A high-resolution thermoelectric module-based calorimeter for measuring the energetics of isolated ventricular trabeculae at body temperature

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Johnston CM, Han JC, Ruddy BP, Nielsen PM, Taberner AJ. A high-resolution thermoelectric module-based calorimeter for measuring the energetics of isolated ventricular trabeculae at body temperature. Am J Physiol Heart Circ Physiol 309: H318–H324, 2015. First published May 22, 2015; doi:10.1152/ajpheart.00194.2015.—Isolated ventricular trabeculae are the most common experimental preparations used in the study of cardiac energetics. However, the experiments have been conducted at subphysiological temperatures. We have overcome this limitation by designing and constructing a novel calorimeter with sufficiently high thermal resolution for simultaneously measuring the heat output and force production of isolated, contracting, ventricular trabeculae at body temperature. This development was largely motivated by the need to better understand cardiac energetics by performing such measurements at body temperature to relate tissue performance to whole heart behavior in vivo. Our approach uses solid-state thermoelectric modules, tailored for both temperature sensing and temperature control. The thermoelectric modules have high sensitivity and low noise, which, when coupled with a multilevel temperature control system, enable an exceptionally high temperature resolution with a noise-equivalent power an order of magnitude greater than those of other existing muscle calorimeters. Our system allows us to rapidly and easily change the experimental temperature without disturbing the state of the muscle. Our calorimeter is useful in many experiments that explore the energetics of normal physiology as well as pathophysiology of cardiac muscle.

NEW & NOTEWORTHY

We have developed a new muscle calorimeter, which has enabled the first simultaneous measurements of the force and heat output of isolated, actively contracting, cardiac trabeculae at body temperature. This was achieved by the use of thermoelectric modules for high-resolution temperature sensing and precise temperature control.

MEASUREMENTS OF THE ENERGETICS of cardiac muscle under controlled experimental conditions, and in particular at body temperature, enable better understanding of cardiac muscle function in health and disease. Studies of force production simultaneous with heat output of cardiac muscle require the use of isolated tissue preparations such as small papillary muscles (2–4, 12, 26, 34) or trabeculae carneae (17–21) as they are sufficiently small to avoid muscle anoxia (14). The use of these tissue preparations, which have approximately one-dimensional arrangements of myocytes, simplifies the interpretation of measurements. Although these tissue studies have improved our understanding of myocardial energetics, previous experiments have been performed only at subphysiological temperatures, typically 10°C below that of body temperature (3, 5, 10, 11, 13, 23, 25, 26, 28, 30, 31). One exception is the study by Widén (39), who made simultaneous force and heat measurements in mouse papillary muscles at body temperature. Her experiments, using nonflow, flat-bed, thermopile system, required the use of relatively large papillary muscles for greater thermal signal, risking tissue anoxia. To prevent anoxia, we use rat ventricular trabeculae of small diameters, continuously superfused with oxygen- and nutrient-rich saline solution.

The fundamental challenge of studying isolated ventricular trabeculae at physiological temperature is their very low rate of heat output, typically in the order of microwatts, and the submillikelvin temperature change that this conveys to the surrounding fluid (7, 37). The need to simultaneously accommodate a mechanical testing apparatus increases the degree of complexity. Although Daut and Elzinga (7) were able to measure the heat output of isolated, continuously superfused trabeculae at 37°C using a thermopile-based calorimeter, they were unable to simultaneously measure their force production because the required multilayer thermal isolation could not accommodate a mechanical testing apparatus. We have overcome these limitations by constructing a temperature-controlled flow-through muscle calorimeter with exceptionally high thermal resolution. In contrast to previous muscle calorimeter designs (16, 36, 37), we have used solid-state thermoelectric modules as both temperature sensors and temperature controllers.

METHODS

Calorimeter design. The measurement chamber of the calorimeter comprises a glass tube, mirror, and thermoelectric modules arranged inside a parylene-coated copper housing as shown in Fig. 1. A square cross-section borosilicate capillary (1 mm inner width, 12 mm long), acting as a conduit for oxygenated Tyrode’s solution, is glued into the housing on top of the thermoelectric modules. A muscle is suspended within this capillary between two platinum hooks. Each hook attaches to a circular cross-section borosilicate capillary (0.7 mm outer diameter, 30 mm long on the upstream side, 12 mm long on the downstream side). The upstream hook capillary is connected to a linear servomotor (Parker Hannifin MX80L) allowing control of the muscle length. The downstream hook (and its associated capillary) is connected to a silicon cantilever beam strain-gauge (Kronex AE801) for force measurement. A microscope graticule is used to make in situ measurements of muscle dimensions. The diameter and length are measured from both top and reflected front views. The reflected front view is made possible by the use of the mirror at 45°. Two platinum electrodes (not shown) are used to field stimulate the muscle. These are located in the liquid path, upstream and downstream of the measurement capillary.
The muscle is superfused with Tyrode’s solution for supply of oxygen and nutrients while removing waste products. The superfusate is oxygenated in an external reservoir, which is controlled to a higher temperature (41°C, at which oxygen solubility is reduced) than that of the experiment (37°C) to prevent oxygen gas bubbles forming in the measurement chamber. Since the sensitivity of the device is dependent on flow rate, the superfusate is gravity fed to ensure steady flow, the rate of which is measured using a flow meter (Sensirion SLI-0430). Outflow is drained using a peristaltic pump (Langer Instruments BQ50-1J).

The rate of heat production (Q˙) of the muscle is estimated from the temperature change (dT) of the superfusate as it passes over the muscle. The meager rate of heat production (~5 μW) limits the temperature change of the superfusate to ~0.7 mK at a flow rate of 0.5 ml/s. The temperature change is measured using a pair of solid-state thermoelectric modules (Watronix inbM1-8-1.3–0.4) designed for pumping heat. The thermoelectric modules are wired to nanovolt amplifiers (EM Electronics A10) with a gain of 10,000 and an equivalent noise resistance of 20 Ω (for comparison, each module has a resistance of 3 Ω).

The calorimeter housing is sandwiched between two temperature-controlled brass blocks (40 mm × 40 mm × 12 mm) as shown in Fig. 1. Each temperature-controlled block has a thermistor (GE Sensing EC95F103V) positioned in its center and a thermoelectric module (Multicomp MCPE1-07107AC-S) on its outer surface. The calorimeter and blocks are mounted on a motorized stage (Newport M-426 stage and Newport 850a motor) to allow movement of the bath to mount the muscle. The motorized stage, in turn, is placed on an optical breadboard.

During experiments, the optical breadboard is enclosed to ensure optical and thermal isolation. The breadboard is mounted 60 mm above an optical table using polyoxymethylene (acetal) legs to limit heat loss to the table. The breadboard and amplifiers for temperature measurement are further enclosed in a Faraday cage that also provides additional isolation of the system from air currents and ambient temperature changes.

A multilevel temperature control system is used with heating of the calorimeter housing itself performed via the temperature-controlled blocks above and below the housing. The inner enclosure is warmed using a heating pad (Omega SRFG-711/2-P) on the underside of the breadboard. The heating pad is driven by a Kepco BOP 100-2M power amplifier, the output of which is controlled using a proportional-integral (PI) controller with feedback from a thermistor located on the top surface of the breadboard. Further control is performed using...
the brass blocks mentioned above. These are driven using Apex Microtechnology PA75CX amplifiers and PI software-based controllers. All control is performed at a rate of 2 Hz.

Temperature control and temperature and force measurement are performed in software written in National Instruments LabVIEW 2013, operating in LabVIEW FPGA, LabVIEW RealTime and LabVIEW for Windows environments.

**Calibration.** The voltage to power sensitivity and transient response of the heat sensors were found by measuring the output of the thermoelectric modules in response to a known heat injection provided by a resistor (1-kΩ 0402 surface-mount package). The resistor was attached to a 0.7-mm outer diameter borosilicate capillary and positioned in the measurement capillary. A 0.5-V square pulse was applied to the resistor and the difference found between the outputs of the upstream and downstream thermoelectric module amplifiers. Figure 3 shows how the resulting signal is divided into three components: an initial flat section (Fig. 3A); a rising exponential first-order step response (Fig. 3B); and a falling exponential first-order response (Fig. 3C). The first section was averaged to give the initial steady-state value, while the other two sections were fitted with exponential step response functions using Matlab software. The difference in steady-state values was used to calculate the voltage to power sensitivity and the time constant was found from the exponential terms. The 90% rise time was also calculated from these traces. Calibration was performed at a range of flow rates and positions of the heat source to ascertain the location of maximum sensitivity within the measurement chamber.

The noise was measured in the absence of any heat source. Ten 100-s signals were recorded at 100 Hz and their power spectral densities were calculated. The resulting spectra were averaged and the root-mean-square magnitude calculated over a 50- to 5-Hz bandwidth. The noise was quantified at 22, 27, and 37°C, enabling the effect of the temperature controllers on the noise to be determined.

**A muscle experiment.** We demonstrate the capability of the calorimeter by measuring, simultaneously, the force and heat rate of an isolated rat cardiac trabecula at 37°C. The experiment was conducted according to protocols approved by the Animal Ethics Committee of the University of Auckland (AEC Approval No. R1057).

Following the methods outlined in Han et al. (17), a free-running trabecula was dissected out of the right ventricle, mounted in the calorimeter, and positioned within the measurement chamber (Fig. 1). The trabecula was superfused with 100%-oxygenated Tyrode solution at a rate of 0.5 μl/s. The superfusate contained 130 mM NaCl, 6 mM KCl, 1 mM MgCl₂, 0.5 mM NaH₂PO₄, 1.5 mM CaCl₂, 10 mM HEPES, and 10 mM glucose with pH corrected to 7.4 using Tris.

The trabecula, at slack length, was electrically stimulated at 5 Hz. It was then gradually stretched to its optimal length (Lₒ, the length at which developed force is maximal). At Lₒ, the trabecula was required to contract at a range of stimulus frequencies (3, 5, 6, and 8 Hz). Experiments were also performed at reduced muscle lengths (84 and 92% Lₒ) at 5-Hz stimulation. The muscle was allowed to rest (unstimulated) between interventions. Force and rate of heat production were continuously recorded throughout the experiment. At the completion of the experiment, the trabecula was removed and the heat artifact arising from electrical stimulation quantified, as a function of stimulus frequency.

The geometry of the trabecula (at Lₒ) was quantified from a photograph of the top view and reflected front view of the muscle. The cross section of the trabecula was assumed to approximate an ellipse with its axes oriented in the vertical and horizontal directions, thereby minimizing the error of estimation arising from the typical assumption that trabeculae have circular cross-sections when measurements are made only in a single plane. Muscle force was converted to stress (in kPa) and the rate of heat production was expressed (in kW/m³) by dividing, respectively, by muscle cross-sectional area and muscle volume (which was calculated from cross-sectional area and muscle length at Lₒ).

### RESULTS

We characterize our calorimeter by quantifying its sensitivity and time response, as functions of superfusate flow rate (see Figs. 4 and 6) and as a function of relative position of the heat source within the calorimeter (see Figs. 5 and 7) to find the location of maximal sensitivity. We also tabulate the noise of the calorimeter (Table 1) and show typical experimental records (see Fig. 8).

**Sensitivity.** The voltage to power sensitivity and temperature to power sensitivity at a range of flow rates are shown in Fig. 4. The voltage to power sensitivity is stated in terms of the unamplified voltage differences between the downstream and upstream thermoelectric modules. The temperature to power sensitivity was calculated using a thermoelectric sensitivity of 280 μV/K, predicted from a finite element model of the system.

The voltage to power sensitivity peaked at ~0.5 μl/s (Fig. 4). Thus this flow rate was chosen to examine the effect of the...
The maximum voltage to power sensitivity was 327 mV/W, which occurred when the heat source was positioned 1.5 mm downstream of the center of the measurement chamber. The center of a trabecula (≈2 mm long and 250 μm in diameter) will typically be located between 0.5 and 1.5 mm downstream from the bath center.

**Transient response.** The time constant and 90% rise time of the calorimeter were found from the same series of experiments as the sensitivities. The dependencies of the transient response parameters on flow rate can be seen in Fig. 6 and those on position in Fig. 7. Changing the position altered the time constant of each of the sensors as the heat source moved closer to or further away from them. As the heater was moved upstream, a simple exponential could no longer describe the response, as the upstream sensor warmed before the downstream sensor. Therefore, only the 90% rise time was reported for changing positions of the heat source. The 90% rise time was 12.9 s at the optimal sensitivity (0.5 μl/s, 1.25 mm downstream from the bath center). The time constant was found to be 5.2 s at the optimal sensitivity.

**Noise.** The root-mean-square voltage noise was calculated by integrating the power spectrum over a bandwidth of 50 mHz to 5 Hz for the thermoelectric modules and over a bandwidth of 50 mHz to 1 Hz for the temperature controllers. The thermoelectric and power sensitivities were used to calculate the temperature and power noise, respectively. The noise present under various experimental conditions is given in Table 1.

The expected voltage noise resulting from Johnson noise was calculated using

$$V_n = \sqrt{4k_B TR \Delta f}$$

where $k_B$ is the Boltzmann constant, $T$ is the temperature, $R$ is the combined resistance of the thermoelectric modules, and the amplifiers and $\Delta f$ is the measurement bandwidth. Over a 5-Hz bandwidth the Johnson noise is 1.93 nV. The measured voltage noise at 22°C (1.91 nV) demonstrates that the resolution is limited by Johnson noise. The temperature isolation system was thus capable of sufficiently minimizing environmental noise to the point that it may be ignored. The temperature controllers were very stable, although there was more noise as the temperature was increased. The presence of liquid flow through the measurement capillary increased the noise slightly when the system temperature was controlled above ambient but had no effect at 22°C. The additional noise measured by the thermoelectric signals as temperature increases is thus likely to be due to disturbances from the temperature controllers and the difference between the temperature of the sensor and that of the incoming liquid.

When liquid was flowing, the measured noise-equivalent temperatures were 0.4, 0.5, and 1.1 μK/√Hz at 22, 27, and 37°C, respectively. The measured noise-equivalent powers were 2.6, 3.3, and 7.1 nW/√Hz, respectively. The expected rate of heat output of a muscle is ~10 μW at 22 and at 27°C and 5 μW at 37°C. Consequently, the signal-to-noise ratios...
over the measurement bandwidth were 1,700, 1,350, and 315 at these temperatures, respectively.

Verification. Our calorimeter was designed to measure simultaneously the heat output and force production of cardiac trabeculae at physiological temperature. The ability of the calorimeter was exemplified by subjecting a rat cardiac trabecula, at its optimal length, to a range of stimulus frequencies, as well as to a range of muscle lengths. The data, normalized to muscle dimensions, can be seen in Fig. 8, which demonstrates the close coupling between mechanics and energetics. Changes in both stress and heat rate with stimulus frequency and muscle length are evidenced.

The results from the trabecula also demonstrate the virtue of providing orthogonal estimates of its dimensions. Its diameters were 0.28 mm (top view) and 0.44 mm (side view), implying an area of 0.097 mm² when assuming an elliptical cross section. In contrast, when assuming a circular cross-section, the estimated cross-sectional area could vary between 64 and 157% of the elliptical area, depending on the direction from which the muscle diameter is estimated. The significance of this may be emphasized by examining the output of the muscle when stimulated at 3 Hz. With the use of both diameters, the muscle stress would be 8.1 kPa and the rate of heat production would be 10.9 kW/m³. However, when calculated using either of the individual diameters, the estimated muscle stress would range from 5.2 to 12.8 kPa and muscle heat output would range from 7.0 to 17.2 kW/m³.

DISCUSSION

Since the first use of isolated cardiac trabeculae by ter Keurs (24) some 35 years ago, there have been several attempts to relate the production of force to the attendant liberation of heat of isolated cardiac trabeculae. In the 1990s, Schramm et al. (35) and Loiselle et al. (29) used the thermopile-based calorimeter of Daut and Elzinga (7) to measure the heat output of trabeculae at 37°C, in the complete absence of force measurement. About two decades later, Han et al. (16) constructed a thermopile-based micromechanocalorimeter that was capable of the first simultaneous measurements of force and heat output of isolated trabeculae. However, comparatively low-resolution temperature sensors were used and the inclusion of a mechanical testing apparatus prevented the use of a thermal control system similar to that of Daut and Elzinga. Consequently, the micromechanocalorimeter was limited to energetic measurements at 22°C.

We have designed, constructed, and tested a thermoelectric module-based calorimeter with an unprecedentedly high thermal resolution. The use of thermoelectric modules offers several advantages. First, it yields a 10-fold improvement in
temperature resolution over our previous micromechanocalorimeter (16, 37) and provides sufficiently low electrical and thermal noise to allow measurements of the extremely small heat output of trabeculae at 37°C, while retaining the ability to measure muscle force. Second, it is mechanically robust, which simplifies device construction and reduces the risk of damage to both the preparation and the instrument when mounting a trabecula. Third, it is easily incorporated into a multilayer temperature-controlled thermal isolation system, which achieves very stable temperature control and enables quick and easy heating and cooling of the calorimeter system. Fourth, it allows inclusion of a mirror oriented at 45° to our calorimeter chamber, which provides estimates of muscle thickness in two orthogonal planes, and thus better estimates of muscle dimensions for accurate normalization of muscle force and heat production. We discuss each of these merits in more details in the following paragraphs.

**Improved thermal resolution.** The micromechanocalorimeter was capable of a noise-equivalent temperature of 4.1 μK/√Hz and a noise-equivalent power of 37.1 nW/√Hz at 22°C (37), but our thermoelectric module-based calorimeter, at 22°C, has resolutions of 0.4 μK/√Hz and 2.6 nW/√Hz, respectively. These values are an order of magnitude better than any previous calorimeter systems (8, 12, 16, 22, 27, 33, 39).

Compared with the micromechanocalorimeter, the inclusion of mechanical testing apparatus to our calorimeter does not death the resolution of thermal measurement. Instead, at body temperature, the noise-equivalent temperature and power of our calorimeter are 1.1 μK/√Hz and 7.1 nW/√Hz, respectively. These values are comparable to those (1.3 μK/√Hz and 7.6 nW/√Hz, respectively) of the calorimeter of Daut and Elzinga (7).

**Multilevel temperature control.** Conventionally, muscle calorimeters have been required to be completely immersed in a temperature controlled water bath or to be in a chamber heated by temperature-controlled water flow (6, 7, 9, 38). In contrast, our calorimeter has a multilevel temperature control system using both thermoelectric modules and a resistive heater. The thermal isolation is capable of limiting environmental noise to such an extent that the control is sufficiently stable to retain high thermal resolutions and allow measurements of the rate of heat output of rat cardiac trabeculae even when experimental temperature is increased. Moreover, the use of thermoelectric modules allows rapid and easy control of temperature with both heating and cooling, thereby simplifying experimental protocols. The high resolutions and tight temperature control will extend the use of the device to higher temperatures.

**Improvements.** The innovative use of thermoelectric modules as both sensors and temperature controllers in this instrument has allowed us to achieve a substantial advance in performance over the previous micromechanocalorimeter. Our thermoelectric modules are significantly more robust than the thin-film thermopiles used in the micromechanocalorimeter, which eases device construction and has resulted in a higher-resolution, more-reliable, calorimeter. Additionally, muscle diameter and length were previously measured in a single plane, and hence trabeculae were assumed to be cylindrical, despite the evidence that trabeculae are typically elliptical in cross section (15). The error in estimating muscle cross-section has been greatly reduced by using a mirror to provide orthogonal views of muscle width, thereby allowing more accurate normalization of muscle force and volumetric heat production.

**Transient response.** The transient response of our calorimeter is not sufficiently rapid to allow partitioning of muscle heat into its “initial” and “recovery” components. This is a consequence of the flow-through design, that is, the superfusate flow rate limits the transient response of the heat sensor. By comparison, the flat-bed thermopile design such as that championed by Colin Gibbs (12) has sufficiently rapid response to resolve twitch heat and divide it into its two components: the
initial heat (reflecting the hydrolysis of ATP primarily by the myosin ATPase, the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase and the Na\(^+-K^+\) pump) and the recovery heat (reflecting the resynthesis of ATP in the mitochondria) (1, 2, 32).

**Conclusion.** Our thermoelectric module-based calorimeter is the first system capable of simultaneously measuring the force and heat output of isolated cardiac trabeculae at body temperature. By performing such measurements at body temperature, in a flow-through system, our calorimeter has extended the range and physiological relevance of previous energetics studies. The ability to perform dynamic mechanical experiments while simultaneously measuring the rate of heat production provides a device that can continue to refine, quantify, and improve our understanding of the energetics of cardiac muscle.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: C.M.J., J.-C.H., B.P.R., P.M.N., and A.J.T. conceived and designed research; C.M.J. and J.-C.H. performed experiments; C.M.J. and J.-C.H. analyzed data; C.M.J., B.P.R., and A.J.T. interpreted results of experiments; C.M.J. prepared figures; C.M.J. drafted manuscript; C.M.J., J.-C.H., B.P.R., P.M.N., and A.J.T. edited and revised manuscript; C.M.J., J.-C.H., B.P.R., P.M.N., and A.J.T. approved final version of manuscript.

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