Muscle heat: a window into the thermodynamics of a molecular machine

Denis Scott Loiselle,1,3 Callum Michael Johnston,1 June-Chiew Han,1 Poul Michael Fønss Nielsen,1,2 and Andrew James Taberner1,2

1Auckland Bioengineering Institute, The University of Auckland, Auckland, New Zealand; 2Department of Engineering Science, The University of Auckland, Auckland, New Zealand; and 3Department of Physiology, The University of Auckland, Auckland, New Zealand

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Loiselle DS, Johnston CM, Han JC, Nielsen PM, Taberner AJ. Muscle heat: a window into the thermodynamics of a molecular machine. Am J Physiol Heart Circ Physiol 310: H311–H325, 2016. First published November 20, 2015; doi:10.1152/ajpheart.00569.2015.—The contraction of muscle is characterized by the development of force and movement (mechanics) together with the generation of heat (metabolism). Heat represents that component of the enthalpy of ATP hydrolysis that is not captured by the microscopic machinery of the cell for the performance of work. It arises from two conceptually and temporally distinct sources: initial metabolism and recovery metabolism. Initial metabolism comprises the hydrolysis of ATP and its rapid regeneration by hydrolysis of phosphocreatine (PCr) in the processes underlying excitation-contraction coupling and subsequent cross-bridge cycling and sliding of the contractile filaments. Recovery metabolism describes those processes, both aerobic (mitochondrial) and anaerobic (cytoplasmic), that produce ATP, thereby allowing the regeneration of PCr from its hydrolysis products. An equivalent partitioning of muscle heat production is often invoked by muscle physiologists. In this formulation, total enthalpy expenditure is separated into external mechanical work (W) and heat (Q). Heat is again partitioned into three conceptually distinct components: basal, activation, and force dependent. In the following mini-review, we trace the development of these ideas in parallel with the development of measurement techniques for separating the various thermal components.

Microcalorimetry; muscle heat production; myothermy; thermopiles

MUSCLE IS A MOLECULAR machine. It operates isothermally, isobarically, and isovolumetrically. At the microscopic level, it directly converts the Free Energy component of the enthalpy of ATP hydrolysis into cyclic attachment and detachment of actomyosin cross bridges, together with transportation of ions up their electrochemical potential gradients. At the macroscopic level, two consequences result: the generation of force and the evolution of heat. Of these two entities, heat is by far the more difficult to quantify and to interpret. In this brief review, we trace the development of techniques to measure muscle heat production from the mid-19th century to the present, providing parallel commentary on the progressive understanding of muscle function. We give particular consideration to the development of techniques of separating the metabolic cost of cross-bridge cycling from the purely “overhead” cost of its activation.

Basic Concepts

The early decades of the 19th century yielded the hard-earned distinction between temperature and heat (in part due to the efforts of Jean Charles Athanase Peltier in France and Thomas Johann Seebeck in Estonia), as well as eventual acceptance of the equivalence of heat and work (in part due to the efforts of the Scot, James Prescott Joule) in a form now stated as the First Law of Thermodynamics, where a change of energy (ΔE) can take one of two forms: the production or absorption of work (W) or the generation or uptake of heat (Q).

$$\Delta E = W + Q$$  \hspace{1cm} (1)

The classical method of measuring heat production is via the use of thermocouples. A thermocouple (Fig. 1) consists of a pair of dissimilar conductors bonded together at both ends. When the two ends are held at different temperatures, and the circuit is interrupted, a voltage arises across the break. The magnitude of the voltage is directly proportional to the difference in temperature between the two ends of the thermocouple. The proportionality constant (which varies with the metals) is known as the Seebeck coefficient.

$$V = \alpha(T_2 - T_1)$$  \hspace{1cm} (2)

By analogy with the early word for what is now called a battery, a collection of thermocouples wired in series is known as a thermopile.

The first reported use of a thermopile to measure the output of heat from an isolated skeletal muscle (see Table 1) is attributed to the German polymath Hermann von Helmholtz in 1848 (137). According to A. V. Hill (71), von Helmholtz used...
three thermocouples in series with a galvanometer and, according to the detailed description provided on page 151 of Kirkes’ Handbook of Physiology (94), the thermopile constructed by von Helmholtz detected an increase of temperature of 0.25 mK in response to a 2- to 3-min tetanus of frog muscle.

It is germane to note that, whereas Fig. 1 shows a voltmeter as a proxy device to determine temperature difference, in practice, von Helmholtz and successors used a low-resistance galvanometer to detect the current drawn from the thermopile. That being the case, a design constraint in the manufacture of thermopiles was to keep their series resistance as low as practicable. A further motivation was to minimise Johnson noise (88), the unavoidable, temperature-dependent, random fluctuations of voltage in any electrical conductor (37, 73), the magnitude of which is proportional to the square root of the thermopile resistance. An obvious way of minimizing thermopile resistance was to minimize the number of thermocouples in series, or to increase their cross-sectional area, both of which reduce the resistance of the thermopile, at the expense of reducing the voltage signal. Hence, a trade-off between sensitivity and signal-to-noise ratio inheres in the design of thermopiles.

**Myometry of Isolated Muscles**

**Flat-bed thermopiles.** The modern era of using thermopiles to measure the heat production of isolated skeletal muscles began in earnest with the description by A. V. Hill of a thermopile, attributed to Blix (16), consisting of five copper-constantan thermocouples arranged in series (72). The device (Fig. 2) is immediately recognisable to anyone who has used a “flat-bed” thermopile. Hill claimed that the instrument was some five times more sensitive than its predecessor and so was capable of detecting a change of temperature of 50 μK (71). Such sensitivity was enhanced by the low resistance presented by only five thermocouples, thereby minimising Johnson noise, and the fact that the muscles of choice were amphibian. This choice of muscle preparation allowed experiments to be performed at 0°C, the unique temperature at which the reference thermocouples can be maintained for extended periods without the need for external thermostatic control. An additional, physiological, advantage obtains at this temperature: “initial heat,” i.e., the alactic, anaerobic heat output from a muscle during, and immediately following, a twitch or tetanic contraction, is well separated temporally from the subsequent lactic and aerobic “recovery heat,” reflecting the relatively high temperature dependence of the kinetic processes underlying the latter thermal sources.

Over the subsequent half-century, A. V. Hill and A. C. Downing continued to make improvements to their galvanometers [see Appendix I of Hill (74)] and thermopiles, including a “protected region” to prevent a cooler segment of muscle moving onto the thermopile during shortening contractions (Fig. 2) and steadily-improving frequency response, largely reflecting progressive reduction of thermopile thickness. Indeed, by the time of publication of Hill’s seminal “thermodynamics” manuscript (75), the time to reach 50% of maximum response following a calibration pulse of heat (some 25 ms, as inferred from Fig. 3) had been reduced 50-fold below that of an instrument used by Hill and Hartree 20 yr earlier (80). At the same time, thermal baseline stability had been improved to the point that aerobic recovery heat could be followed confidently through its full 30-min time course following a 12 s tetanus at 0°C, a remarkable accomplishment, even in an “ice-bath” (82). The thermopiles, constructed by Downing (74), consisted of constantan and manganin wire, rolled to 15-μm thickness, mounted on mica, and insulated with Bakelite varnish. Some of these instruments were still in use at University College (London) until recent times, which underscores our contention that it would be difficult to overstate the contribution of these two men to the development of myothermic techniques.

**A PAEAN TO A. V. HILL.** However, A. V. Hill’s contributions go well beyond thermometric techniques. He made a seminal contribution to the understanding of “Brownian motion” (what is now known as “Johnson noise,” see above) in moving magnet galvanometers of the type constructed by Downing (73). His 1938 paper on skeletal muscle energetics (75) remains a classic in the field. In that paper, he confirmed Fenn’s discovery (38, 39) that release of a muscle during a steady-state tetanus led to an increment of heat production above its isometric level. The magnitude of the thermal increment was proportional to x, the extent of shortening (ergo: the concept of “shortening heat”), whereas its rate was inversely proportional to the afterload, P. These results, relating force, shortening, and heat led to Hill’s famous “Characteristic Equation”; the first quantitative (if phenomenological) thermodynamic model (Eq. 3).

\[
(P + a)V = b(P_o - P)
\]

where V is velocity, \( P_o \) is peak isometric force, and \( b \) is a constant whose units are those of velocity. Whereas Hill himself would ultimately find that \( a \), the constant of proportionality in the expression for shortening heat, \( ax \), was not strictly constant, and whereas the model would ultimately be superceded by A. F. Huxley’s “sliding filament theory” (86), the “Characteristic Equation” still finds wide use today in describing that most fundamental of all the properties of a muscle: its force-velocity relation.

As mentioned above, Downing continually strove to improve the frequency response of both his galvanometers and his thermopiles. These improvements were paralleled by Hill’s laborious “long-hand” corrections for the rate of heat loss from the muscle-thermopile system (74) (preceding by decades the...
development of mathematical deconvolution techniques), which revealed that heat was evolved “...during the short interval between a shock and the moment when the contraction begins...”. This observation led Hill to introduce the notion of “activation heat” (76). The combination of previously revealed “shortening heat” with recently revealed “activation heat” led Hill to propose that muscle heat production consisted of two parts: the heat of activation (A) and the heat of shortening (\(\alpha x\)):

\[ E = A + \alpha x \]  

This formulation (76) provided an obvious method of estimating the magnitude of activation heat: progressive reduction of the extent of shortening. Whereas Hill was aware of the risk of overestimation of A using this technique, he was equally concerned at the risk of causing irreversible damage by stretching the muscle to the point at which active force was eliminated, requiring \(\sim 100\%\) extension, as shown by Ramsey and Street for single semitendinosus fibers of the frog (121). The variability of estimates left Hill reluctant to make a definitive statement. Nevertheless, he estimated activation heat to comprise one-third to one-half of the total heat in a twitch, independent of muscle length, a range of values that was to prove, yet again, his prescience.

### Table 1. Noteworthy milestones in the history of muscle myothermia

<table>
<thead>
<tr>
<th>Year</th>
<th>Author (Ref. No.)</th>
<th>Technique</th>
<th>Species</th>
<th>Muscle</th>
<th>Temperature, °C</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1848</td>
<td>von Helmholtz (137)</td>
<td>Thermopile 16 Sb-Bi couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>5–18</td>
<td>Oxygen or nitrogen</td>
</tr>
<tr>
<td>1902</td>
<td>Blix (16)</td>
<td>Thermopile 5 Cu-Constantan* couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>0, 10, 13, 25.5</td>
<td>Isotonic heat exceeds isometric heat</td>
</tr>
<tr>
<td>1911</td>
<td>A. V. Hill (72)</td>
<td>Ibid</td>
<td>Frog</td>
<td>Sartorius</td>
<td>0</td>
<td>First smooth muscle heat measurements</td>
</tr>
<tr>
<td>1920</td>
<td>A. V. Hill and Hartree (80)</td>
<td>Thermopile 50 Au and Ni couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>20–22</td>
<td>Stretch increases basal heat rate</td>
</tr>
<tr>
<td>1923</td>
<td>Penn (38)</td>
<td>Ag-constantan</td>
<td>Frog</td>
<td>Sartorius</td>
<td>20</td>
<td>Stretch increases basal heat rate</td>
</tr>
<tr>
<td>1930</td>
<td>Bozler (18a)</td>
<td>Curved thermopile</td>
<td>Snail</td>
<td>Retractor pharynx</td>
<td>0</td>
<td>Protected region added to thermopile</td>
</tr>
<tr>
<td>1932</td>
<td>Feng</td>
<td>Thermopile 70 Manganin-Constantan couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>0</td>
<td>Shortening heat revealed and mathematical model presented</td>
</tr>
<tr>
<td>1937</td>
<td>A. V. Hill (74) and Downing</td>
<td>34 or 42 Constantan-manganin and 28 Constantan-Fe</td>
<td>Frog</td>
<td>Sartorius</td>
<td>0</td>
<td>Recovery heat measured</td>
</tr>
<tr>
<td>1938</td>
<td>A. V. Hill (75)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>11–13</td>
<td>First single-fiber length-tension relation</td>
</tr>
<tr>
<td>1940</td>
<td>D. K. Hill (82)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Frog</td>
<td>Semitendinosus</td>
<td>0</td>
<td>Activation heat estimated</td>
</tr>
<tr>
<td>1949</td>
<td>A. V. Hill (76)</td>
<td>Thermopile 70 Manganin-Constantan couples</td>
<td>Toad</td>
<td>Seminembranosus</td>
<td>0</td>
<td>Sliding-filament mathematical model of Hill’s 1938 results</td>
</tr>
<tr>
<td>1957</td>
<td>A. F. Huxley (86)</td>
<td>Thermopile 70 Manganin-Constantan couples</td>
<td>Frog</td>
<td>Sartorius</td>
<td>0</td>
<td>Mathematical model of Hill’s 1938 results</td>
</tr>
<tr>
<td>1961</td>
<td>Neill et al. (109)</td>
<td>Thermistors in aorta and great cardiac vein</td>
<td>Dog</td>
<td>Closed-chest whole hearts</td>
<td>37</td>
<td>First measurement of whole heart heat</td>
</tr>
<tr>
<td>1965</td>
<td>Ricchiuti and Mommaerts (123)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Rabbit</td>
<td>RV papillary</td>
<td>20</td>
<td>First cardiac muscle heat measurements</td>
</tr>
<tr>
<td>1967</td>
<td>Gibbs et al. (54)</td>
<td>Thermomper 50 Ag-constantan couples</td>
<td>Rabbit</td>
<td>Whole heart</td>
<td>20</td>
<td>Integrating thermopile</td>
</tr>
<tr>
<td>1968</td>
<td>Wilkie (141)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Rabbit</td>
<td>Whole heart</td>
<td>20</td>
<td>Dewar flask calorimetry</td>
</tr>
<tr>
<td>1971</td>
<td>McDonald (105)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Rabbit</td>
<td>Whole heart</td>
<td>20</td>
<td>Golay cell</td>
</tr>
<tr>
<td>1971</td>
<td>Fraser (42)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Dog</td>
<td>Whole heart</td>
<td>20</td>
<td>Novel method of calibration of thermocouples</td>
</tr>
<tr>
<td>1972 and 1975</td>
<td>Kretzchmar and Wilkie (97, 98)</td>
<td>Thermopile 50 Ag-constantan couples</td>
<td>Rabbit</td>
<td>Whole heart</td>
<td>20</td>
<td>Vacuum-deposition thin-film thermocouples</td>
</tr>
<tr>
<td>1977</td>
<td>Mulieri et al. (108)</td>
<td>Thermopile 14–20 Bi-Sb couples</td>
<td>Rabbit</td>
<td>Interventricular septum</td>
<td>35</td>
<td>Perfused tissue</td>
</tr>
<tr>
<td>1982</td>
<td>Ponce-Hornos et al. (31)</td>
<td>Thermistors</td>
<td>Rabbit</td>
<td>Interventricular septum</td>
<td>35</td>
<td>First single-fiber heat recording</td>
</tr>
<tr>
<td>1983</td>
<td>Curtin et al. (31)</td>
<td>20 Constantan-Chromel‡ couples</td>
<td>Frog</td>
<td>Tibialis anterior</td>
<td>3–20</td>
<td>First single-fiber heat recording</td>
</tr>
<tr>
<td>1988</td>
<td>Daut and Elzinga (32, 33)</td>
<td>6 Chromel-constantan thermocouples</td>
<td>Guinea-pig</td>
<td>RV trabeculae</td>
<td>37</td>
<td>Flow-through microcalorimetry (sans mechanics)</td>
</tr>
<tr>
<td>2005</td>
<td>Taberner et al. (133)</td>
<td>2 Infra-red thermocouples</td>
<td>Rat</td>
<td>RV trabeculae</td>
<td>20</td>
<td>Flow-through microcalorimetry (fixed-end twitches)</td>
</tr>
<tr>
<td>2009</td>
<td>Taberner et al. (134)</td>
<td>2 Infra-red thermocouples</td>
<td>Rat</td>
<td>RV trabeculae</td>
<td>20</td>
<td>Flow-through microcalorimetry (work-loops)</td>
</tr>
<tr>
<td>2011</td>
<td>Taberner et al. (132)</td>
<td>2 Infra-red thermocouples</td>
<td>Rat</td>
<td>RV trabeculae</td>
<td>20</td>
<td>Flow-through microcalorimetry (with mechanics)</td>
</tr>
<tr>
<td>2014</td>
<td>Johnston et al. (90)</td>
<td>Peltier heat pumps</td>
<td>Rat</td>
<td>RV</td>
<td>37</td>
<td>Flow-through microcalorimetry (with mechanics)</td>
</tr>
</tbody>
</table>

RV, right ventricle. *Constantan: copper (55%) - nickel (45%) alloy. †Manganin: copper (86%) - manganese (12%) - nickel (2%) alloy. ‡Chromel: nickel (90%) - chromium (10%) alloy.
Fig. 2. Schematic of the 1st thermopile to have a “protected region” (thermocouples in series between A and F) electrically isolated from an unprotected region of identical thermocouples (between D and E). Shortening of the muscle from its cooler tendinous end (A) avoided thermal contamination of the voltage output from the protected region. [Reproduced from Fig. 4 of A. V. Hill (74) with permission of Proc R Soc Lond B Biol Sci and The Royal Society via RightsLink.]

physical response, was proposed by Gibbs and Ricchiuti (56) and subsequently extensively tested by Gibbs et al. (55). The conceptual model underlying this investigation, attributed to Hill (79), is given in Eq. 5:

$$Q = A + \alpha x_1 + W + h(P)$$  \hspace{1cm} (5)

where the subscripts signify, respectively, internal shortening by, and work against, the series elastic component and $h(P)$ represents tension-related heat. Using Sartorius muscles at 0°C, these authors varied the interval between two consecutive stimuli until mechanical fusion was achieved. At that point, the contributions of the three right-most terms in Eq. 5 were assumed to be identical between the two twitches. Hence, the increment of heat associated with the second pulse provided an estimate of $A$: 40% of the total heat in an isometric twitch, rising to 45% at room temperature. At either temperature, activation heat appeared to be independent of muscle length.

To compare the magnitude of activation heat in frog and toad Sartorius muscles (at 6°–7°C), Chapman and Gibbs (23) subjected them to isometric contractions, progressively reducing muscle length until developed force was negligible. Whether plotted as functions of force or force-time integral, intersection with the heat axis produced estimates of activation heat in the vicinity of 50% of that recorded at $L_o$. By this time, the number of distinct contributors to total energy expenditure ($E$) had inflated, as shown in Eq. 6:

$$E = A + k_1 W + \alpha x + k_2 \int P dt + k_3 \int x dt$$  \hspace{1cm} (6)

where $W$ is now external work, the $k$-terms are constants, and the rightmost term was proposed by Jöbsis and Duffield (87). Dissatisfied with that state of confusion, Chapman and Gibbs proffered a radical model for the initial energy of a muscle twitch (22):

$$E = A + \Delta H \int k \cdot n \ dt$$  \hspace{1cm} (7)

where $A$ is activation heat, $n$ is the instantaneous number of cross-bridge links between actin and myosin, $\Delta H$ is the molar enthalpy of ATP hydrolysis, and $k$ is a constant quantifying the rate of dissociation of cross-bridge links between actin and myosin, which was assumed to vary with species, muscle, temperature, and inherent actomyosin ATPase activity. The (long overdue) incorporation of the fundamental thermodynamic assumptions of A. F. Huxley’s “sliding filament model” (86) is apparent in the second term on the right-hand side.

This much-simplified conceptual formulation resonated nicely with the contemporaneous results of Homsher et al. (84) and Smith (126), both of whom exploited the ability to reversibly lengthen the semitendinosus muscles of the frog to the point where active force became negligible (121). Both studies revealed that the right-hand term of Eq. 7 was a simple linear function of tension. Its intercept with the ordinate provided estimates of $A$ of 30% ($n = 35$) and 26% ($n = 43$), respectively. It is seldom that a mathematical model receives such immediate and gratifyingly consistent experimental corroboration.

THERMOPILE CALIBRATION. To this juncture, thermopiles had generally been calibrated by rapid transference between two water reservoirs held at different temperatures (typically 0°C and room temperature). However, an entirely new approach, which obviated the need to measure either the flux of heat or the difference of temperature, was developed by Kretzschmar and Wilkie (97, 98). This method capitalizes on another fundamental property of dissimilar conductors, namely, the Peltier effect. The phenomenon is the converse of the Seebeck effect; i.e., if current is passed through a circuit comprising a thermopile, heat is produced at one junction and absorbed at the other. Kretzschmar and Wilkie placed identical metal (copper) blocks

Fig. 3. Schematic of “second generation” integrating thermopile. A: thermopile, silver strip and muscle in cross-section. B: top view of the muscle lying on the silver strip, above the embedded chromel heat-calibration wire. [Reproduced from Davey et al. (36) with permission of the Copyright Clearance Center].

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of known mass and thermal capacity on “hot” and “cold” junctions of a flat-bed thermopile. Passage of current through the circuit caused the temperature of the “hot” junction (and its added copper mass) to rise and that of the “cold” junction (and its identical copper mass) to fall until a steady state of temperature-difference prevailed. At this point, heating was discontinued and the system allowed to cool, while the voltage was monitored. From the accompanying exponential rates of decline of temperature with time, the Seebeck coefficient could be calculated (98). The near-perfect linearity of the system [see Fig. 1A of Ref. 97] meant that only a single copper block needed to be used and, since thermal equilibrium is established much more quickly at the reference than at the active junctions (81), it was placed at the latter location.

The integrating thermopile. ENTHALPY OF PHOSPHOCREATINE HYDROLYSIS. To link, quantitatively, the biochemical and biophysical energetics of muscle contraction, the existing thin, multicouple thermopiles, with their focus on high sensitivity and rapid frequency response were unsuitable. What was required, instead, was a device with a negligible rate of heat loss and a stable thermal baseline over extended periods, to capture all of the heat released over the entire length of the muscle throughout any contractile sequence, including trains of 30 or more twitches. To that end, Wilkie (141) developed the “integrating thermopile,” which he described as “… resembling a calorimeter opened out flat so that the muscle can lie along its surface.” The surface, mimicking the dimensions of a frog sartorius muscle, consisted of a 250-μm-thick strip of silver, thereby ensuring rapid conduction of heat from the muscle to the thermocouples and rapid equilibration of longitudinal temperature gradients. The device was calibrated by Joule heating of a fine constantan wire running the length of the silver. Muscle heat production, corrected for (slow) heat loss, was inferred from the output of a pair of chromel-constantan couples, with attendant increase of series resistance, was made by winding a continuous length of constantan wire around a pair of threaded preanodised mandrels. One-half of the resulting longitudinal helix was masked and the other half etched electrolytically and electroplated with silver. Compared with the authors’ conventional flat-bed thermopiles, consisting of 32 active silver-constantan junctions, whose resistance was 27.5 Ω and output 0.815 mV/°C, the new wire-wound “trough” design comprised 50 active junctions with resistance ~1,000 Ω and output 3.25 mV/°C. This transition to many more thermocouples, with attendant increase of series resistance, was made possible by the commercial availability of the Astrobotella Nanovolt amplifier whose maximum gain was 106 with a flat frequency response from DC to 200 Hz. With that development, coupled with the greater ease of “wire-wound” construction, galvanometry largely faded from the field.

ACTIVATION HEAT (CARDIAC MUSCLE). Upon changing focus to the energetics of cardiac muscle, Gibbs and colleagues did not have the luxury of stretching papillary muscle beyond L0 (the length at which active force development is optimal). Hence, they were obliged to use a protocol in which force was progressively reduced by shortening muscles to lengths less than L0. Whereas this risked contamination of microscopic cross-bridge activity, undetectable macroscopically, at short lengths (76), the estimate of activation heat as a proportion of total heat in a twitch was ~23% (54, 122), thereby giving further credence to the conceptual model represented by Eq. 7.

Gibbs and Vaughan (57) also adopted the “preshortening” technique. Because of the absolute reliance of cardiac contractility on the presence of extracellular calcium ion (124), extra-cellular Ca2+ concentration became the variable of interest. Nominally Ca2+-free superfusate roughly halved the magnitude of activation heat whereas raising the temperature from 18 to 32°C reduced it by an equivalent fraction. In their comprehensive discussion, the authors review the evidence that activation heat probably reflects, in large part, the energetic cost of returning “trigger Ca2+” to the sarcoplasmic reticulum during relaxation. Hence, attention turned to the effect of known inotropic agents on activation heat, with Gibbs demonstrating data analysis. Experiments were performed at 27°C. Gibbs and Loiselle (52) subsequently used this integrating thermopile, as well as one of slightly higher sensitivity, to examine the effects of temperature (19 vs. 29°C) on the same type of muscle preparation. Further experiments characterising the energetics of smooth muscles have been performed using rat anococcygeus muscle (138) and longitudinal muscle from the rabbit urinary bladder (111, 139, 140). The brevity of this list highlights the existence of a largely unexplored field of muscle energetics. The seminal study of smooth muscle heat production, by the pharynx retractor muscle of the edible snail (Helix pomatia), was performed by Bozler (18a).

Wire-wound electroplated thermopiles. Until the mid-1960s, myothermic studies focussed almost exclusively on skeletal muscles from the frog, “the Old Martyr of Science” (83). However, at that time, interest expanded to include cardiac muscle, where preparations of suitable size were presented by papillary muscles from small mammals. Despite the suitability of size, their cylindrical shape ensured poor contact with the “flat-bed” nature of existing thermopiles. To overcome that issue, Ricciuti and Mommaerts (123) moulded wire-wound thermopiles to contain a centrally located hemicylindrical trough, as shown in Fig. 4. The thermopiles were constructed by winding a continuous length of constantan wire around a pair of threaded preanodised mandrels. One-half of the resulting longitudinal helix was masked and the other half etched electrolytically and electroplated with silver. Compared with the authors’ conventional flat-bed thermopiles, consisting of 32 active silver-constantan junctions, whose resistance was 27.5 Ω and output 0.815 mV/°C, the new wire-wound “trough” design comprised 50 active junctions with resistance ~1,000 Ω and output 3.25 mV/°C. This transition to many more thermocouples, with attendant increase of series resistance, was made possible by the commercial availability of the Astrobotella Nanovolt amplifier whose maximum gain was 106 with a flat frequency response from DC to 200 Hz. With that development, coupled with the greater ease of “wire-wound” construction, galvanometry largely faded from the field.
the potentiating effect of catecholamines (44, 51) and elevated concentration of extracellular Ca\(^{2+}\) (43).

Later investigators adopted various methods to avoid using the preshortening protocol. Thus Alpert et al. (1) eliminated tension (and, by extension, tension-dependent heat) in isometrically contracting rabbit papillary muscles at 21°C, by using a mixture of 2,3-butanedione monoxime (BDM) and hyperosmotic mannitol. These authors found tension-independent heat to be reduced at reduced muscle lengths and increased at increasing pacing frequencies when using hypertonic solutions ranging from 2 to 2.5 normal osmolarity. Given that hyperosmotic superfusion had long been recognised to increase intracellular Ca\(^{2+}\) concentration and heat production [see Loiselle et al. (102) and references therein], this result remains contentious.

In contrast to the “BDM and mannitol” approach, Gibbs et al. (53) developed a purely biophysical technique, “latency relaxation,” in which a papillary muscle at optimal length was rapidly shortened during the latent period between delivery of the electrical stimulus and the onset of measureable shortening or force production. By grading the extent of shortening, a heat-stress relation was generated. The intercept of this relation yielded an estimate of activation heat that was ~50% higher than the value arising from the preshortening protocol, accounting for 30% of the heat in a maximal isometric contraction at optimal length. This proportion approximated that found earlier in skeletal muscle [see ACTIVATION HEAT: SKELETAL MUSCLE] but was substantially higher than that reported by Alpert et al. (1).

Kiyooka et al. (95) capitalised on Suga’s “Pressure-Volume-Area” (PVA) concept (see FLOW-THROUGH MICROCALIMETRY) in a study using the blood-circulated, cross-perfused, dog heart. By subtracting the heart-rate-independent metabolic rate during K\(^+\) arrest [as determined in a previous study (110)] from the intercept of the V\(_{O2}\)-PVA relation, Kiyooka et al. (95) generated an estimate of activation heat. Contrary to suggestions by both A. V. Hill (76) and Gibbs and Vaughan (57), these authors found this component of cardiac energy expenditure to increase nearly four-fold as pacing frequency was increased from 60 min\(^{-1}\) to 180 min\(^{-1}\). Clearly, the jury remains “out” on most of the important details of activation heat in cardiac muscle: its magnitude, length dependence, and heart-rate dependence.

WORK AND EFFICIENCY (CARDIAC MUSCLE). Despite uncertainty concerning the contribution of activation heat, interest progressively shifted to quantifying the efficiency of afterloaded isotonic contractions, by analogy with the efficiency of the heart performing pressure-volume work. Efficiency (\(\varepsilon\)) is given by the ratio of external work (\(W\)) to enthalpy (\(\Delta H\)):

\[
\varepsilon = \frac{W}{\Delta H}
\]

where, in the case of isolated muscles, \(W\) is the product of afterload and extent of shortening, and enthalpy is the sum of heat (\(Q\)) and work. Over subsequent years, Gibbs and colleagues, using predominantly wire-wound thermopiles (24, 45, 46, 48, 49, 100), examined the influence of various factors anticipated to affect efficiency. In every case examined, the dependence of efficiency on afterload varied roughly parabolically, being optimal in the vicinity of 0.3 peak isometric force (49) and peaking at a value of 10 to 15%. Somewhat paradoxically, agents such as ouabain (50), catecholamines (44), caffeine, or elevated extracellular Ca\(^{2+}\) concentration (25), all of which were found to increase contractility, tended to diminish contractile efficiency because of their pronounced potentiation of the activation heat component. Somewhat later, Barclay and colleagues developed software to drive papillary muscles through isotonic work-loops, comparing the effects of “realis-
tic” and “sinusoidal” strain patterns on heat production (15, 106, 107) and clarifying the distinction between cross-bridge and mitochondrial efficiencies (12, 13).

**Vacuum-deposition, thin-film, thermopiles.** In the continual pursuit of improved performance and simplified methods of construction, the wire-wound thermopiles of Ricchiuti gave way to vacuum-deposition techniques. Mulieri et al. (108), using reversible copper masks, evaporated bismuth and antimony onto thin, planar mica sheets. Whereas mica rendered the thermopiles brittle, the thinness of the sheet, combined with the Bi-Sb couples, considerably reduced the thermal lag, while allowing an excellent signal-to-noise ratio. Despite the availability of the recently published “Peltier cooling” approach of Kretzschmar and Wilkie (97, 98), the authors continued to calibrate their new thermopiles by rapid transference between two water baths of different temperature. Regardless of this detail, vacuum deposition has become the thermopile fabrication technique of choice. For the interested reader, attention is drawn to the highly pertinent publication by Barclay (6), published during the period of revision of the current manuscript.

**Single-fiber experiments.** The vacuum-deposition thermopiles of Mulieri et al. (108) were developed primarily for use with thin rabbit right-ventricular papillary muscles, although the authors pointed out that they were also suitable for thermal measurements of bundles of skeletal muscle fibers. However, the ultimate refinement, in this direction, measurement of the heat produced by a single anterior tibialis muscle fiber from the frog, was nevertheless made by Curtin et al. (30, 31) using thermopiles of the Hill-Downing type modified along the lines described by Howarth et al. (85). Very low heat capacity was achieved by using 20 brazed constantan-chromel thermocouples, flattened to 8-µm thickness, insulated between two 6-µm-thick layers of Kapton film (Fig. 5). With the use of a galvanometer arrangement similar to that described by Howarth (85), sensitivity was such that the voltage output of a single fiber from the frog anterior tibialis muscle at 15°C was ~0.5 µV in response to a single stimulus.

A decade would pass before single fiber experiments were again performed, now using bismuth-antimony couples in thermopiles of the Mulieri-type (108) and low-noise amplifiers. Thus Barclay et al. (9) examined the differential effects of mild fatigue on cross-bridge and non-cross-bridge force and heat production of single frog fibers as well as small bundles of fibers. Their measurements implied that non-cross-bridge heat (estimated by extrapolation to 0% “filament overlap”) contributing 25–30% of the heat generated at 100% filament overlap. Shortly thereafter, Buschman et al. (19, 20) characterised the mechanical and thermal behavior, at room temperature, of single slow-twitch and fast-twitch fibers dissected from the iliofibularis muscles of the African clawed frog *Xenopus laevis*. Yet again, a principal focus was the separation of muscle heat into its force-independent (activation) and force-dependent (actomyosin ATPase) components (21). The results were strikingly fiber-type dependent, activation heat accounting for ~30% of total heat production in type 1 fibers but slightly over 50% in fibers of type 3.

Subsequent substitution of melinex (polyethylene terephthalate) sheets (of thickness 12 µm) by the group of Barclay (4, 5, 7, 8, 10, 11, 14, 27) greatly reduced the brittleness and fragility of mica-based vacuum-deposition thermopiles without loss of thermal sensitivity or reduction of frequency response. As with all flat-bed thermopiles, the frequency response was nevertheless diminished somewhat, as were the rates of diffusive exchange of oxygen and carbon-dioxide, by the layer of solution adhering to the muscle as shown schematically in Fig. 5b.

**The Golay cell.** A variation on the technique of measuring the heat produced by muscles resting on a planar surface was introduced by Fraser and Carlson (41) and Fraser (42) and subsequently replicated by Kobayashi and Sugi (96). These authors constructed infra-red radiometers based on the Golay cell, a device consisting of a gas-filled cavity and a diaphragm that absorbs radiation in the infrared region of the electromagnetic spectrum. Absorption raises the temperature of the diaphragm and that of the gas, thereby causing it to expand and distend the diaphragm, the extent of which can be measured opto-electronically. The infra-red region of the thermal spectrum generated by contraction of a muscle was captured from...
a (7 mm²) region of the surface of the muscle frog sartorius at temperatures of 15°C (Fraser) and 23 to 26°C (Kobayashi and Sugi). The frequency response and signal-to-noise ratio of the devices were comparable to, or better than, those of contemporary flat-bed thermopiles. That the infra-red myometer did not find wider usage can probably be attributed to the size of the Golay cell and the resulting large surface area of muscle required.

Cardiac Muscle Myometry

Unperfused whole hearts: thermopiles. At the suggestion of A. V. Hill, Fischer (using Downing’s flat-bed thermopiles) attempted to measure the heat production of working hearts (either intact or split longitudinally and flattened) from the frog, eel and tortoise (40). Needless to say, “muscle movement” was an issue. Temperature changes in the order of 1 mK were recorded, but “No definite conclusions could be drawn from the[se] results.” (p. 333). A decade earlier, Snyder (127) had used the terrapin heart, maintained at 10 to 12°C in a “thermos” food jar...obtained in the shops . . .”. “Experiments were performed between one and four o’clock in the morning when the [street] car service was reduced to one car every 30 min [so that] satisfactory observations could be made.” Snyder used two different, 30-thermocouple, thermopile designs: “. . . one [in which] the instrument is made to clasp the tissue, the other [in which] the tissue is made to clasp the instrument.” In keeping with the contemporary practice, the current output from the thermopiles was detected by a galvanometer. Early experiments (127) focused primarily on the timing of heat production with respect to the contraction of the heart beat, but the quality of the records remains impressive. By the following year (128), Snyder’s interest had shifted to separating “initial heat” from “total heat” in a single heartbeat, using the improved “Einthoven galvanometer”, thereby allowing him the privilege of coining the phrase “thermocardio-gram” (129, 130).

Perfused whole hearts. In every study referenced above, the muscle preparation was nonperfused. Hence, except for the aforementioned single-fiber studies (9, 19–21, 31), the issue of the adequacy of oxygen supply to the muscle core by diffusion from its surface(s) continued to concern experimentalists. For example, this concern motivated Kiriazis and Gibbs (91–93) to split papillary muscles longitudinally to increase the rate of radial diffusion of oxygen from the superfusate to the muscle core. However, substantially preceding this novel undertaking, investigators began to explore the feasibility of measuring the heat production of the perfused whole heart preparation. The first success was that of Neill et al. (109) who determined the rate of removal of heat by the coronary circulation from measurements of coronary flow and the veno-arterial temperature gradient, using thermistor-tipped catheters placed in the ascending aorta and great cardiac vein. In situ experiments were conducted in anaesthetized, closed-chest dogs, and a wide range of hemodynamic conditions was explored. The production of heat was attributed exclusively to the left ventricle. A decade later, McDonald (105) simplified the experimental protocol by developing a Dewar-flask calorimeter to measure the heat output of isolated, isovolumically contracting, Langendorff-perfused rabbit hearts. To be able to separate tension-dependent and tension-independent heat, so that his results could be compared with published papillary muscle studies, McDonald used a simple spherical model of the heart to estimate wall tension from left ventricular balloon pressure, revealing that some 50% of total heat production arose from force-independent sources, a value rather higher than those

Fig. 6. Schematic cutaway diagram depicting, in exploded view, an “on-line calorimeter.” Note the triangular-shaped, arterially-perfused, rabbit interventricular septum located between a pair of lower stainless steel (S.S.) hooks, which double as stimulus electrodes, and an upper connection to a force transducer. After closure, the calorimeter is immersed in a thermally isolated constant-temperature water bath. Two temperature-sensitive units, each comprising 31 thermosensitive junctions are connected in series and lie between the inner chamber and the copper heat sink. [Reproduced from Fig. 1 of Ponce-Hornos et al. (119) with permission purchased from Am J Physiol Heart Circ Physiol via RightsLink.]
reported for isolated papillary muscles. Coulson and Rusy (29), extended the “Dewar flask” technique by supplementing thermocouples with polarographic \( \text{PO}_2 \) electrodes placed in the arterial and venous lines, thereby allowing simultaneous measurements of heat and oxygen consumption. Comparable systems were subsequently used by Theisohon et al. (136) to study the mecano-energetics of the isolated rabbit heart, as well as by Coulson (28) in an extensive exploration of the effects of varying left ventricular volume, and hence peak left ventricular pressure, on heat production. Coulson arrived at a remarkably similar estimate of the proportion of energy expenditure unrelated to contraction that MacDonald had observed earlier.

**Perfused interventricular septa.** Somewhat later, an innovative contribution to the field of myocardial myometry was provided by Ponce-Hornos et al. (119). This group initially developed a method to measure myocardial heat production in the isolated, arterially-perfused, interventricular septum of the rabbit (Fig. 6). Perfusion of the septum via its own circulation achieved a compromise between the isolated heart and its isolated muscles. It ensured adequacy of oxygenation while allowing ready control of perfusate constituents including \( \text{PO}_2 \).

In addition, the effluent could be collected and analyzed for lactate production to quantify the extent of anaerobic metabolism. The fundamental shortcoming of the tissue preparation reflected its triangular shape, the inability to make unambiguous estimates of mechanical performance. Nevertheless, the measurements of both resting metabolism and heat per twitch aligned well with thermometric estimates. The technique immediately produced a flurry of publications (18, 114–116, 120). It was later extended to accommodate the perfused rat whole heart, as well as either its isolated left ventricle or right atrium (125), complete with indwelling left ventricular balloon to alter mechanical energy demand (17, 26, 103, 104, 125) and, subsequently, the mouse heart (118), culminating in a demonstration of measuring the heat production of aliquots of isolated cardiac myocytes (117). Interestingly, the technique has not been adopted by other groups in the interim.

**Flow-through microcalorimetry.** The historical trajectory of myothermic measurement techniques, traced above, can be viewed as a continual striving for balance among three (often competing) requirements: resolution of heat measurements, adequacy of thermal frequency response, and adequacy of oxygenation of the muscle preparation. In general, flat-bed thermopiles, when used to study either isolated whole skeletal muscles or isolated papillary muscles, achieved the first two of these requirements. However, except for the study of single skeletal fibers frogs (9, 19–21, 30, 31), or Barclay’s studies of bundles of fibers from the hindlimb muscles of the mouse (2–5, 7, 8, 14, 27, 99), simultaneous achievement of all three requirements has been rare.

**THE AMSTERDAM MICROCALORIMETER.** A fundamental change of approach to thermal measurements, at least for studies of cardiac muscle, was taken by Daut and Elzinga (32), who developed a flow-through microcalorimeter designed to allow the measurement of heat production by thin cardiac trabeculae isolated from the right ventricles of guinea pigs. Because of the linear arrangement of their myocytes, trabeculae are ideal for mechanical measurements (135). Because of their small radii, trabeculae superfused with oxygenated saline solutions are unlikely to experience oxygen diffusion insufficiency, even at high rates of stimulation (63). The design of the “Amsterdam calorimeter” was conceptually simple: hexagonal upstream and downstream arrays of thermocouples were embedded in the walls of an acrylic tube of inside diameter 0.8 mm. When a trabecula was placed inside the tube, mid-way between the two thermocouple arrays, and a flow of superfusate commenced, the downstream array of thermocouples reported a higher voltage than the upstream one (Fig. 7). Knowing the Seebeck coefficients of the thermocouples, and the rate of flow of superfusate, the rate of heat production by the trabecula could be calculated. The technique has been variously used to determine the time and \( \text{PO}_2 \) dependence of cardiac resting heat production (33), and the effects on muscle heat production of metabolic substrate (34), inhibition of the sarcolemmal Na\(^+\)-K\(^+\) ATPase (33, 35), hyperosmolality (102), pharmacological agents (60), and chemical skinning (101).

**THE AUCKLAND MICROCALORIMETER.** Despite this list of achievements, the “Amsterdam microcalorimeter” suffered from the inability to make mechanical measurements simultaneously with thermal ones. This limitation has recently been overcome by Taberner and colleagues. The devices designed and constructed by this group are similarly “flow-through” but open-ended (133). The first version (Fig. 8) used thin-film infra-red thermopile sensors in “conduction mode” (64) and allowed the heat output of rat right-ventricular trabeculae undergoing fixed-end contractions to be made (65). Subsequent modifications to both hardware and software (132) achieved both truly isometric contractions as well as isotonic “workloop” contractions at various afterloads. Nevertheless, it re-

![Fig. 7. Schematic of the Perspex flow-through recording chamber (inner diameter: 0.8 mm; outer diameter 1.0 mm) of the “Amsterdam” calorimeter, comprising hexagonal upstream and downstream arrays of chromel-constantan thermocouples (for clarity, only a single couple is displayed) embedded, at a 4-mm separation, into the 100-µm thick walls of the tube, thereby having no contact with the 37°C superfusate. [Reproduced from Fig. 1 of Daut and Elzinga (32), with permission of J Physiol, John Wiley and Sons, and the Copyright Clearance Center.]

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mains that any flow-through device necessarily sacrifices frequency-response vis-à-vis that of any of the flat-bed thermopiles aside from Wilkie’s “integrating thermopile.” Indeed, the flow-through design renders the separation of initial and recovery heat unlikely in the Auckland device.

An early use of the instrument aimed to understand the thermodynamic basis of Suga’s “PVA efficiency” (131), in which the numerator of Eq. 8 comprises an arbitrarily defined segment of the triangular region lying between the end-systolic and end-diastolic pressure-volume relations of the heart. This investigation was motivated by the yawning conceptual and quantitative disparities between the resulting load-independent, constant value (typically 40%) of “PVA efficiency,” and the much lower and load-dependent values reported by Gibbs et al. (46). Clarification was achieved upon demonstration that the “work” term in Suga’s formulation (i.e., the numerator of Eq. 8) contained a variable proportion of heat production. This rendered its numeric value both inflated and afterload invariant, leading to “The demise of isoefficiency” (67, 68).

A subsequent undertaking addressed a further issue of fundamental thermodynamics: published claims of greatly increased cardiac efficiency in animals fed diets supplemented with omega-3 fish oils (112, 113). Despite careful recapitulation of dietary protocols, results from trabecula experiments in the flow-through microcalorimeter showed no effect of fish oil supplementation on load-dependent cardiac efficiency (58) nor did those from isolated working whole hearts (59).

The microcalorimeter provided the ideal tool with which to explore interventricular differences in both isometric and isotonic performance of isolated trabeculae. Whereas there was no difference in mechanical performance, the elevated activation heat of specimens from the left ventricle rendered their contractile performance less efficient (66). More recent studies have shown that streptozotocin-induced type I diabetes has no effect on the energetic performance of ventricular trabeculae (70) despite causing the peak efficiency of the isolated whole heart to shift to lower values of afterload (62). In contrast, the force-length work output of trabeculae from spontaneously hypertensive rats, whether at “failing” or “nonfailing” age, has been shown to decrease vis-à-vis that of age-matched control animals. Since the decrement of work exceeded that of heat production, contractile efficiency declined (69). The extent of decline mirrored the increase in mass of the heart (61).
A PARADIGM SHIFT. However whether in the “Amsterdam” or “Auckland” microcalorimeters, the basic modus operandi is to infer muscle heat production from the increase of temperature of superfusate flowing at a known rate between upstream and downstream arrays of thermocouples. We now report a further paradigm shift in the measurement of muscle heat production: adoption of thermoelectric modules (Peltier heat pumps) as the thermal sensors per se.

The use of heat-pumps as thermal sensors is certainly not unknown, since they have long been employed in the practice of isothermal titration calorimetry. Their use in muscle myometry reflects a shift of objective from maximizing the temperature increase and corresponding voltage signal, to maximizing the signal-to-noise ratio. Heat pumps typically comprise few thermopiles with a relatively small gap between the hot and cold junctions. This decreases the overall Seebeck coefficient of the sensor but also decreases the electrical resistance, thereby decreasing Johnson noise (90). Common-mode noise that is introduced during the measurement of the thermoelectric voltages can be minimized by measuring independently the upstream and downstream signals, and calculating the difference using software. The “Peltier microcalorimeter” resulting from this latest paradigm shift (Fig. 9) retains the same fundamental “flow-through” design. Whereas it has lower sensitivity than the previous “Auckland microcalorimeter,” implementation of flow-control and improved temperature-control has achieved a signal-to-noise ratio of 1,700, an order of magnitude improvement.

![Diagram](https://example.com/diagram.png)

Fig. 9. The latest version of the measurement chamber of the “Auckland Microcalorimeter” in which thermopile chips have been replaced by thermoelectric modules, located at the bottom and outside the borosilicate tube. When in use, a coverslip is placed atop the copper housing to reduce air currents. Note the mirror, oriented at 45°, to allow the diameter of the trabecula to be estimated in 2 orthogonal planes. [Reproduced from Fig. 1 of Johnston et al. 2015 (89) under the aegis of the Rights of Authors of APS Articles.]

![Graph](https://example.com/graph.png)

Fig. 10. Heat output from a trabecula during the performance of 7 after-loaded isotonic contractions of progressively diminishing magnitude, bracketed by isometric contractions, at optimal muscle length and at 37°C. A: original data (courtesy of Toan Pham). B: data corrected for 1.5-μW linear baseline drift during the 30-min measurement period.

![Diagram](https://example.com/diagram.png)

Fig. 11. Schematic representation of partitioning of cardiac muscle enthalpy expenditure (ΔH), as a function of relative afterload, into 4 components, the relative contributions of which vary with muscle type and experimental protocols. The work component is zero when the muscle is undergoing isometric contractions. The horizontal dotted lines indicate uncertainty regarding the dependence on afterload of both force-independent (activation) heat and the rate of basal heat production.
over the original “Auckland microcalorimeter” (90), providing thermal resolution of 10 nW.

A further refinement of the “Auckland microcalorimeter” is the use of additional Peltier modules for temperature control purposes, thereby avoiding the need to immerse the calorimeter in a temperature-controlled water bath, or to use a pump to drive temperature-controlled water around a chamber surrounding the calorimeter. Instead, the device is placed on a vibration-isolation table under an airtight, insulated hood and the temperature of the air therein is controlled. An example of the stability of the thermal baseline underpinning lengthy measurement periods is shown in Fig. 10. The improved temperature controllability of the surrounds, combined with the increased signal-to-noise ratio of the “Auckland Peltier microcalorimeter” has thereby enabled the first simultaneous measurements of the heat production, force output and mechanical efficiency of isolated cardiac trabeculae at body temperature (89, 134).

Summary

In summary, we have traced the history of myothermic measurements from its infancy, early in the 20th century, characterised by relatively large muscle preparations contracting at temperatures scarcely above that of freezing, to the present day, when the heat output of preparations some 10 to 50 times smaller can be quantified at temperatures some 40 K higher. We conclude with a graphical representation (Fig. 11) of our view of muscle energetics, [based on equivalent pictorial representation by Gibbs and colleagues (46, 47, 49)], as encapsulated in Eq. 9:

$$\Delta H = Q_B + Q_A + Q_F + W$$

where the subscripts B, A, and F denote “basal”, “activation,” and “force-dependent” heat, respectively.

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Present address of C. Johnston: Heart Science Centre, Imperial College London, London, UK.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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