A Flowthrough Infusion Calorimeter for Measuring Muscle Energetics: Design and Performance

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Abstract—The heat produced during muscle contraction arises from distinct underlying cellular processes. While current calorimeters can measure total muscle heat production, none can allow accurate filtration and infusion of chemical drugs to probe the thermodynamic mechanisms that underlie various heart diseases. We have constructed a calorimeter that incorporates an infusion system for exposing muscle samples to time-varying concentrations of pharmacological agents. The design of the instrument is informed by the development and the use of analytical lumped parameter and finite-element models. This new infusion calorimeter uses thermopile-based heat sensors to achieve a noise-equivalent power of 2.6 nW·Hz−1/2 and a signal-to-noise ratio of up to approximately 1700. We report on the modeling and optimization techniques that were used to inform our design. We characterize the instrument’s performance and demonstrate its utility by exposing muscle to pharmacological interventions that mimic two cardiac disease conditions, acute force impairment and dynamic contracture.

Index Terms—Calorimeter, energetics, muscle, thermopile.

I. INTRODUCTION

MUSCLE is a thermodynamic machine that converts chemical enthalpy into mechanical work. The efficiency with which it does so is modest (~15 %) [1]–[3]; the majority of the change in enthalpy is liberated as heat. The heat produced by muscle tissues arises from the various cellular processes that underlie muscle function. These processes are involved in general maintenance of the muscle tissues (“basal heat”), activating the muscle to a state where it is ready to produce force (“activation heat”), and the production of force and work (“cross-bridge heat”). Each of these processes can be perturbed by physiological and pharmacological interventions and can become dysfunctional in disease.

For more than a century, measurements of heat production have provided useful insight into the characteristics of the muscle tissue, in health and disease [5]. A variety of calorimeters have been constructed to measure the heat production of muscle samples maintained by a supply of oxygen and nutrients [3], [6], [7]. However, it is only relatively recently that flowthrough calorimeters have been constructed in a form that allows concurrent measurements of heat and force [1], [4].

Despite recent advances in the design of calorimeters for the study of cardiac energetics, no previously reported calorimeters have allowed muscle samples to be systematically exposed to pharmacological interventions that mimic many cardiac disease conditions. The development of this capability would allow the use of system identification techniques to probe, individually, the processes that underlie muscle contraction and thus advance our understanding of the cellular mechanisms behind cardiac failure.

To address this deficit, we have recently constructed a new flowthrough infusion calorimeter that allows us to examine the energetic processes within cardiac muscle samples by perturbing the chemical composition of the fluids to which they are exposed [8]. Our calorimeter is unique in allowing the muscle heat rate to be measured simultaneously with force and shortening during systematic pharmacological interventions.

Here, we report in detail on its design and construction and quantify its performance. We develop equations and models that describe the physics of the heat measurement technique, predict the performance of different design options, and thereby identify the design of a suitable device. The new flowthrough infusion calorimeter successfully allows us to quantitify the energetic processes within cardiac muscle samples by perturbing the chemical composition of the fluids to which they are exposed. This is demonstrated in a series of experiments in which: 1) we sequentially inhibit and engage the contractile processes in a sample of cardiac tissue, by chemical intervention, and 2) we measure the maximum total heat production of a muscle as it transits into chemically induced contracture. These two experiments mimic cardiac disease states where the molecular force-producing elements are impaired or challenged.

II. DESIGN CONSIDERATIONS

A. Measurement Principle

Our muscle calorimeter continues the flowthrough differential approach that has been used successfully in previous devices [1], [4], [7], [9]. In these instruments, the rate of heat production of a muscle sample is inferred from the temperature increase it imparts to a fluid flowing along its length. A muscle sample is advanced into the center of a “measurement chamber” (Fig. 1) comprising a 1-mm square cross sectional borosilicate glass tube filled with flowing oxygenated superfusate. Platinum hooks secure the ends of the muscle to a linear positioner (upstream) and force transducer (downstream) via quartz tubes. Temperature sensors are placed in proximity to the external surface of the glass tube, upstream and downstream of the muscle. The muscle heat rate is inferred from the difference between the temperature estimates gathered at these two locations.

Others have developed custom thin-film thermopile temperature sensors for use in microfluidic drug reaction calorimeters [10], [11]. These sensors can provide resolutions in the 10 nW range, but suffer from the fragility of the substrates on which they are constructed and are not conducive to the open-ended configuration required for this application. We have previously reported on our own novel use of thin-film thermopiles (designed for use in infrared thermometers but used by us as conduction-mode temperature sensors [6]) and on our experimental development of vapor–pressure–temperature sensors [12], [13]. While both of these techniques have proven feasible and useful, they are limited by the fragility and inferior reliability of the sensors.

In contrast, thermoelectric modules (TEMs; often referred to as “Peltier coolers” or “thermoelectric heat pumps”) are a robust and readily available alternative form of thermopile. These sensors have found use in balance calorimeters for RF power loss measurement [14], [15] and in isothermal titration calorimeters for drug discovery [16], [17]. In this paper, we report on our recent use of TEMs as temperature sensors in our flowthrough infusion calorimeter.

TEMs are thermopiles comprising alternating pillars of two thermocouple materials (typically alloys of bismuth telluride) thermally arranged in parallel but electrically connected in series. While they are primarily intended to actively pump heat, they can also be used as sensors that provide a voltage in proportion to the temperature difference developed between their two faces. By bonding one face of a TEM to the surface of the glass tube and its other surface to a reference material (copper housing in Fig. 2), the temperature difference between the glass tube and the reference material can be inferred from the thermopile voltage. The difference between the upstream and downstream sensor voltage signals is used to infer muscle heat output, with the added advantage that common-mode noise is removed.

TEMs of a variety of dimensions are readily available. In order to select thermopiles with dimensions and materials suitable for use in a flowthrough calorimeter, we turned to mathematical modeling methods. Others have developed models of heat flow in TEMs being used in applications for power generation [18], [19]. However, in this application, no electrical power is demanded by the amplifying electronics, and the flowthrough configuration of our device demands the consideration of additional physics. Thus, we first developed a lumped parameter model of our proposed design to narrow our selection of thermopile devices and then used finite-element methods to determine an appropriate final selection and arrangement in a calorimeter. Our goal was to determine a calorimeter design that provided a high signal-to-noise ratio (SNR) but was also relatively straightforward to assemble, and robust.

B. Analytical Modeling

The voltage generated by a thermopile sensor arises from the temperature difference developed between its faces and is proportional to the number of thermocouple pillar pairs in the array. The total thermal resistance of the thermopile should be high compared with the thermal resistance between the thermopile and the heat source, in order to maximize the temperature gradient across the sensor and thus the signal. The thermal resistances of the pillars add in parallel—increasing their number or area decreases the total thermal resistance.
At the same time, the electrical noise generated in the thermopile is proportional to the square root of their total series electrical resistance, which increases with the number of pillars and decreases with their cross-sectional area.

A lumped parameter analytical model was thus used to investigate how these thermopile parameters together determine the sensitivity and noise parameters of the proposed design and its resulting SNR. The model examined the heat flow path between the fluid and the copper housing across the downstream thermopile as shown in Fig. 2, and the corresponding temperature differences that arise. A steady-state model with adiabatic boundaries was considered, with heat permitted to flow into this region at rate \( \dot{Q} \). A portion of this heat is conducted through the thermopile, whereas the remainder is transported from the fluid outlet. The thermopile substrate’s thermal conductivity was at least 20-fold higher than the glass and the fluid and was thus modeled as a thermal conductor of infinite conductivity; the conduction of heat in the \( x \)-direction, except via the substrate, was not considered in this model. The fluid temperature was assumed to be constant in the \( y \)- and \( z \)-directions, and the copper housing was taken to be a perfect heat sink. All temperatures are reported relative to the housing.

The temperature difference between the fluid and the substrate at any point may be found by performing a heat balance across the total fluid volume above the thermopile. Solving for the substrate temperature gives

\[
T_s = \frac{\dot{Q} R_p}{\dot{m} C_p R_p} \exp \left( -\frac{X}{\dot{m} C_p R_g} \right)
\]

where \( T_f \) and \( T_s \) are the fluid and substrate temperature, respectively, \( x \) is the distance from the left-hand end of the thermopile, \( \dot{m} \) is the fluid mass flow rate, \( C_p \) is the fluid specific heat capacity, \( X \) is the total length, and \( R_g \) is the absolute thermal resistance of the glass. The rate of energy entering the fluid volume is equal to the rate of energy output of the fluid, i.e., \( \dot{m} C_p T_f(0) = \dot{Q} \).

The substrate temperature may be found by considering a heat balance across the total fluid volume above the thermopile. Solving for the substrate temperature gives

\[
T_s = \frac{\dot{Q} R_p}{\dot{m} C_p R_p} \exp \left( -\frac{X}{\dot{m} C_p R_g} \right)
\]

where \( R_p \) is the absolute thermal resistance of the pillars and

\[
\varepsilon = 1 - \exp \left( -\frac{1}{\dot{m} C_p R_g} \right)
\]

where \( \varepsilon \) is the heat exchanger effectiveness and reflects the efficiency of heat transfer between the fluid and the substrate if the substrate is assumed to be a heat sink, i.e., \( R_g \) is zero.

Given the model assumptions, the temperature difference across the pillars \( dT \) is equal to the substrate temperature. The dependence of \( dT \) on the absolute thermal resistance of the pillars and the glass is shown in Fig. 3. As expected, the temperature difference is maximized by using a system with a high combined pillar thermal resistance and a low glass thermal resistance. However, we must also take into account the effect of these design choices on the corresponding electrical properties of the sensor.

The voltage signal arising in the sensor may be estimated by multiplying the temperature difference by the number of thermocouples \( n \) and their Seebeck coefficient \( S \). The dominant noise source in thermocouples is typically the Johnson noise. Thus, the SNR may be found by dividing the expected voltage difference by the Johnson noise, such that

\[
\text{SNR} = \frac{n S dT}{\sqrt{4 k_B T R_p \Delta f}}
\]

where \( k_B \) is the Boltzmann constant, \( T \) is the absolute temperature, \( R_p \) is the electrical resistance, and \( \Delta f \) is the measurement bandwidth.

Combining (2) and (4), in addition to the electrical resistance as a function of the number of thermocouples, the ratio of pillar area \( (A_p) \) to height \( (Z_p) \) and the pillar’s electrical resistivity \( (\rho) \) gives

\[
\text{SNR} = \frac{\dot{Q} S \sqrt{R_p}}{\sqrt{16 k_B T R_p \Delta f}} \cdot \frac{\varepsilon \sqrt{Z_p}}{1 + \dot{m} C_p R_p \varepsilon}
\]

where \( \kappa_p \) is the thermal conductivity of the pillars.

The dependence of the SNR on the number of pillars and their form factor is displayed in Fig. 4. For a sensor of given length \( X \), the maximum SNR is independent of the pillar geometry. The maxima occur at the same pillar thermal resistances; i.e., there is an ideal thermal resistance for any combination of volumetric flow rate and specific heat capacity of the fluid. The thermal resistance at which the maximum occurs depends on \( R_g \), but the effect is minimal over a relatively wide range. \( R_g \) can be up to 30% of \( R_p \) with only a 5% change in the value of \( R_p \) at which the maximum SNR occurs. The dependence of the SNR on the product of the mass flow rate and the specific heat capacity for a range of pillar thermal resistances is shown in Fig. 5.

Equation (5) can be rewritten as

\[
\text{SNR} = \frac{\dot{Q}}{2} \frac{\sqrt{Z T}}{4 k_B \Delta f T^2 R_p} \cdot \frac{\varepsilon \sqrt{Z_p}}{1 + \dot{m} C_p R_p \varepsilon}
\]
TABLE I
THERMOPILE SPECIFICATIONS

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Module</th>
<th>Dimensions X (mm) • Y (mm) • Z (mm)</th>
<th>Number of Thermocouples</th>
<th>Resistance (Ω)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laird</td>
<td>OT08-0203</td>
<td>3.4 • 3.4 • 0.50 • 3.4 • 1.61 • 0.50</td>
<td>4</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>OT08-0305</td>
<td>4.9 • 4.9 • 0.50 • 4.9 • 4.9 • 0.50</td>
<td>10</td>
<td>1.43</td>
</tr>
<tr>
<td>Micropelt</td>
<td>MPC-D303</td>
<td>0.83 • 2.42 • 0.53 • 0.83 • 1.18 • 0.53</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>MPG-D651</td>
<td>2.50 • 3.35 • 0.53 • 2.50 • 2.50 • 0.53</td>
<td>286</td>
<td>185</td>
</tr>
<tr>
<td>Watronix</td>
<td>inbM1-4-1.3-0.4</td>
<td>2.26 • 2.08 • 0.50 • 2.08 • 1.14 • 0.50</td>
<td>4</td>
<td>2.7</td>
</tr>
<tr>
<td></td>
<td>inbM1-4-1.6-1.3</td>
<td>4.18 • 3.34 • 0.50 • 4.18 • 2.18 • 0.50</td>
<td>4</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>inbM1-8-1.3-0.4</td>
<td>2.04 • 3.22 • 0.50 • 2.04 • 2.04 • 0.50</td>
<td>8</td>
<td>2.97</td>
</tr>
</tbody>
</table>

Fig. 4. Predicted SNR across a thermopile depending upon the number of thermocouples and the ratio of pillar area ($A_p$) to pillar height ($Z_p$). $A_p/Z_p$ varies logarithmically from 10 to 1000 nm. The thermal resistance of the glass was 100 K/W, the fluid flow rate was 0.5 μL/s, the muscle heat output was 5 μW, and the bandwidth was 5 Hz.

where $G_p$ is the thermal conductance of the pillar layer and $ZT$ is the dimensionless thermoelectric figure of merit often used in the temperature control literature [20]. $Z$ is the ratio of the square of the Seebeck coefficient to the product of the resistivity and the thermal conductivity and is closely linked to the efficiency of thermoelectric devices. A more efficient thermoelectric material therefore will also be a better power sensor.

The denominator of the second term in (6) is the phonon noise, a result of the random movement of energy carriers between the sensor and its environment [21], [22]. The movement occurs even when the sensor is in equilibrium with the environment, thus reflecting the absolute limit on thermal power measurement.

Using this model, a range of commercially available TEM families were assessed. Table I lists the properties of the sensors, all of which use bismuth telluride thermocouples. Bismuth telluride is commonly used for its high thermoelectric figure of merit ($ZT = 0.80$ at 25 °C).

The SNR of these various sensor designs is shown in Fig. 6 as a function of the module length. While the sensors themselves are available only in discrete lengths (filled symbols), this approach allows us to identify for each family an “optimal” length beyond which the SNR is not enhanced by the use of additional thermopile pillars.

Fig. 6 shows that the Laird and Watronix thermopile families provide similar peak SNR. These thermopiles have a few
tall thermocouple pillars, whereas the Micropelt sensors are of a radically different design, comprising many very short pillars that may be more suitable for other applications such as energy harvesting. The results show that the peak SNR is obtained at a thermopile length between 2 mm and 3 mm.

### C. Finite-Element Modeling

The analytical model developed in Section II-B provides a useful design guidance but makes a number of simplifying assumptions that require further consideration. In particular, the effects of the assumptions regarding heat loss to the surrounding air and heat conduction in the fluid, and the effect of the location of the temperature sensors, are best examined by using finite-element methods. In addition, the use of finite-element methods allows us to consider the potential advantage of locating additional sensors on the side faces of the glass tube.

Geometric models of the TEMs and the muscle calorimeter were generated in Solidworks and then imported into ANSYS CFX for the analysis. Models were meshed using up to 850000 tetrahedral elements. The calorimeters were modeled with up to three TEMs (on the bottom and two side faces) located upstream and downstream of the muscle heat-source. Fluid flow and muscle heat output were set at 0.5 μL/s and 5 μW, respectively. The flow was laminar as Reynolds’s number was approximately 0.5. The top and bottom surfaces of the calorimeter block were held at ambient temperature, whereas the sides of the calorimeter were modeled as adiabatic boundaries. The fluid and thermal flow equations were solved to steady state.

![Fig. 7. Predicted steady-state temperature increases down the center line of the calorimeter. The fluid flow rate was 0.5 μL/s and the muscle heat output was 5 μW.](image)

An example of the steady-state temperature distribution predicted by the finite-element model is shown in Fig. 7. While the maximum temperature change of the muscle approached 3 mK, the temperature increase of the downstream thermopile was no more than 1 mK across all of the analyses. The temperature difference between the top and bottom substrates was extracted from the model at the upstream and downstream locations. The difference between the downstream and upstream temperature differences was then calculated, allowing the expected voltage signal to be found. A Seebeck coefficient of 202 μV/K was assumed for the bismuth telluride thermocouples (Laird Technologies, 2013). The voltage signal was then divided by the Johnson noise to give the expected SNR of each design.

Each of the thermopiles listed in Table I was examined using this finite-element modeling approach, with the results (FEM SNR) shown in Table II. The general trends revealed by this analysis are similar to the analytical predictions but have much smaller SNRs, as the analysis now takes into account heat loss.

### Table II

**Thermopile Modeling Results**

<table>
<thead>
<tr>
<th>Module</th>
<th># of modules per end</th>
<th>$\Delta T$ (mK)</th>
<th>$V_r$ (μV)$^a$</th>
<th>$V_\nu$ (μV)$^b$</th>
<th>Analytical SNR</th>
<th>FEM SNR</th>
<th>Amp-limited SNR$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laird OT08-0203</td>
<td>1</td>
<td>0.64</td>
<td>0.74</td>
<td>0.3</td>
<td>4790</td>
<td>2000</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.46</td>
<td>1.08</td>
<td>0.43</td>
<td>4720</td>
<td>1920</td>
<td>440</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.36</td>
<td>1.24</td>
<td>0.52</td>
<td>4440</td>
<td>1720</td>
<td>470</td>
</tr>
<tr>
<td>Laird OT08-0305</td>
<td>1</td>
<td>0.46</td>
<td>1.06</td>
<td>0.43</td>
<td>4720</td>
<td>1910</td>
<td>430</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.32</td>
<td>1.46</td>
<td>0.6</td>
<td>4160</td>
<td>1670</td>
<td>520</td>
</tr>
<tr>
<td>Micropelt MPC-D303</td>
<td>1</td>
<td>0.15</td>
<td>0.17</td>
<td>0.22</td>
<td>3000</td>
<td>460</td>
<td>60</td>
</tr>
<tr>
<td>Micropelt MPG-D651</td>
<td>1</td>
<td>0.006</td>
<td>0.52</td>
<td>5.52</td>
<td>2520</td>
<td>70</td>
<td>60</td>
</tr>
<tr>
<td>Watronix inbM1-4-1.3-0.4</td>
<td>1</td>
<td>0.95</td>
<td>1.11</td>
<td>0.46</td>
<td>4030</td>
<td>1670</td>
<td>410</td>
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<tr>
<td>Watronix inbM1-4-1.6-1.3</td>
<td>1</td>
<td>0.39</td>
<td>0.45</td>
<td>0.25</td>
<td>4660</td>
<td>1280</td>
<td>180</td>
</tr>
<tr>
<td>Watronix inbM1-8-1.3-0.4</td>
<td>1</td>
<td>0.76</td>
<td>1.77</td>
<td>0.7</td>
<td>4650</td>
<td>1770</td>
<td>630</td>
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<tr>
<td></td>
<td>2</td>
<td>0.65</td>
<td>3.03</td>
<td>0.99</td>
<td>4820</td>
<td>2450</td>
<td>1170</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.54</td>
<td>3.76</td>
<td>1.21</td>
<td>4650</td>
<td>2390</td>
<td>1330</td>
</tr>
</tbody>
</table>

$^a$ $V_r$ indicates the expected signal voltage
$^b$ $V_\nu$ indicates the expected Johnson noise.
$^c$ Amp-limited SNR indicates the SNR when considering both the noise-equivalent resistances of the amplifiers and the resistances of the TEMs.
into the surrounding air, and heat conduction within the fluid. For example, the analytical model gave an SNR of 4650 for a single Watronix inbM1-8-1.3-0.4 thermopile; the equivalent finite-element model predicted an SNR of 1770.

In practice, the amplifiers used to measure the output of the thermopiles also inject noise into the measurement circuit. Consequently, the amplifier-limited SNR must also be considered. The nanovolt amplifiers chosen for use in this device (EM electronics A10) provided input noise equivalent to a 20 Ω resistor, which can be considered in series with the thermopile resistance. The corresponding “Amp-limited SNR” is shown in the final column of Table II.

This analysis revealed that the Watronix inbM1-8-1.3-0.4 thermopiles provided a superior performance to the other options. Even the use of a single thermopile, located at each temperature measurement point, upstream and downstream of the muscle, provides an SNR of 630, when taking into account the amplifier noise. The 2 mm × 2 mm substrate dimensions are a good match to the outer dimensions of the glass tube. The use of two or three sensors at the temperature measurement positions further increases the SNR to 1170 and 1330, respectively, at the expense of construction complexity.

III. METHODS

A. Muscle Calorimeter

The analyses detailed in Section II allowed us to select a design for our calorimeter that provides enhanced performance over previous devices, yet was relatively simple to construct and robust against regular use. Our analysis had indicated that the Watronix inbM1-8-1.3-0.4 thermopiles would provide an SNR of at least 630. To minimize construction complexity, we thus chose to design a calorimeter with only a single thermopile located upstream and downstream of the muscle.

The selected thermopiles (Watronix inbM1-8-1.3-0.4) were placed 4 mm apart with their measurement junctions attached to the lower surface of a glass tube of square cross-section, as shown in Fig. 8. The reference end of the thermopiles was adhered to a parylene-coated gold-plated copper housing using ceramic-loaded epoxy adhesive.

The thermopile voltages were amplified by preamplifiers (EM Electronics A10, gain = 10000) and digitized at a rate of 50 kHz, using simultaneous-sampling 24-bit analog-to-digital converters (National Instruments NI 9239), and electronically filtered and downsampled to 10 Hz bandwidth. The difference between the upstream and downstream voltages was computed in software and divided by the flow-rate-dependent sensitivity (in V/W) to provide an estimate of the muscle heat rate.

Platinum electrodes (not shown) placed at each end of the measurement chamber enabled muscle contraction via field stimulation. As in our previous devices, quartz tubes (0.7 mm OD) and platinum hooks were used to secure the muscle to a linear positioner (upstream) and interferometric force transducer (downstream). The custom-made force transducer (see [1] for details) comprised a cantilever of stainless steel (1 mm width, 8 mm length, and 0.15 mm thickness). The deflection of the cantilever was measured by a laser interferometer (Keysight Technologies) to 0.31 nm resolution, providing an estimate of muscle force. This type of force transducer is robust, provides high resolution, and does not inject heat into the calorimeter environment. All softwares were developed in the LabVIEW programming environment (National Instruments).

B. Fluid-Titration and Mixing System

One of the key requirements for the instrument was the ability to infuse a concentrated pharmacological agent into a flowing, oxygenated Tyrode (saline) solution. This was achieved using two synchronized pumping systems; their outflows were combined just prior to entry into the calorimeter body, in order to allow the heat of dilution to dissipate before the fluid reached the temperature sensors and muscle sample (see Fig. 9). Since the sensitivity of the calorimeter is dependent on flow rate, care was taken to ensure that the flow rate was smooth and constant.

The Tyrode solution fluid flow control system comprised a vertical linear motor (Parker-Daedel MX80L with pneumatic counterbalance (not shown)) that controlled the elevation of a heated water-jacketed oxygenation reservoir. Fluid drained from the chamber into the mixer via a flow sensor (Sensirion SLI-0430), oxygen-impervious tubing (Tygon), and a flow restrictor (200 μm diameter × 30 mm length). The flow rate of the Tyrode solution was measured to 16-bit resolution at a rate of 10 Hz and transferred to a control computer by the RS-422 protocol. A software-based feedback algorithm adjusted the height of the oxygenation chamber to achieve the desired fluid flow rate, in the range 0.4 μL/s to 0.6 μL/s.

The concentrate was delivered by a computer-controllable syringe pump (PhD Ultra, Harvard Apparatus) from a 50 μL gas-tight syringe (Hamilton, #1705) through 200 μm-inner diameter flexible fused silica tubing (Polymicro). The concentrate typically was delivered at a rate of 20 nL/s and mixed with the Tyrode solution which was flowing at a rate...
KCl; 1.5 mmol/L MgCl₂; 0.5 mmol/L NaH₂PO₄; 1.5 mmol/L superfusate (Tyrode solution: 130 mmol/L NaCl; 6 mmol/L KCl; 1.5 mmol/L MgCl₂; 0.5 mmol/L NaH₂PO₄; 1.5 mmol/L CaCl₂; 10 mmol/L HEPES; and 10 mmol/L glucose with pH corrected to 7.4 using Tris). For measurements of barium-induced muscle contracture, the concentration of CaCl₂ in the Tyrode solution was reduced to 0.1 mmol/L. The Tyrode solution was vigorously bubbled with 100 % O₂, at 41 °C, and passed along the length of the muscle at a precisely controlled rate in the range of 0.5 μL/s to 0.55 μL/s.

Each trabecula was electrically stimulated at 3 Hz (amplitude between 6 V and 10 V and duration from 6 ms to 10 ms) until fully recovered from dissection, and an optimal calorimeter chamber temperature was achieved. Trabeculae were considered to have recovered when force production reached a steady state, and reducing the stimulus voltage or stimulus duration to typical supra-threshold values (6 V and 6 ms) had no impact on the force production. Muscle volume was estimated by using a microscope graticule to estimate the length and two orthogonal diameters of the specimen.

### E. Experiments

1) **Calorimeter Performance:** The sensitivity, noise, and time response of the calorimeter were determined at an operating temperature of 37 °C. Calibration was performed by liberating a square wave of electrical power from a thin-film resistor (1 kΩ, 0402) that was soldered to a flexible circuit film and placed in the center of the measurement chamber. The difference between the thermopile voltages was determined while the Tyrode solution was passed through the calorimeter at 0.5 μL/s, 0.52 μL/s, and 0.55 μL/s.

The noise of the voltage signal was measured in the absence of any heat source. Ten signals (each with a period of 100 s) were recorded and their power spectral densities were calculated. The power spectral densities were averaged, and the rms magnitude of the resulting signal was calculated over a 50-mHz to 5 Hz bandwidth. These experiments were performed with and without fluid flow at room temperature (approximately 23 °C, although the temperature was not explicitly controlled), 27 °C, and 37 °C.

The variability of the flow rate of the Tyrode solution was quantified by the amplitude spectral density of the flow rate measured over a bandwidth of 50 mHz to 1 Hz. The variability of flow from the syringe pump was not directly measured but its contribution to overall flow noise is likely to be negligible.

2) **Heat of Mixing:** One of the motivations for constructing this instrument was the need to quantify the contribution of activation heat to total muscle heat production at 37 °C. [“Activation heat” is a term that refers to the energetic cost of engaging and maintaining the cellular processes that put the muscle in a state where it is able to produce force; the energetic cost of muscle force-production itself (“cross-bridge heat”) sums with this to give the total heat (above resting heat) evolved by the muscle during force production.] However, before conducting these measurements, it was first necessary to explore the possible effect of the heat of dilution or mixing.

First, the Tyrode solution was flowed into the calorimeter to establish a baseline value for subsequent infusions. Second, we infused, at 20 nL/s, a concentrate containing a 10 % solution of dimethylsulfoxide (DMSO)—a biologically compatible solvent that is convenient for use with salt compounds. After initiating infusion, we looked for any change in the temperature signals detected in the calorimeter, which might indicate the presence of the heat of dilution. Next, we infused into the calorimeter a 10 % DMSO solution containing 0.375 mmol/L blebbistatin, an inhibitor of the muscle cross-bridge force production cycle [24]. After it had mixed with the Tyrode solution, the final concentration of blebbistatin was 15 μmol L⁻¹. We recorded any consequent changes to heat production. Finally, the infusion was ceased, allowing the Tyrode solution alone to flow into the measurement chamber. The duration of each intervention was 30 min.
3) Heat of Activation: Using a second muscle, we then conducted a series of measurements of muscle heat production in the absence and presence of blebbistatin. A muscle was mounted in the calorimeter and allowed to settle to steady-state force during exposure to the Tyrode solution. The muscle was then stretched to its optimal length $L_o$—the length beyond which no further active force could be developed without additional “resting” (i.e., passive) force. Muscle heat rate was measured over a period of 60 s, as the muscle was stimulated at a rate of 5 Hz. Muscle length was shortened in five steps to approximately 0.77 $L_o$—a length at which no active force was produced during a muscle twitch. Subsequently, the muscle was stretched back to $L_o$, and the experimental protocol was repeated during the infusion of DMSO and blebbistatin.

4) Heat of Forced Contracture: We conducted a third muscle experiment to determine the total heat rate of a muscle during a chemically initiated sustained contracture. This experiment was designed to reveal the oxidative contribution to heat release during the life-threatening condition of malignant hyperthermia [25]. This experiment was conducted at 32 °C, to reproduce the temperature typically experienced by peripheral muscle tissue, in vivo [26], and to reduce the risk of the irreversible muscle damage that often ensues at the body temperature [27].

First, a solution containing 35 mmol/L CaCl$_2$ was supplied to the measurement chamber via the infusion syringe pump at a rate of 20 nL/s to provide a calcium concentration of 1.5 mmol/L when mixed with the Tyrode solution. The total heat rate and force were recorded as the muscle was stimulated at a rate of 1 Hz. Once a steady state was achieved, stimulation was ceased, and the calcium solution in the syringe pump was replaced with a solution containing 37.5 mmol/L BaCl$_2$ to provide 1.5 mmol/L barium concentration in the measurement chamber. The muscle force and heat production were recorded simultaneously.

### IV. RESULTS

#### A. Calorimeter Performance

The calibration of the calorimeter revealed its sensitivity at a flow rate of 0.5 μL/s to be 3850 V/W and to decline with increasing flow rate (Fig. 10). The step response time of the calorimeter at this flow rate was similar to that of our previous designs (approximately 15 s).

#### B. Heat of Mixing

In this experiment, the heat measurement system was nulled with the muscle at rest in the center of the chamber [Fig. 11(a)]. Upon the arrival of the DMSO solution in the measurement chamber, a small increase (0.11 μW) in heat rate was detected [Fig. 11(b)]. Addition of blebbistatin to the DMSO made no appreciable increase in heat rate [Fig. 11(c)]. Thus, the heat of mixing (for this experiment) was 0.11 μW and was accounted for in subsequent analyses. Finally, the infusion was terminated, allowing a normal Tyrode solution to superfuse the muscle. The resulting muscle heat rate is shown in Fig. 11(d).

#### C. Heat of Activation

The heat rate of activation was found to be invariant with a muscle length (Fig. 12) at approximately 1 μW. The active heat rate was found to increase to approximately 3.6 μW when this muscle was stretched to $L_o$. Thus, at optimal length, approximately 27% of the energy consumed by this

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**Fig. 10.** Thermal sensitivity as a function of superfusate flow rate.

**Fig. 11.** Heat rate and force measurements during (a)-(c) resting and (d) actively contracting states of a superfused muscle. (a) During Tyrode infusion. (b) During infusion of DMSO. (c) During infusion of DMSO and blebbistatin. (d) During a typical 180 s period of muscle heat-production at a stimulus frequency of 5 Hz. Arrival (blue arrows) of the infused mixture into the measurement chamber and its exit (red arrows).
muscle was involved with activating the contractile machinery, whereas the remainder of the heat was associated with producing force.

**D. Heat of Forced Contracture**

The active heat rate during 1 Hz electrical stimulation was approximately 2.5 μW (Fig. 13). When electrical stimulation was ceased, the heat rate began to decline back toward its “resting” level. Upon infusion of BaCl₂, the muscle heat rate increased to approximately 8 μW as the muscle progressively transitioned into contracture. During this period, the muscle began to spontaneously twitch, whereas the resting force continuously increased, in accord with early observations [28]. After approximately 2000 s of infusion, the muscle was in complete contracture. When normalized to the muscle volume, the heat rate amounted to 48 kW/m³. When the infusion was halted, the muscle heat rate gradually declined to its resting value.

**V. DISCUSSION AND CONCLUSION**

The sensitivity of this calorimeter exceeds (by up to 60%) that reported for all of our previous calorimeters. This is due, in part, to our use of low-resistance TEMs and low-noise preamplifiers for temperature sensing but may also owe to our use of copper-coated polyimide film for making electrical connection to the heat source during calibration. It is probable that with this approach, the very low thermal conductivity of the traces in the circuit prevents heat conduction from the resistor through the electrical connections, leading to more heat conducting directly into the superfusate solution, and a larger response from the downstream temperature sensor. Consequently, our calorimeter can resolve muscle heat rate to approximately 6 nW; given a maximum measured heat rate of 8 μW, this corresponds to an SNR of approximately 1350.

The response time of the calorimeter at this flow rate is similar to that of our previous designs (~6 s) but could be decreased by increasing the flow rate, at the expense of sensitivity [6]. The calorimeter thus has a much lower bandwidth than the dynamics of muscle energetics and cannot fully resolve the time course of heat-release during each twitch [Fig. 13 (insets)]. Yet, it may be possible to deconvolve the output signal (representing the heat accumulated across several seconds) with the calorimeter impulse response function to reveal more information to a higher temporal resolution.

The oxygenation chamber can contain a sufficient Tyrode solution for 24 h of continuous experimentation. The syringe pump can contain up to 50 μL of concentrate, at an infusion rate of 20 nL/s; this implies a total possible infusion time of 2500 s (approximately 40 min). With the use of a two-way tap, the syringe can be refilled and infusion resumed in a matter of seconds. Although the speed of the syringe pump can be changed several times per second, the time delay for the infused fluid to progress to the measurement chamber may allow a significant diffusion of concentrate into the preceding or following Tyrode solution. The potential for this effect to occur has not yet been examined.

The heat of dilution in the mixing experiment was consistent and small in comparison with the heat evolved by the muscle (Fig. 11). However, it may be necessary to determine whether this heat rate is similar for other infusions at other concentrations, on a case-by-case basis. In future, we will examine whether forced air flow could be used to improve the coupling between the mixer and the surrounding temperature-controlled air.

The direct measurements of heat of activation by this method have revealed a new finding that the heat of activation in the cardiac muscle is independent of the muscle length. This finding has important implications for our interpretation of muscle heat during work production [29].

Finally, the barium experiment has provided us with an estimate of the total heat output of a muscle during chemically induced contracture. The heat rate of 48 W/m³ is similar to that reported in other studies of cardiac tissues subjected to high-frequency electrical stimulation [30].

We are now equipped with a unique infusion calorimeter, through which we can infuse pharmacological drugs, for understanding muscle energetics under various interventions. We plan to use this device to study the effect of drug treatments on diseased cardiac and skeletal muscle tissues. We also plan to integrate this calorimeter design into a fluorescence imaging system and a brightfield microscope to measure even more muscle characteristic parameters, including intracellular calcium release and sarcomere shortening.
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