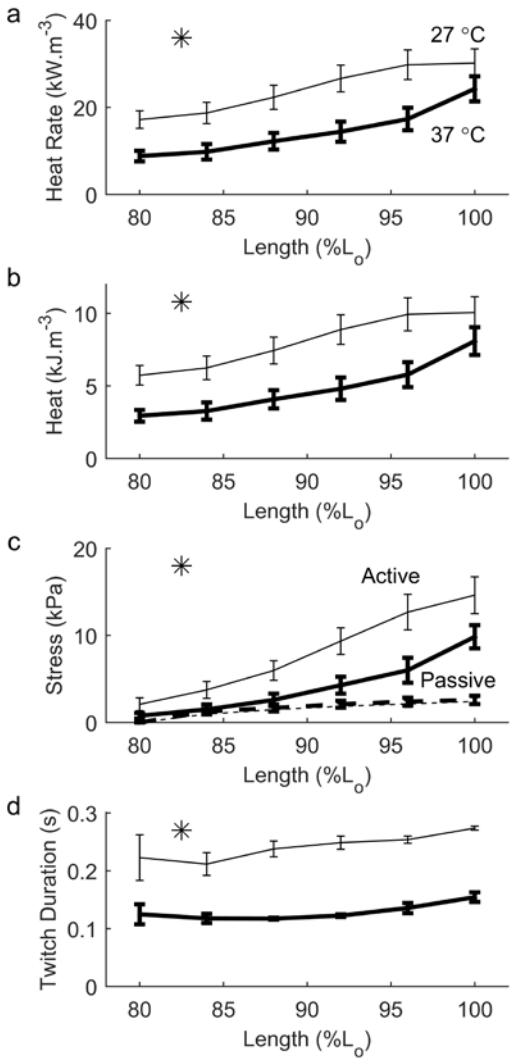

Fig. 3 Steady-state active heat rate (a), heat per twitch (b), active and diastolic stress (c), and twitch duration (d) as functions of muscle length for trabeculae stimulated at 3 Hz. The symbol * indicates a statistically significant effect of temperature as a function of muscle length.

Active heat versus stress relationships

The data from the frequency-change and pre-shortening protocols were combined to populate the heat versus active stress relations. The relations at both temperatures were fitted using linear regression as no statistically significant improvement was observed when fitting with a

quadratic function. Fig. 4 shows the relationships between heat and stress at 27 °C and 37 °C, and the effect of temperature on the heat-intercepts (i.e., activation heat) and slopes (i.e., the inverse of the crossbridge economy) of the relations. The slopes of the average relations were independent of temperature (Fig. 4c). Activation heat was lower at the higher temperature (Fig. 4d). The Q_{10} temperature coefficient of activation heat (the factor by which the activation heat changes with a 10 °C increase of temperature) was calculated to be 0.64 ± 0.10 over this temperature range.

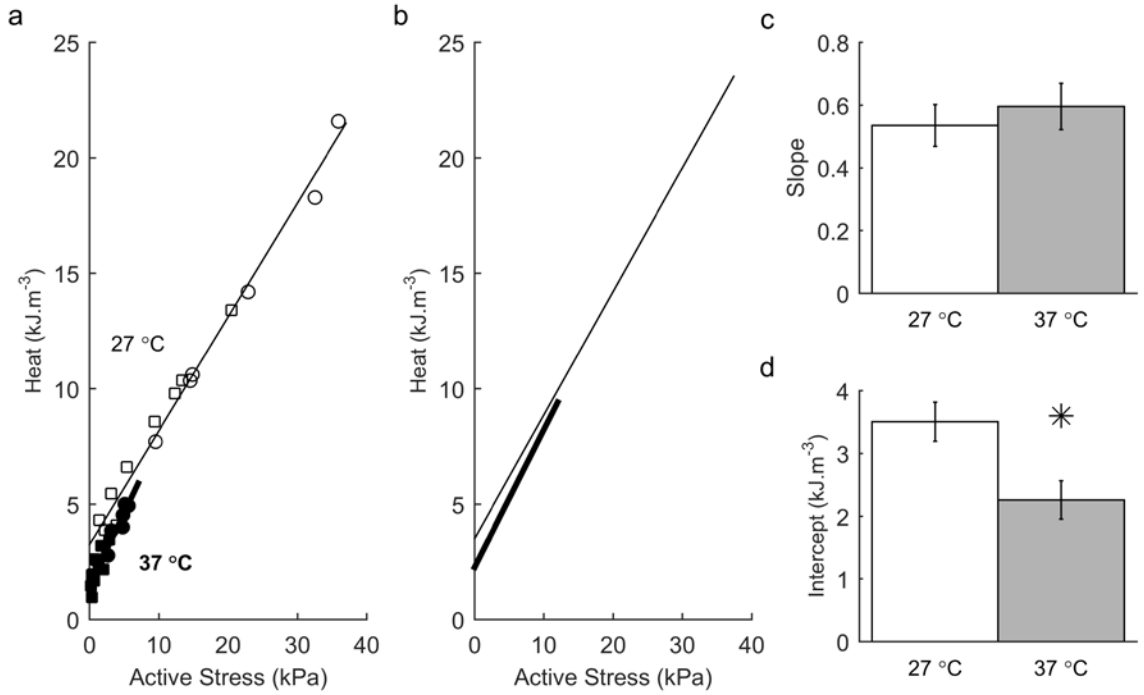

Fig. 4 Relationship between heat per twitch and stress at 37 °C compared with that at 27 °C. (a) Heat versus active stress relations of a representative trabecula obtained from changes in stimulus frequency (circles) and muscle length (squares) at 27 °C (thin lines) and at 37 °C (thick lines). (b) Heat versus stress relations averaged over six trabeculae (see Methods). (c) Mean ± SE of the slopes (i.e., inverse of the crossbridge economy) of the heat versus stress relations. (d) Mean ± SE of the intercepts (i.e., activation heat) of the heat versus stress relations. The asterisk indicates a statistically significant effect of temperature.

Discussion

Our study has three novel aspects. These have been made possible by recent development of a novel calorimeter, which provides simultaneous measurements of heat and stress at body temperature (31), thus allowing us to make the first measurement of the heat-stress relation of isolated cardiac muscle at body temperature. From the inverse of the slope of the resulting normothermic heat-stress relation, we quantify crossbridge economy (i.e., the stress development per unit energetic cost of the contractile apparatus) and, from its intercept, an estimate of activation heat (i.e., energy output from Ca^{2+} cycling and restoration of membrane potential). We have thus extended earlier findings of an inverse dependence of cardiac activation heat on temperature, measured under hypothermic conditions, to body temperature. In so doing, we are able to comment on the results of Suga *et al.* (53) where no change in either the VO_2-intercept or the slope of the VO_2-PVA relation was observed between 27 °C and 37 °C in the isolated, cross-perfused, dog heart.

It has long been recognized that hypothermia increases the force output of the whole heart (19) as well as that of its isolated tissues (8). Our results from rat trabeculae (Figs 1 and 2) are consistent with these observations. In addition, we find that increasing stimulus frequency decreases heat per twitch output, as well as abbreviating twitch duration (Fig. 2), results which are in accord with those of Han *et al.* (22), Kassiri *et al.* (33), and Varian and Janssen (57). Our results showing the potentiating effects of muscle length on heat per twitch, stress, and twitch duration (Fig. 3) are also consistent with those of previous studies (26, 30, 45). Finally, an increase of temperature causes a downward shift of each of the aforementioned relations (Figs 2-3), results which are also in agreement with reports in the literature (6, 14, 18, 30, 41).

Adequacy of oxygen supply The increase of diastolic stress (Fig. 1) and the decreased force and heat production (Fig. 2) observed at 37 °C, when pacing at high stimulus frequencies, is not a consequence of insufficient diffusive oxygen supply to the trabeculae. We provide three supporting pieces of evidence for this conclusion in the following.

Firstly, as has been shown by Han *et al.* (22), the increase of diastolic stress at high stimulus frequency is attained with negligible metabolic expenditure, due to the presence of attached, but non-cycling (latched), and non-ATP-hydrolyzing, crossbridges. These authors provided further evidence showing that increased diastolic stress is not due to a deficiency of either oxygen or glucose supply.

Secondly, the critical muscle radius to ensure adequacy of oxygen diffusion has been calculated to be 140 μm (37 °C, 10 Hz) by Han *et al.* (21) and approximately 300 μm (37 °C, 8 Hz) by Barclay (5). All of the trabeculae used in the above experiments (up to 8 Hz) were smaller than both of these critical radii.

Thirdly, we performed a simple Hill-type diffusion calculation of the adequacy of O_2 supply to the largest trabecular used (see Appendix), which predicted a critical extracellular partial pressure of oxygen of 50 kPa (0.49 atm) (29). We continuously supplied 100% O_2 (101 kPa; 1.00 atm) to the trabecula. Previous mathematical modelling of oxygen delivery in the microcalorimeter (21) showed that downstream PO_2 at the muscle surface is reduced only to 61 kPa (0.60 atm) (calculated using an extremely high value of $\dot{V}O_2$: 0.18 mol·m^{-3}·s^{-1}, and an uncharacteristically lengthy trabecula: 4 mm). Since this value is 20 % higher than the critical

PO_2 of 50 kPa, we are confident that even the largest trabecula that we used was adequately supplied with oxygen.

Crossbridge economy We index 'crossbridge economy' as the inverse of the slope of the heat-stress relation. It represents the energy cost of stress development by the contractile machinery. (Note that our 'economy' and Suga's 'efficiency' are incommensurate.) We find that crossbridge economy is not significantly different between 27 °C (crossbridge economy of 1.84 ± 0.21) and 37 °C (1.68 ± 0.23) (Fig. 4). Temperature-independent crossbridge economy is consistent with literature results from isolated muscles at sub-physiological temperatures (6, 41) and from whole hearts cooled below physiological temperature (53). A fixed stress production for a given expenditure of energy thus appears to be an underlying property of the myosin ATPase, independent of temperature.

Methods of Estimating Activation Heat Activation heat reflects the metabolic costs of removal of calcium from the cytoplasm and restoration of membrane potential after activation (10, 17). Therefore its measurement requires a means of eliminating crossbridge cycling. Unlike the situation with skeletal muscle, cardiac muscle cannot be stretched beyond optimal length to the point of zero stress production without causing irreversible damage making any estimates of the activation heat quite uncertain (17). All methods of estimation must thus be restricted to the ascending limb of the force-length relation. Since we have recently published a review that both details and critiques these methods (39), we will do little more than list them here.

The pre-shortening method takes advantage of the Frank-Starling law by progressively reducing muscle length from its optimal value to the point where no macroscopic force production can be measured. A line is then fitted to the heat-stress relation to predict the intercept at zero force. The stimulus frequency-independence of the heat-stress relation (15, 22, 38, 41) may be used to further populate the relation. Compared with other methods (see below), the pre-shortening method confounds the measurement of true activation heat in two ways. The first is that, even in the absence of macroscopic force production, there may still be residual crossbridge cycling, thereby potentially resulting in an overestimation of the activation heat. Secondly, since it has been shown that the magnitude of Ca^{2+} release is muscle length-dependent (1, 12, 57), the magnitude of activation heat may be likewise.

Pharmacological methods have also been used whereby contraction is hindered through the use of hypertonic solutions, with attendant risk of irreversibility (2, 3, 47), or putative crossbridge inhibitors (e.g. BDM), whose actions are seldom specific (4, 9, 48). In order to avoid these concerns, Gibbs *et al.* (17) developed the 'latency release' method in which a muscle is stimulated at optimal length before being rapidly shortened 15 ms after the onset of contraction and then re-stretched prior to the next stimulus. Different methods for populating the heat-stress curve have been found to have consistent effect on the estimation of activation heat, i.e., the method will simply cause a consistent offset, meaning that comparative studies can still be performed (17). Therefore, despite the uncertainties in each of these techniques, we opted to use the pre-shortening in the current study.

The Temperature-Dependence of Activation Heat We observed a decrease in activation heat with an increase of temperature from 27 °C to 37 °C (Fig 4(d)), in accordance with

previous studies performed at hypothermic temperatures (6, 16, 18, 41). The cellular explanation is that the open-time of sarcoplasmic reticular calcium channels is reduced at higher temperatures due to an increased rate of closure, thus reducing the amount of calcium released (52). Reduced calcium release in turn lowers the activity of SERCA, thereby reducing its net hydrolysis of ATP (49) and, hence, activation heat. Since less Ca^{2+} is available to bind to Troponin-C, twitch stress is also reduced.

Numerous investigators have estimated activation energy, using intact hearts, from the intercept of the linear relationship between oxygen consumption (VO_2) and pressure-volume area (PVA). However, the intercept of the VO_2-PVA relation comprises basal oxygen consumption in addition to activation energy. Literature values for the intercept of the VO_2-PVA relation at body temperature show a wide range of values (5 kJ/m^3 to 17 kJ/m^3) (46, 50, 54–56). The use of isolated muscle in our calorimeter removes a major component of uncertainty, since our heat measurements are strictly suprabasal, and thus provide more direct and less ambiguous estimates of activation heat.

Comparison with the results of Suga's et al. Suga *et al.* (53) previously found that the VO_2-intercept of the VO_2-PVA relation is independent of temperature when lowering from 37 °C to 27 °C. In contrast, we find activation heat to be temperature-dependent. There are a number of differences between our approaches that may account for this discrepancy. Firstly, two different contraction regimes were employed. Suga *et al.* used ejecting contractions to generate PVA as opposed to our use of isometric contractions. However, Suga *et al.* (54) had previously shown that the linear relation between oxygen consumption and PVA is independent of the mode of contraction. That is, both isovolumic contractions and ejecting

contractions yield the same VO_2-PVA relation. Thus, it is unlikely that these different contraction regimes is the cause of the discrepant results.

Alternatively, the difference in temperature response of the activation heat may be attributed to species differences, given the differences in action potential shape and calcium handling between canine and rat myocytes (28, 32, 58). However, when examining other metrics arising from the heat-stress relation, the effect of temperature is the same. Thus, our results from rat trabeculae, showing temperature-independence of crossbridge economy, is consistent with those obtained from papillary muscles of the cat and rat (6, 41). Additionally, no difference was observed in the crossbridge economy between rat, guinea pig and cat indicating that species differences may not be a major determinant of cardiac energetics.

In our view, the most likely explanation for the difference between our results and those of Suga *et al.* arises from the fact that the VO_2-intercept of the VO_2-PVA relation represents the sum of activation and basal metabolism Thus the result observed by Suga *et al.* may be due to an increase in basal metabolism with increased temperature that is sufficiently large to offset a decrease in activation metabolism. The Q_{10} of basal metabolism of rat myocardium has been calculated to be approximately 1.3 (42, 43). This result, in combination with our measured Q_{10} of 0.64, indicates that a temperature-dependent increase of basal metabolism may be sufficient to offset any decrease of activation VO_2 with temperature. We therefore conclude that our hypothesis is valid: activation metabolism continues to decline as temperature is increased from 27 °C to 37 °C.

Conclusions

We report the first investigation of the heat-stress relation of isolated cardiac muscle at body temperature. We find its slope to be temperature-insensitive. We find the activation heat versus temperature relation to retain its inverse nature, evident at sub-physiological temperatures, at body temperature.

Appendix

Hill's diffusion calculation

According to Hill's diffusion equation, the partial pressure of extracellular oxygen (PO_2) at which the core of a preparation of elliptical cross section becomes anoxic is given by:

$$PO_2 = (\frac{\dot{VO}_2}{2K})/(\frac{4}{D_L^2} + \frac{4}{D_S^2})$$

We examined the largest trabecula used (largest diameter (D_L): 420 µm, smallest diameter (D_s): 250 µm) at the highest stimulus frequency used (8 Hz). The maximal rate of oxygen consumption ($\dot{VO}_2$) is equal to the sum of the active and basal rates of metabolism. The rate of active metabolism (0.0980 $mol \cdot m^{-3} \cdot s^{-1}$) was calculated from its rate of active heat rate output (50 $kW \cdot m^{-3}$; Fig. 2). The basal metabolic rate (0.0510 $mol \cdot m^{-3} \cdot s^{-1}$) was calculated from the basal heat rate reported by Loiselle et al. (40) (26 $kW \cdot m^{-3}$; see their Table 1). Krogh's diffusion constant (K) at 37 °C is given by van der Laarse et al. (36) as 1.72×10^{-14} $mol \cdot m^{-1} \cdot Pa^{-1} \cdot s^{-1}$. Using these values, and the above equation, the calculated extracellular critical PO_2 is 50 kPa (or 0.49 atm).

Acknowledgements

Callum Johnston is currently affiliated with the Heart Science Centre, Imperial College London, London, United Kingdom.

Grants

This work was supported by the Marsden Fund, from Government funding, administered by the Royal Society of New Zealand. Callum M. Johnston was supported by a University of Auckland Doctoral Scholarship. June-Chiew Han is supported by a New Zealand Heart Foundation Research Fellowship. Aspects of this work were partly supported by a Project Grant from the New Zealand Heart Foundation.

Disclosures

Nothing to disclose.

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Figure captions

Fig. 1 A subset of typical experimental records of stress production (top) and rate of heat production (bottom) of a trabecula at 27 °C (a) and 37 °C (b) in response to various stimulus frequencies and muscle lengths.

Fig. 2 Steady-state heat rate (a), heat per twitch (b), active and diastolic stresses (c), and twitch duration (d) at optimal muscle length, as functions of stimulus frequency. The asterisk indicates statistically significant effect of temperature at the two stimulus frequencies (1 Hz and 3 Hz) common to the two temperatures (27 °C and 37 °C).

Fig. 3 Steady-state active heat rate (a), heat per twitch (b), active and diastolic stress (c), and twitch duration (d) as functions of muscle length for trabeculae stimulated at 3 Hz. The symbol * indicates a statistically significant effect of temperature as a function of muscle length.

Fig. 4 Relationship between heat per twitch and stress at 37 °C compared with that at 27 °C. (a) Heat versus active stress relations of a representative trabecula obtained from changes in stimulus frequency (circles) and muscle length (squares) at 27 °C (thin lines) and at 37 °C (thick lines). (b) Heat versus stress relations averaged over six trabeculae (see Methods). (c) Mean ± SE of the slopes (i.e., inverse of the crossbridge economy) of the heat versus stress relations. (d) Mean ± SE of the intercepts (i.e., activation heat) of the heat versus stress relations. The asterisk indicates a statistically significant effect of temperature.