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A work-loop calorimeter for measuring the force-length-heat relationship of working excised cardiac muscle fibers

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Abstract— **Isolated cardiac trabeculae are convenient specimens with which to study the properties of cardiac muscle under a variety of controlled conditions *in vitro*. We have developed an instrument for measuring the mechanical and energetic properties of continuously-superfused cardiac trabeculae. Our instrument is capable of dynamically transitioning between fixed-length, isometric and isotonic modes of control during the time-course of a muscle twitch, allowing us to impart force-length work-loops that mimic the behaviour of cardiac muscle *in vivo*. Simultaneously, sensitive temperature transducers quantify muscle heat production. The combination of these interventions and measurements yields unique insight into the energetic efficiency of living cardiac muscle.**

I. Introduction

THE heart is a thermodynamic machine. With every beat, it performs work by creating a pressure-volume cycle that results in blood being pumped around the vasculature. Throughout this process, individual cardiac muscle fibres describe a force-length cycle while liberating energy in the form of heat. In order to gain insight into the complex thermo-mechanical processes occurring within the muscle, both the mechanical and metabolic events, i.e. the force-length cycle and heat production, should ideally be measured simultaneously.

Cardiac trabeculae are the smallest naturally arising collections of linearly arranged myocytes in the heart and are accordingly a convenient preparation for *in vitro* studies of function of intact heart muscle *in vivo*. We have previously developed an instrument [1, 2] that is capable of measuring simultaneously the heat and force production, and dynamic stiffness of continuously superfused cardiac trabeculae under a variety of conditions [3-5].

In this paper, we report on a new instrument (the "work-loop calorimeter") which is capable of measuring the heat production of continuously superfused cardiac trabeculae being subjected to force-length work-loops that mimic the behavior of cardiac muscle *in vivo*. This instrument allows us to control the force-length trajectory described by the muscle with every twitch, while recording the muscle heat production. From these, we can determine the relationship between the force-length relationship and heat production of respiring cardiac trabeculae and gain insight into their energetic efficiency under a variety of physiological and pharmacological interventions.

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II. Materials and Methods

A. Muscle preparation and superfusate solutions

Wistar rats were anaesthetized using isofluorane prior to decapitation, thoracotomy and cardiectomy (as approved by The University of Auckland Animal Ethics Committee). The excised heart was quickly plunged into a cold dissection solution. The aorta was cannulated and the coronary vasculature was Langendorff-perfused with dissection solution, which contained (in mM): 130 NaCl, 6 KCl, 1 $MgCl_2$, 0.5 NaH_2PO_4, 0.3 $CaCl_2$, 10 HEPES, 10 glucose, and 20 BDM (2,3-butanedione monoxime), had a pH of 7.4 (adjusted using Tris) and was vigorously bubbled with 100% O_2, at 22-23^{o}C. An unbranched and geometrically-uniform trabecula (diameter: 270 µm, length: 2.5 mm) was dissected from the right-ventricular inner wall. It was then transferred to the work-loop calorimeter containing superfusate of composition identical to that of the dissection solution except for the absence of BDM and an increase of Ca^{2+} concentration to 2 mM.

B. Heat measurement

The principle of operation of the microcalorimeter that resides at the center of our instrument has been described in detail elsewhere [6, 7]. Briefly, two thermopile arrays, each consisting of three sensors of 100 thermocouples deposited on a Si_3N_4 membrane, are mounted 4 mm apart close to the outer surfaces of a glass tube (Fig. 1, top-left). The three sensors in each array are each mounted at the bottom and on both sides.

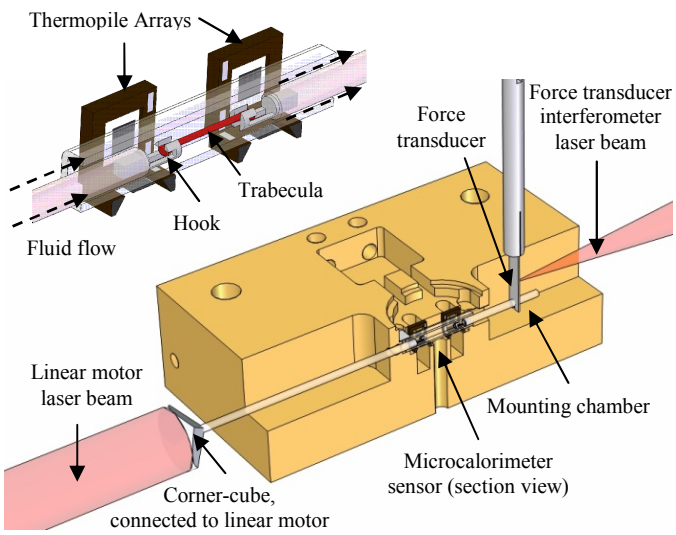

Fig. 1. Schematic diagram of the work-loop calorimeter showing a cut-away view of its components (lower panel), and a close-up cut-away view of the microcalorimeter (upper panel) showing the upstream and downstream thermopiles arrays, and a trabecula mounted onto the hooks. The broken arrows indicate the direction of flow of superfusate.

The trabecula, positioned in the center of the microcalorimeter measurement chamber midway between the non-contact thermopile arrays, was electrically stimulated (2 V, 5 ms pulses) to contract at 1 Hz via a platinum electrode located in the muscle-mounting chamber. Stimulus heat artifact quantified in the absence of trabecula, was negligible. Oxygen- and nutrient-rich superfusate flowed past the trabecula and was warmed by the heat of the muscle. The rate of heat production of the trabecula was estimated from the difference between the voltage signals from the up- and down-stream thermopiles, with a sensitivity of 3.0 V/W (at a measured fluid flow rate of ~0.7 µL/s). The thermopile voltages are measured using a synchronized pair of computer-interrogated nanovoltmeters (Agilent 34420). Muscle heat is the heat rate divided by stimulus frequency. The entire device is contained within a thermally-, optically-, and acoustically-insulated enclosure on a vibration-free optical table.

C. Work-loop controller

The work-loop system comprises a linear motor (Parker-Daedel) for muscle-length perturbation and a custom stainless-steel cantilever for force transduction (transducer stiffness ≈ 300 N/m). Platinum hooks secure the ends of the muscle to the linear motor (upstream) and cantilever (downstream) via 0.7 mm OD quartz tubes. The linear motor is driven by a custom-made low-noise amplifier.

Muscle length perturbation and force transducer deflection are determined by a two-channel heterodyne laser interferometer (Agilent), with ~0.3 nm resolution and 20 kHz bandwidth. The laser interferometer transfers position and force estimates via parallel digital outputs to a computer running a control system in LabVIEW9.0 RealTime. A second desktop computer provides a user interface and data-logging facilities in the WindowsXP operating system. Experimental parameters (force, length, thermopile voltage signal) and inputs (twitch-frequency -voltage and -period, and length perturbation) are collected in, and controlled by, software written in LabVIEW 9.0 and LabVIEW SignalExpress. Muscle twitches are elicited by a stimulation pulse generated in the real-time control computer, and delivered in field-stimulation mode via a platinum electrode.

A length-force control system is implemented in LabVIEW RealTime. A 20 kHz PID algorithm with lead-lag compensation seamlessly transitions between *fixed-end*, *isometric* and *isotonic* modes of control. In *fixed-end* mode, the linear motor holds one end of the muscle in constant position; any force developed by the muscle bends the force transducer, allowing the muscle to shorten by approximately 10 µm. In *isometric* mode, muscle length is servo-controlled to a constant value during force development by moving the linear motor by a distance equivalent to the force-transducer displacement. In *isotonic* mode, muscle force is feedback-controlled to a constant value by varying muscle length. Separate sets of control constants are implemented for each mode, with bumpless transition between modes.

During work-loop experiments, the controller operates in two additional modes: *half-loop*, and *full-loop*. In *half-loop* mode (Fig 2a), the controller allows electrically-elicited isometric (at length L_0) muscle twitch force to develop to a user-defined level *f*, whereupon the muscle is allowed to shorten, and then re-lengthen, at constant force. In *full-loop* mode (Fig. 2b) the controller mimics the force-length behavior of cardiac muscle in the heart. Upon elicitation of a muscle twitch, isometric force is allowed to develop to a user-selected level *f* (phase 1). The controller then transitions to isotonic mode, thereby allowing the muscle to shorten until muscle force can no longer be sustained without re-lengthening (phase 2). At this point (length L_{min}), the controller operates in isometric mode until the twitch-force diminishes (phase 3), whereupon the muscle is gently re-stretched to its initial length, at a user-defined velocity (phase 4).

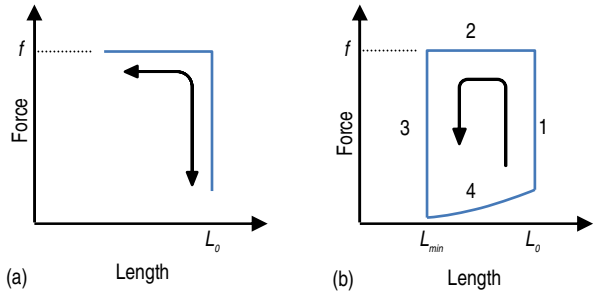

Fig. 2. Schematic illustration of the half (a) and full (b) work-loop. Solid blue lines represent the force-length trajectory during a loop; numbers represent the four sequential phases of a full work-loop.

III. Results

The trabecula, contracting at 1 Hz, was sequentially subjected to the three different control modes: *fixed-end*, *isometric* and *isotonic*. The *full work-loop* control mode consists of a combination of *isometric* and *isotonic* phases. Fig. 3*A* (top) shows the displacements of the motor while Fig. 3*A* (bottom) shows the steady-state muscle twitch force during the various control modes. In this example, muscle force was clamped at 1.1 mN during the *full work-loop* contraction, while the muscle was able to produce force of 3.5 mN during *fixed-end* contraction. Fig. 3*B* shows muscle heat rate during the entire periods of the various control modes. Stimulation was then turned off to allow the trabecula to rest. The heat rate of the trabecula during this quiescent period (labeled 'q') was less compared to those during contraction modes (labeled i – iv).

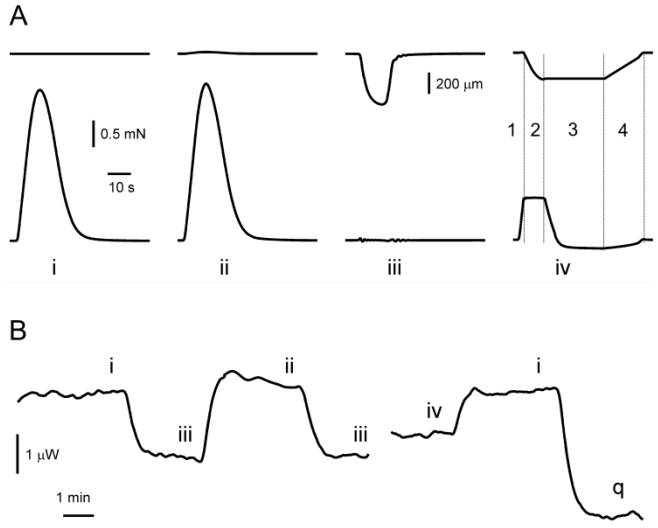

Fig. 3. *A*: experimental measurements of motor position (top) and muscle force (bottom) at various control modes (i: *fixed-end*, ii: *isometric*, iii: *zero-force isotonic*, iv: *full workloop*). In the *full workloop* mode (iv), the thin lines indicate the time points at which transitions between isometric (regions 1 and 3) and isotonic (2 and 4) modes were made. *B*: experimental measurements of muscle heat rate at various modes of contraction. The Roman numerals i-iv correspond to those in *A*, and the letter 'q' represents period of quiescence (resting) for the muscle.

Fig. 4 shows a parametric plot of muscle force versus motor displacement. During *fixed-end* mode, the motor was stationary. During *isometric* mode, the motor actuated in the positive direction (away from the force transducer) in order to compensate for the slight diminution of muscle length (~20 μm) admitted by the force transducer. During zero-force *isotonic* mode, the motor traveled in the negative direction and returned to keep muscle force zero. During *full work-loop* mode, motor transitioned between isometric and isotonic modes. In this example, f = 1.1 mN, L_0 = 0, and L_{min} = -240 μm.

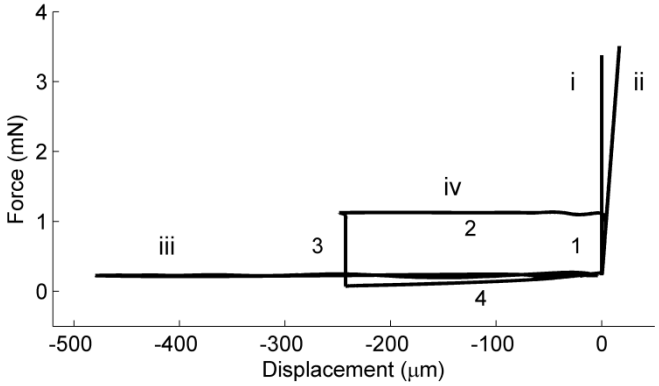

Fig. 4. Muscle force production as a function of motor displacement. The Roman numerals i-iv and the numbers 1-4 correspond to those in Fig. 3.

IV. Discussion

Replicating the contractile pattern of the heart *in vivo* on a cardiac trabecula *in vitro* requires precise transitioning, both temporally and spatially, between isometric and isotonic contraction modes (Figs. 3*A* and 4). Our work-loop calorimeter achieves this end via laser-interferometer feedback from both the linear-motor and the force transducer (Fig. 1). Simultaneously, the heat output of the trabecula (Fig. 3*B*), which is of the order of 1 μW, can be measured by arrays of non-contact thermopiles. For the full work-loop shown in Fig. 4, we estimated that the mechanical efficiency of the trabecula was 13 %. Our value is within the range reported in the literature [8-13].

V. Conclusion

Our work-loop microcalorimeter is capable of driving cardiac trabeculae to perform a range of force-length work-loops. Coupled with muscle heat measurement, it is a unique device with which to extend our understanding of cardiac energetic efficiency.

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