

## COMPARISON OF THE EFFICIENCY OF RAT PAPILLARY MUSCLES DURING AFTERLOADED ISOTONIC CONTRACTIONS AND CONTRACTIONS WITH SINUSOIDAL LENGTH CHANGES

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### Summary

The results of previous studies suggest that the maximum mechanical efficiency of rat papillary muscles is lower during a contraction protocol involving sinusoidal length changes than during one involving afterloaded isotonic contractions. The aim of this study was to compare directly the efficiency of isolated rat papillary muscle preparations in isotonic and sinusoidal contraction protocols. Experiments were performed *in vitro* (27 °C) using left ventricular papillary muscles from adult rats. Each preparation performed three contraction protocols: (i) low-frequency afterloaded isotonic contractions (10 twitches at 0.2 Hz), (ii) sinusoidal length change contractions with phasic stimulation (40 twitches at 2 Hz) and (iii) high-frequency afterloaded isotonic contractions (40 twitches at 2 Hz). The first two protocols resembled those used in previous studies and the third combined the characteristics of the first two. The parameters for each protocol were adjusted to those that gave maximum efficiency. For the afterloaded isotonic protocols, the afterload was set to 0.3 of the maximum developed force. The sinusoidal length change protocol incorporated a cycle amplitude of  $\pm 5\%$  resting length and a stimulus phase of  $-10^\circ$ . Measurements of force output, muscle length change and muscle temperature change were used to calculate the work and heat produced during and after each protocol.

Net mechanical efficiency was defined as the proportion of the energy (enthalpy) liberated by the muscle that appeared as work. The efficiency in the low-frequency, isotonic contraction protocol was  $21.1 \pm 1.4\%$  (mean  $\pm$  S.E.M.,  $N=6$ ) and that in the sinusoidal protocol was  $13.2 \pm 0.7\%$ , consistent with previous results. This difference was not due to the higher frequency or greater number of twitches because efficiency in the high-frequency, isotonic protocol was  $21.5 \pm 1.0\%$ .

Although these results apparently confirm that efficiency is protocol-dependent, additional experiments designed to measure work output unambiguously indicated that the method used to calculate work output in isotonic contractions overestimated actual work output. When net work output, which excludes work done by parallel elastic elements, rather than total work output was used to determine efficiency in afterloaded isotonic contractions, efficiency was similar to that for sinusoidal contractions. The maximum net mechanical efficiency of rat papillary muscles performing afterloaded isotonic or sinusoidal length change contractions was between 10 and 15%.

Key words: mechanical efficiency, heat production, enthalpy output, work loop, series elastic compliance, cardiac muscle, rat.

### Introduction

Many investigations of ventricular energetics have used isolated papillary muscles as a model of ventricular muscle. These muscles have myocytes arranged in parallel along their length, can be readily dissected and can have ties or clips attached to either end, allowing them to be attached to apparatus for measuring mechanical properties. The most common protocols used *in vitro* have involved either isometric or afterloaded isotonic contractions, with muscle length set to that giving maximum active force output. As a model of ventricular function, isometric contraction protocols are less than ideal since, unlike the contracting ventricle, no mechanical work is performed during contraction. However,

even afterloaded isotonic contractions produce no net work because the muscle shortens and lengthens against a constant force (Gibbs et al., 1967). Consequently, the amount of work done on the muscle to lengthen it is the same as that done by the muscle during shortening. Another criticism of the afterloaded isotonic protocol is that the combination of force and length changes is unrealistic. In an effort to approach a more realistic pattern of length changes, a number of recent studies, using cardiac and skeletal muscle preparations, have used sinusoidal length change protocols (Baxi et al., 2000; Curtin and Woledge, 1993; Josephson, 1985; Syme, 1994). An advantage of this protocol is that, with appropriate selection of

cycle frequency and timing of stimulation, net work is performed and the force–length diagrams resemble the pressure–volume diagrams of the intact ventricle (Baxi et al., 2000).

The efficiency of muscular contraction is, in general terms, the ratio of work output to energy cost. In studies using isotonic contractions, work output is defined as the product of shortening force and distance shortened (Gibbs et al., 1967; Hartree and Hill, 1928; Kiriazis and Gibbs, 1995; Sonnenblick, 1962). In other words, the work output during only the shortening phase of the contraction is calculated. Using this definition, previous studies using afterloaded isotonic contraction protocols have reported maximum net mechanical efficiency values of between 20 and 25%, achieved when shortening against a load of approximately  $0.3P_0$  (where  $P_0$  is maximum isometric twitch force) (Gibbs and Chapman, 1979a; Kiriazis and Gibbs, 1995). Twitch frequency in these protocols was typically approximately 0.2 Hz, and energy use was measured from a total of 10–20 twitches. In a recent study using a sinusoidal length change protocol, a considerably lower maximum efficiency (approximately 15%) was found (Baxi et al., 2000). However, the protocol used in that study involved higher twitch frequencies (1–4 Hz) and more contractions (40) than in the studies that used afterloaded isotonic contractions. An additional variable was that, although both the isotonic and sinusoidal experiments were performed in the same laboratory, different heat recording technologies and different calibration techniques were used in the two studies. Thus, it is unclear whether the differing efficiencies reflected the pattern of length changes or whether the higher frequency and greater number of contractions used by Baxi et al. (Baxi et al., 2000) may also have influenced efficiency.

Another factor clouding the comparison between results from the two protocols was that different definitions of work output were, necessarily, used in afterloaded isotonic and sinusoidal protocols. In sinusoidal protocols, net work output is calculated by determining the area enclosed by a plot of force output as a function of muscle length. Net work output is the difference between the work done by the muscle during shortening and that done on the muscle to re-lengthen it. In contrast to the sinusoidal protocol, there is no net work output in an afterloaded isotonic contraction, and the work output is taken as the work performed during the shortening phase alone (Gibbs et al., 1967; Hartree and Hill, 1928; Hill, 1949; Sonnenblick, 1962).

The primary aims of the present study were (i) to confirm the different efficiency values in sinusoidal and isotonic contractions by performing both protocols on the same preparation using the same apparatus and (ii) to determine whether the contraction frequency or number of contractions over which energy output is measured affects efficiency.

In addition, two other factors that can influence estimates of efficiency were assessed: (i) the assumption that all the work done on the muscle to re-lengthen it is ultimately converted into heat in the muscle, and (ii) whether appropriate definitions

of work output were used in the two types of contraction protocol.

## Materials and methods

### *Preparation*

Adult male rats (Sprague-Dawley strain; 12–16 weeks old) were killed by cervical dislocation whilst under chloroform anaesthesia. The heart was rapidly excised and immersed in a series of beakers containing oxygenated (95% O<sub>2</sub>/5% CO<sub>2</sub>) Krebs–Henseleit solution (composition in mmol l<sup>-1</sup>: 118 NaCl, 4.75 KCl, 1.18 KH<sub>2</sub>PO<sub>4</sub>, 1.18 MgSO<sub>4</sub>, 24.8 NaHCO<sub>3</sub>, 1.6 CaCl<sub>2</sub> and 10 glucose) at room temperature (approximately 22 °C), where it was gently massaged to remove remaining blood. The heart was then back-perfused through the coronary circulation with 10 ml of oxygenated Krebs–Henseleit solution (at room temperature) containing 30 mmol l<sup>-1</sup> 2,3-butanedione monoxime (BDM; Sigma Chemical Co., St Louis, MO, USA). This rapidly caused the heart to cease contractile activity. The heart remained immersed in the BDM–Krebs–Henseleit solution throughout the dissection period. The use of BDM during dissection does not affect the mechanical or energetic properties of the preparation once the BDM has been washed out (Kiriazis and Gibbs, 1995; Mulieri et al., 1989). Suitable papillary muscles from the left ventricle were trimmed and/or split if necessary, and the underside of the selected papillary muscle was separated from adhering tissue. Each end of the muscle preparation was tied with a silk tie incorporating a platinum wire loop. Note that the loops were attached to the papillary muscle itself, i.e. no tendon or ventricular wall was included in the preparation. The preparation was maintained under a light tension while being dissected from the ventricular wall.

All procedures involved in the handling and care of animals were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and were approved by the Monash University Animal Welfare Committee.

### *Experimental recordings*

Details of recording muscle heat output, length changes and force have been described previously (Barclay, 1994; Barclay et al., 1995; Baxi et al., 2000) and are outlined only briefly here. The major difference between the present study and earlier ones from this laboratory is that a horizontally mounted thermopile was used instead of the traditional, vertical arrangement. The advantages of the new thermopile included greater thermal stability and the ability to position preparations more accurately along the active thermocouples. The new system also incorporated low-compliance connections (platinum loops) between the preparation and recording apparatus and a more sensitive force transducer suitable for use with smaller, split papillary muscle preparations. The system allowed muscle preparations to be immersed in oxygenated saline during non-recording periods.

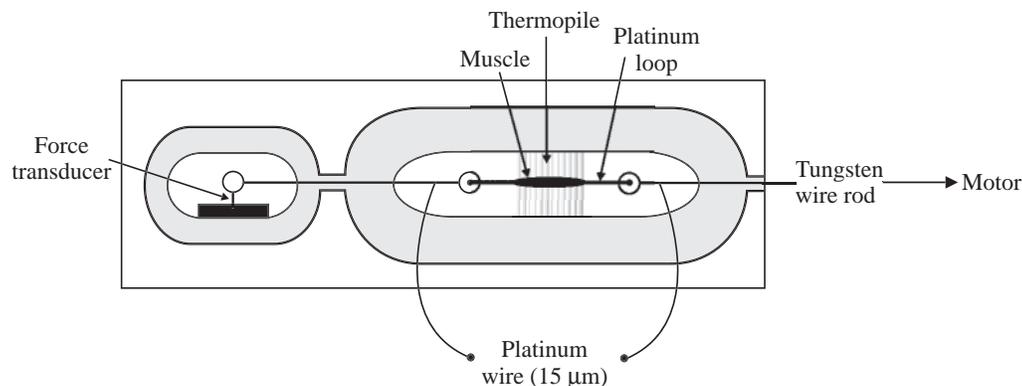


Fig. 1. Diagram of the arrangement of a muscle on the recording apparatus. The preparation lay along the central region of the thermopile, overlying the active thermocouples. Tungsten wire rods provided low-compliance links between the preparation and the recording apparatus. At each end of the preparation, one end of a platinum wire loop was bound to the muscle and the other end of the loop was placed over a hook in the end of the tungsten rod. The muscle was stimulated *via* two fine platinum wire electrodes soldered to the tungsten rods. The fine wire of the electrodes (15  $\mu\text{m}$  in diameter) prevented them from interfering with either the movement of the muscle or the recording of force.

The thermopile was mounted in an aluminium frame. Frame temperature was kept constant by circulating water from a 40 l reservoir through channels in the frame. The temperature of the water in the reservoir was maintained at 27 °C by a thermostatically controlled heater connected in series with a cooler. This arrangement was designed to minimise temperature oscillations inherent in thermostatically controlled systems.

Each muscle preparation was mounted between a semiconductor force transducer (AE801, SensoNor, Norway) and a servo-controlled lever (300H, Cambridge Technology, Inc., Watertown, MA, USA) (Fig. 1). The lever was used both to control and to measure muscle length changes. Rods constructed of fine tungsten wire provided low-compliance links between the preparation and the recording apparatus. The platinum loops tied to the ends of the preparation were placed over hooks at the ends of the connecting rods (Fig. 1). The muscle was stimulated *via* platinum wire electrodes attached to each tungsten connecting rod.

Stimulus pulse amplitude and duration were the minimum required to elicit maximum twitch force. Stimulus pulses were typically of 1–2 ms duration with an amplitude of 5–7 V. Stimulus heat per pulse was calculated from measurements of the heat produced during stimulation after the muscle had been rendered inexcitable with 20 mmol l<sup>-1</sup> procaine. Stimulus heat accounted for less than 2% of the total heat recorded in these experiments.

Two thermopiles were used in this study. These had active recording regions of 4 and 5 mm, contained 16 and 20 antimony/bismuth thermocouples and produced 1.09 mV °C<sup>-1</sup> and 1.24 mV °C<sup>-1</sup>, respectively. These thermopiles differed slightly from those described previously (Barclay et al., 1995) in that the tabs connecting the thermopile to the recording circuitry were made of antimony rather than copper. Antimony evaporates at a lower temperature than copper, which allowed the tabs to be vacuum-deposited on the Melinex substratum with less likelihood of thermal damage to the polymer. Leads

were attached to the antimony tabs using a conductive epoxy resin (Conductive Epoxy, CircuitWorks, Chemtronics, USA).

Data were recorded using a laboratory computer and a multi-function data-acquisition board (DAS1802AO, Keithley Instruments, Cleveland, OH, USA) using software developed using Test Point (Capital Equipment Corporation, Burlington, MA, USA). Force, length and temperature signals were sampled at 110 Hz, digitised and stored on disk.

During analysis of the recordings, thermopile output was filtered using a low-pass, digital filter (cut-off frequency 10 Hz; see pp. 558–559 in Press et al., 1992), corrected for heat lost from the muscle during recording and multiplied by the muscle heat capacity to give heat output. Heat loss and muscle heat capacity were determined from the time course of cooling and the steady-state temperature reached after a period of Peltier heating (Kretzschmar and Wilkie, 1972; Woledge et al., 1985).

#### Experimental protocols

Following dissection, muscle preparations performed isometric contractions for 1.5 h at a rate of 0.2 Hz to allow mechanical performance to stabilise. The lengths at which active isometric force ( $L_{\text{max}}$ ) and sinusoidal work output ( $L_{\text{opt}}$ ) were maximal were determined. The isometric force–length relationship was determined using 10 twitches at 0.2 Hz at each length. The mean maximum active force in the last five twitches was calculated. Sinusoidal work output was determined using a series of 10 contractions at 2 Hz during which length was altered in a sinusoidal pattern, also at 2 Hz; i.e. the muscle performed one contraction in each length cycle. The amplitude and stimulus phase used were  $\pm 5\%$  initial length and  $-10^\circ$ , respectively. Stimulus phase was defined as the deviation (in degrees) from the maximum-length point in each cycle. The mean work performed in the last five contractions at each length was calculated. In this study,  $L_{\text{max}}$  and  $L_{\text{opt}}$  were found to be the same. This finding differs from that in previous studies in which  $L_{\text{opt}}$  was slightly less than  $L_{\text{max}}$  (Baxi et al., 2000; Layland et al., 1995).

The present study was designed to compare the efficiency of papillary muscles performing isotonic and sinusoidal contractions. Three contraction protocols were selected: the afterloaded isotonic protocol used by Kiriazis and Gibbs (Kiriazis and Gibbs, 1995) (10 twitches at 0.2 Hz), the sinusoidal length change protocol used by Baxi et al. (Baxi et al., 2000) (40 twitches at 2 Hz) and a protocol that combined isotonic contractions with the higher twitch frequency and greater number of contractions used in the sinusoidal protocol.

The afterload for the isotonic contractions was  $0.3P_0$ , where  $P_0$  is the maximum isometric twitch force (excluding passive force). The pattern of length changes required to generate the isotonic contractions was determined using an adaptive force control algorithm (Peterson et al., 1989). Briefly, the pattern of length changes required to produce the desired isotonic contraction was determined in a series of contractions performed before the recording run. To determine the correct pattern of length changes, force output during a twitch was compared with the desired time course of force output, and the difference between the two was used to modify the applied muscle length changes. The desired time course of force output consisted of the muscle contracting isometrically until the force reached  $0.3P_0$ , then maintaining this force while the muscle shortened and then re-lengthened to the initial length, and finally relaxing isometrically. Between five and 10 twitches were sufficient to refine the length changes so that an isotonic force recording was produced. The main difference in the way we applied this technique and a conventional isotonic lever or ergometer was that this method produced isotonic contractions in which the relative afterload was constant but the absolute afterload varied between contractions in a series of twitches, reflecting the changes in the active force-generating capacity of the muscles. In other words, the afterload in each twitch was 0.3 times the isometric force that could be developed at that time in the contraction series.

Experimental recordings of force, length and temperature change were made with the solution drained from the thermopile chamber. Stimulation of the muscle ceased prior to drainage to allow any heat generated by the muscle during stimulation to dissipate into the solution. Following drainage, any remaining droplets of solution on the thermopile were carefully blotted using a small piece of tissue. The preparation remained unstimulated for 8 min prior to the start of the experimental protocols, allowing thermopile output to stabilise.

### Calculations

#### Data normalisation

At the conclusion of each experiment, the preparation was removed from the experimental apparatus, and the ends of the muscle beyond the ties were removed. The muscle was lightly blotted using filter paper, and its mass was determined using an electronic balance. The mean cross-sectional area of each muscle was calculated [mass/(length×density)], assuming a density of  $1.06 \text{ g cm}^{-3}$  (Hill, 1931). All forces were normalised by cross-sectional area.

A previous study from this laboratory (Baxi et al., 2000) used a mathematical model to assess the adequacy of diffusive  $\text{O}_2$  supply to isolated papillary muscles. That analysis indicated that  $\text{O}_2$  supply was adequate to meet the muscles' metabolic requirements at the contraction rates and temperature employed in the present experiments. The preparations used in the present study were much smaller (cross-sectional area  $0.57 \pm 0.06 \text{ mm}^2$ , mean  $\pm$  S.E.M.,  $N=6$ ) than those used as the basis of the previous model ( $0.98 \pm 0.05 \text{ mm}^2$ , mean  $\pm$  S.E.M.,  $N=8$ ; Baxi et al., 2000), providing further confidence that  $\text{O}_2$  supply would not have limited muscle metabolism.

#### Net mechanical efficiency

Net mechanical efficiency ( $\epsilon_{\text{Net}}$ ) was defined as the percentage of the total, suprabasal enthalpy output ( $H_{\text{Total}}$ ) that appeared as external, mechanical work:

$$\epsilon_{\text{Net}} = \frac{W_{\text{Total}}}{H_{\text{Total}}} \times 100,$$

where  $W_{\text{Total}}$  was the sum of the work output produced in each contraction in the series. It should be noted that work output was calculated differently in the isotonic and sinusoidal protocols. Following the example of Kiriazis and Gibbs (Kiriazis and Gibbs, 1995), work output in isotonic contractions was defined as the product of the total afterload (i.e. active force+passive force) and shortening amplitude. For the sinusoidal protocol, net work output was determined by integrating force with respect to muscle length change, which is equivalent to the area enclosed by the 'work loop' formed when force is plotted as a function of change in muscle length. Note that, in an isotonic contraction, there is no net work output over the complete cycle because shortening and re-lengthening take place against the afterload. Instead, the work output, as defined, is the work output during just the shortening phase of the isotonic contraction.

$H_{\text{Total}}$  included all the enthalpy, in excess of the basal enthalpy output, produced during and after the series of contractions and, thus, included both initial and recovery metabolisms. Enthalpy output was also determined differently in the two protocols. During the sinusoidal length change protocol, energy was liberated from the muscle as both heat and work, and the enthalpy output was the sum of the total heat produced and the total work (net) performed. In isotonic contractions, it was assumed that an amount of heat equivalent to the work done during shortening was liberated in the muscle as a consequence of doing work on the muscle to re-lengthen it (Gibbs et al., 1967; Hill, 1949). Therefore, the total enthalpy output was equivalent to the total heat output (i.e. the heat output included a component equivalent in magnitude to the work done).

#### Conversion of mechanical energy into thermal energy in isotonic contractions

An experiment was designed to determine whether all the work done to re-lengthen the muscle in an isotonic contraction

was dissipated in the muscle as thermal energy. Each muscle performed two series of contractions. One consisted of isotonic contractions (20 twitches at a frequency of 1 Hz with an afterload of  $0.3P_0$ ) and the other was a similar series in which lengthening was delayed until force had decreased to resting levels (Fig. 2). In the latter protocol, the work done during shortening was the same as in the normal isotonic protocol but, by delaying lengthening until the force output had decreased to resting levels, there was net work output; in contrast to the normal isotonic contractions, the heat output did not include a component resulting from re-lengthening against the afterload. Therefore, the total enthalpy output for this protocol was the sum of the work and heat outputs. Comparison of the total enthalpy output in the two protocols enabled the assumption that all the work done on the muscle during lengthening reappeared as thermal energy in the isotonic protocol to be assessed.

#### Statistical analyses

All data are presented as means  $\pm 1$  S.E.M. Data were analysed using analysis of variance (ANOVA) with repeated measures (i.e. the multiple-comparisons version of a paired test). *Post-hoc* testing was performed using the least significant difference (LSD) test. Data were included in the analysis only when all relevant protocols were successfully completed on any one preparation. All decisions concerning statistical significance were made at the 95% confidence level.

### Results

The primary aim of this study was to compare the efficiency of rat cardiac papillary muscle preparations performing isotonic and sinusoidal contractions. General characteristics of the papillary muscle preparations used in this part of the study are shown in Table 1.

#### Recordings during different contraction protocols

Typical recordings from all three contraction protocols are illustrated in Fig. 3. For each protocol, length change, force output, muscle temperature change and muscle heat output are shown. The mechanistic difference between the isotonic and sinusoidal protocols was that in the former force output was controlled, whereas in the latter muscle length was controlled. A notable difference in the recordings is that the magnitudes of the temperature changes in the two high-frequency protocols were substantially greater than in the 0.2 Hz protocol. Small alterations in the temperature baseline (typically with a magnitude of less than  $2.0 \times 10^{-4} \text{ }^\circ\text{C}$ ) were occasionally observed in the recordings. The high-frequency recordings were much less sensitive to these small alterations than the low-frequency recordings because of the greater magnitude of temperature change.

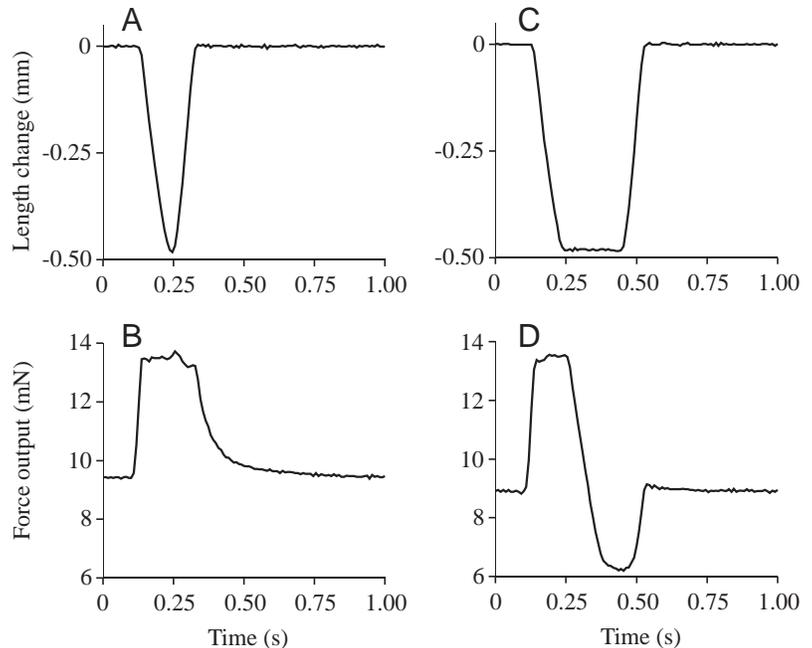


Fig. 2. Recordings illustrating the protocol used to check the work and enthalpy output during isotonic contractions. The left-hand panels show the length change (A) and force output (B) in an isotonic contraction with an afterload of  $0.3P_0$ , where  $P_0$  is the maximum isometric twitch force (excluding passive force). The right-hand panels show a contraction in which muscle length (C) was held at the shortest length until active force generation (D) had ceased. Only then was the length returned to the starting length.

Cyclic changes in muscle temperature are particularly clear in the recordings from the 0.2 Hz twitch frequency protocol (Fig. 3A). There is an abrupt increase in temperature during each twitch followed by a slow decline in temperature during the interval before the next twitch. The decline in temperature reflects heat being lost from the thermopile into the frame at a greater rate than that at which the muscle produced heat. This is compensated for when the correction for heat loss is made (Fig. 3, bottom panels). In the last half of the low-frequency isotonic contraction protocol, the cyclic temperature changes in successive cycles were the same, indicating that the muscle had achieved an energetic steady state (Paul, 1983). This state was not quite achieved in the two high-frequency protocols. In all three protocols, muscle temperature returned to its pre-

Table 1. Characteristics of papillary muscle preparations

Wet mass (mg)	3.00 $\pm$ 0.32
$L_{\max}$ and $L_{\text{opt}}$ (mm)	4.95 $\pm$ 0.20
Cross-sectional area at $L_{\max}$ ( $\text{mm}^2$ )	0.57 $\pm$ 0.06
Passive force at $L_{\max}$ (mN)	3.9 $\pm$ 0.7
Isometric active force at $L_{\max}$ (mN)	16.6 $\pm$ 1.2
Active stress at $L_{\max}$ ( $\text{mN mm}^{-2}$ )	29.8 $\pm$ 2.1
Passive force/active force at $L_{\max}$	0.24 $\pm$ 0.04

All values are mean  $\pm 1$  S.E.M. ( $N=6$ ).

$L_{\max}$ , muscle length at which active isometric force was maximal;  
 $L_{\text{opt}}$ , muscle length at which sinusoidal work output was maximal.

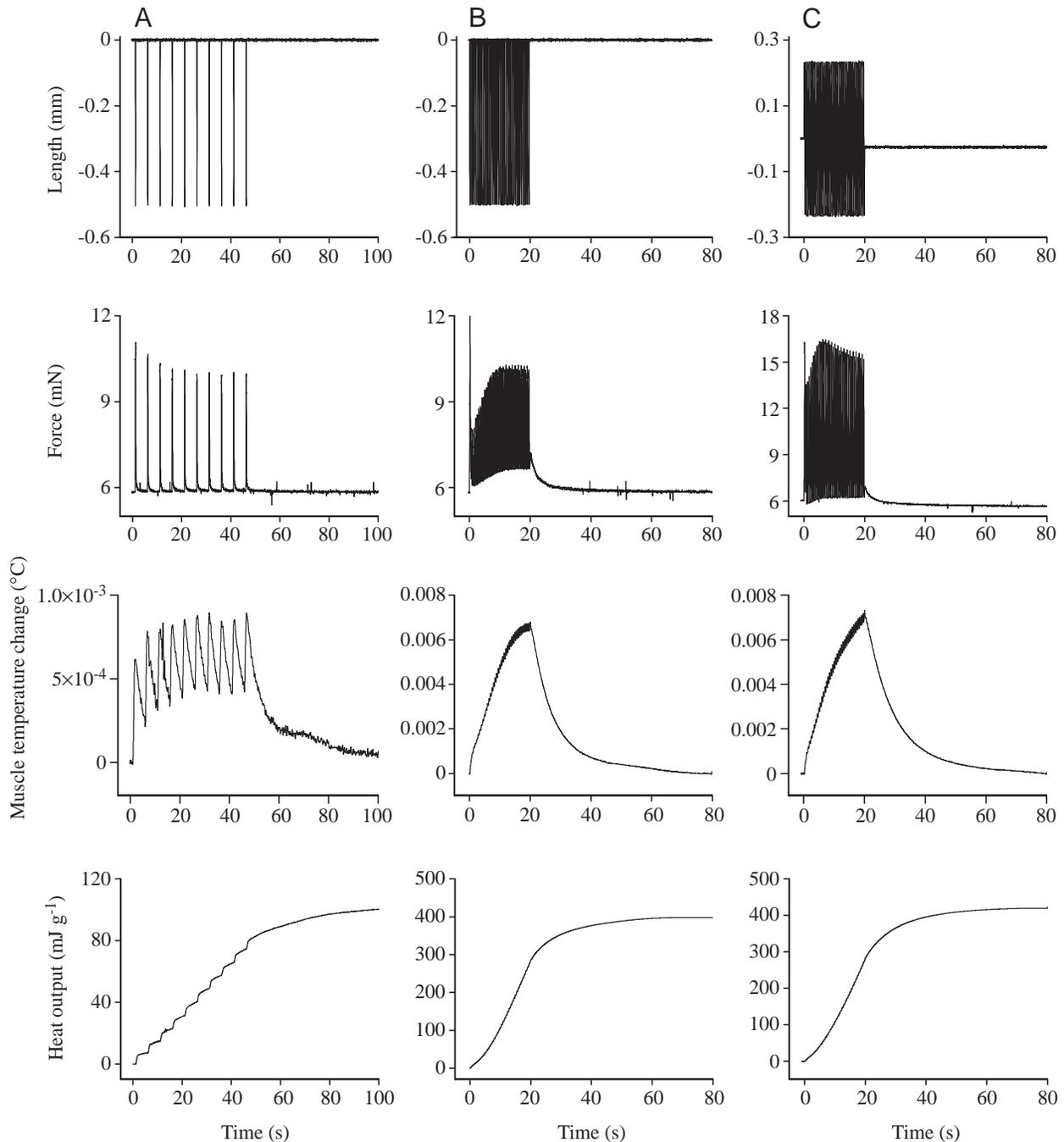


Fig. 3. Examples of recordings made during the three contraction protocols. Recordings are shown for (A) low-frequency isotonic contractions (10 twitches at 0.2 Hz), (B) high-frequency isotonic contractions (40 twitches at 2 Hz) and (C) sinusoidal contractions (40 twitches at 2 Hz). For each protocol, recordings are shown of (from top to bottom) change in muscle length, force output, change in muscle temperature and cumulative heat output. All recordings were made on the same muscle (mass, 2.0 mg; length, 4.6 mm; cross-sectional area, 0.41 mm<sup>2</sup>).

stimulation value, indicating that all recovery metabolism was complete in less than 1 min after completion of the contractions.

#### *Comparison of low-frequency isotonic protocol and sinusoidal length change protocol*

The nature of the afterloaded isotonic contraction is such that little or no net work is performed. This is illustrated in Fig. 4, in which force is plotted as a function of change in

muscle length for both types of contraction. In the sinusoidal protocol (Fig. 4B), the force during shortening was substantially greater than that during the re-lengthening phase, resulting in a loop, in which time progressed anticlockwise, indicating that there was net work output. In contrast, in the isotonic contraction, the force was the same during shortening and lengthening, so no loop was formed, which indicates no net work output (Fig. 4A). Therefore, work performed in an

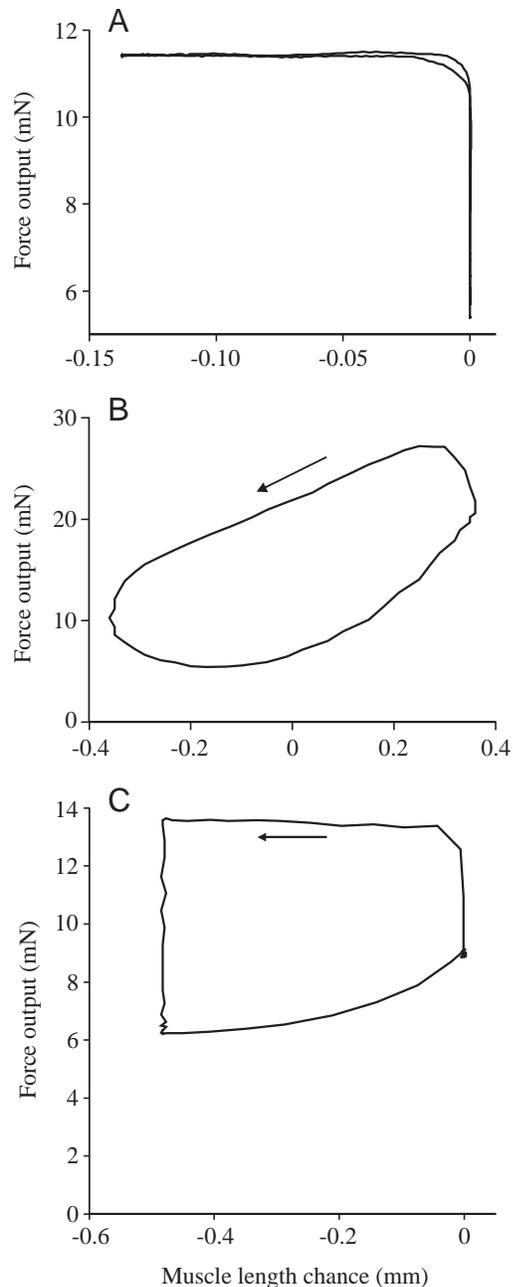


Fig. 4. Examples of work loops formed during isotonic (A) and sinusoidal (B) contractions. Work loops were produced by plotting force with respect to change in muscle length. Contraction rate was 2 Hz for sinusoidal contractions and 0.2 Hz for the isotonic twitches. Sinusoidal contractions resulted in work loops enclosing a substantial area, indicating net work output. Isotonic contractions did not form work loops, indicating that little or no net work was performed by the muscle. (C) An example of the work loop formed when re-lengthening in an isotonic contraction was delayed until active force production had ceased. The contraction started at the longest length and force increased with no change in muscle length until the force matched the afterload. The muscle then shortened with a constant force output, before being allowed to relax while muscle length was held at the shortest length. The passive muscle was then stretched back to its initial length. Time progresses around the loops in an anticlockwise direction (arrows in B and C).

isotonic contraction is expressed as the work performed during just the shortening phase.

During the contraction protocols used to determine efficiency, the total work output was much greater in the sinusoidal protocol, during which 40 twitches were performed, than during the low-frequency isotonic protocol, in which only 10 twitches were performed (Table 2). However, more work was performed in each low-frequency isotonic twitch than in each twitch with sinusoidal length changes. The total enthalpy output in the sinusoidal protocol (=total heat+total work) was also much greater than that in the low-frequency isotonic protocol (total enthalpy=total heat) but, when normalised by the number of contractions, the energy cost per twitch did not differ between the two protocols (Table 2). As reported previously, in separate studies, the net efficiency of the muscles was greater during isotonic contractions than during sinusoidal contractions. In the 0.2 Hz isotonic contractions, the mean value of  $\epsilon_{\text{Net}}$  was  $21.1 \pm 1.4\%$ , whereas in the sinusoidal protocol  $\epsilon_{\text{Net}}$  was  $13.2 \pm 0.7\%$ . It should be noted that, in each of these protocols, the loads, the stimulus timing and the length changes used were those previously shown to be required to produce maximum efficiency in the respective protocol.

The third protocol used isotonic contractions combined with the twitch frequency and number of contractions used for the sinusoidal protocol. The notable result was that, with this protocol,  $\epsilon_{\text{Net}}$  was  $21.5 \pm 1.0\%$ , which did not differ from that in the low-frequency isotonic protocol but was significantly greater than that in the sinusoidal protocol. Therefore, the difference in  $\epsilon_{\text{Net}}$  between the isotonic and sinusoidal contractions was not simply due to the different contraction frequency or number of twitches.

This study used an adaptive force algorithm to control the muscle length changes, giving a constant relative afterload of  $0.3P_0$ . Comparison of the present results with those of previous studies (Gibbs and Chapman, 1979b; Kiriazis and Gibbs, 1995) indicates that the values of the energetic variables measured from a protocol in which the relative afterload was constant between contractions (present study) were the same as those in which the absolute afterload was constant. The mean active force using the present method was only approximately 5% greater than what would have been recorded if the absolute afterload force had been constant and equal to 0.3 of the steady state, isometric twitch force.

#### *Conversion of mechanical energy into thermal energy in isotonic contractions*

A series of experiments was performed to determine (i) whether the work output in isotonic contractions was really equivalent to total force  $\times$  distance shortened and (ii) whether all the work performed on a muscle during isotonic lengthening (which is equal to the work done during shortening) was dissipated in the muscle as thermal energy. The experiments involved comparing work output calculated in the usual isotonic way with that determined from the area of the work loop formed when re-lengthening was delayed until force had

Table 2. Mean values of energetic variables for the three contraction protocols

	Low-frequency isotonic	Sinusoidal	High-frequency isotonic
Contraction frequency (Hz)	0.2	2	2
Number of twitches	10	40	40
Total work output (mJ g <sup>-1</sup> )	19.5±1.6 <sup>a</sup>	51.5±3.1 <sup>b</sup>	60.6±7.8 <sup>b</sup>
Total heat output (mJ g <sup>-1</sup> )	93.0±6.6 <sup>a</sup>	349.1±32.9 <sup>b</sup>	283.6±33.6 <sup>c</sup>
Work per twitch (mJ g <sup>-1</sup> )	1.95±0.16 <sup>a</sup>	1.29±0.08 <sup>b</sup>	1.52±0.20 <sup>b</sup>
Heat per twitch (mJ g <sup>-1</sup> )	—	8.73±0.82	—
Enthalpy per twitch (mJ g <sup>-1</sup> )	9.30±0.66 <sup>a</sup>	10.01±0.89 <sup>a</sup>	7.09±0.84 <sup>b</sup>
Net efficiency (%)	21.1±1.4 <sup>a</sup>	13.2±0.7 <sup>b</sup>	21.5±1.0 <sup>a</sup>

All values are mean ± 1 S.E.M. (N=6).

Letters indicate significance difference ( $P < 0.05$ ). For each variable, values labelled a differ from those labelled b and c, and those labelled b differ from those labelled c.

For isotonic protocols, values for heat output per twitch are not listed since, by definition, heat output per twitch and enthalpy output per twitch are the same.

relaxed to passive values. Mean values for the protocols are displayed in Table 3.

When work output was calculated as total force × distance shortened, the work per twitch was, as expected, the same in the two contraction protocols. However, work output calculated from the area of the work loops in the delayed re-lengthening contractions was significantly less than that calculated as total force × shortening amplitude (Table 3). The difference between the two methods for calculating work was that the work loop method took account of the work that had to be done on the muscle to re-lengthen it; the net work output is the difference between the work performed during shortening and the work required to re-lengthen the muscle. A comparison of the enthalpy output for these two protocols provided a thermodynamic assessment of which method of calculating work was correct.

The enthalpy output in response to the normal isotonic protocol was compared with the enthalpy output calculated for the protocol in which lengthening was delayed (Table 3). The enthalpy output of the delayed lengthening protocol was equal to the heat + work, but work could be calculated two ways: (i) as total force × shortening amplitude and (ii) as the area of the

work loop. If work was calculated using the first of these methods, then the enthalpy output in the delayed lengthening protocol was significantly greater than that in the normal isotonic protocol. However, when the second method of calculating work (work loop area) was used, then there was no significant difference between the enthalpy output in the two protocols (Table 3). This is consistent with the idea that the area of the work loop was the accurate index of work output. If this is so, then it can also be concluded that all the work done during re-lengthening in the normal isotonic contractions was dissipated in the muscle as heat.

### Discussion

The initial set of experiments in this study confirmed that the efficiency, as previously defined, of papillary muscles is different when performing sinusoidal contractions and when performing isotonic contractions. The subsequent experiments provided information that can be used to investigate the basis of this difference. Specifically, the final set of experiments indicated that the method of calculating work used in the isotonic contractions probably overestimated work output and,

Table 3. Work and enthalpy outputs for normal and delayed-lengthening isotonic contractions

	Calculation	Contraction type	
		Normal isotonic	Delayed lengthening
Work output (mJ g <sup>-1</sup> twitch <sup>-1</sup> )	Work= $P \times \Delta L$	1.61±0.17 <sup>a</sup>	1.48±0.22 <sup>a</sup>
	Work= $\int PdL$	—	0.81±0.15 <sup>b</sup>
Enthalpy output (mJ g <sup>-1</sup> twitch <sup>-1</sup> )	H=total heat	7.06±1.10 <sup>c</sup>	—
	H=( $P \times \Delta L$ )+heat	—	8.33±1.15 <sup>d</sup>
	H= $\int PdL$ +heat	—	7.64±1.14 <sup>c</sup>

All values are mean ± 1 S.E.M. (N=5).

Letters indicate significant differences using repeated-measures comparison: a differs from b, and c differs from d.

$P \times \Delta L$ =total afterload × amplitude of shortening;  $\int PdL$ =force integrated with respect to length change=area enclosed by work loop;  $L$ , muscle length.

$H$ , enthalpy output; enthalpy is heat output for normal isotonic contractions and (heat+work) for isotonic contractions with delayed lengthening.

thus, efficiency in these contractions. Both these issues are addressed in the following discussion.

*What is the correct method to calculate work output in isotonic contractions?*

Work output calculated as total force  $\times$  shortening amplitude was substantially greater than that calculated from the area of the work loop in the modified isotonic protocol. The difference between these two estimates of work output is that the former includes the work done by elastic elements in parallel with the contractile elements. In contrast, using the area of a work loop to measure work output recognises that work must be done on the muscle to re-lengthen parallel elastic elements (assuming that there is no contractile activity during re-lengthening), so they contribute no net work output or may even absorb energy (Baxi et al., 2000; Josephson, 1985). It is interesting to note that, in early studies on the energetics of muscles during isotonic contractions, explicit corrections were made for the underlying passive force (Gibbs and Gibson, 1970; Gibbs et al., 1967; Hill, 1949) so that work output was calculated in a manner analogous to the work loop method used with sinusoidal contractions. Using such an analysis yielded maximum efficiency values of between 10 and 15%.

Support for the idea that the work output during isotonic shortening, excluding parallel elastic work, did correspond to the area of the work loop in the delayed lengthening contractions was provided by observations of the enthalpy output in the two isotonic protocols (i.e. normal isotonic twitches and those with delayed re-lengthening; Fig. 4C). In the delayed re-lengthening protocol, the heat output was less than that in the normal isotonic protocol. However, when the sum of the heat and work produced in the delayed re-lengthening protocol was calculated, it was the same as the total heat produced in the normal isotonic protocol. This is what would be expected if (i) the work output during shortening in the isotonic protocol, excluding work done by parallel elastic elements, was equivalent to the work loop area in the delayed re-lengthening protocol, and (ii) all the work required to stretch the muscle in the normal isotonic contractions was dissipated as heat in the muscle (Gibbs et al., 1967; Hill, 1949). If, however, the enthalpy output in the delayed re-lengthening protocol was calculated using the product of total force and shortening amplitude (the definition used in previous studies), then the calculated enthalpy output was greater than the enthalpy output measured in the normal isotonic protocol.

If calculating work as total force  $\times$  distance shortened overestimated the actual work output, then efficiency would also have been overestimated. The data in Table 3 indicate that, in the preparations used in this study, the values of total force  $\times$  distance shortened were approximately twofold greater than the work loop area. Correcting for an error of this magnitude reduces the efficiency for isotonic contractions in this study from a mean value of approximately 21 to 12%, a value similar to that determined for the sinusoidal contractions. This revised value is also similar to those quoted in early studies on

papillary muscle energetics in which appropriate corrections were made to the work output (Gibbs and Gibson, 1970; Gibbs et al., 1967). In addition, the maximum efficiency of frog ventricular muscle, determined using a work loop technique, was reported to be 13% (Syme, 1994), again similar to the values in the present study if work output is calculated on the basis of active force output alone.

In a very recent study investigating changes in the energetics of rat papillary muscles with age, Kiriazis and Gibbs (Kiriazis and Gibbs, 2000) presented data for work output calculated both without and with a correction for changes in passive force during shortening in isotonic contractions. Making an estimate from their data indicates that maximum efficiency was decreased from approximately 25 to 19% when the total work was replaced by the estimated net work output. The magnitude of the difference between the two methods for calculating work was smaller in that study than ours, which must be due to the passive force at  $L_{\max}$  being lower in the work of Kiriazis and Gibbs (Kiriazis and Gibbs, 2000) than in the present study. However, the results of those authors support the idea that the maximum efficiency of papillary muscles is likely to be less than 20%.

*Comparison with efficiency of whole hearts*

There is extensive evidence to indicate that the efficiency of animal (Elzinga and Westerhof, 1980) and human (for a review, see Gibbs and Barclay, 1995) hearts is likely to be at least 20%. If the efficiency of papillary muscle determined in the present study is an accurate index of the efficiency of ventricular muscle, then for the efficiency of the ventricle to be substantially higher than the net efficiency of the muscles suggests that some other process contributes to power output of the ventricles but without using metabolic energy from ventricular muscle. If, for instance, the energy required to stretch the ventricular muscle during diastole was ultimately derived from a source other than contraction of cardiac muscle, then the efficiency of the heart would be greater than that of the muscle itself. An alternative possibility is that neither of the contraction protocols used in the present study could produce efficiency values as high as those for cardiac muscle *in vivo*. To test this possibility, similar experiments should be performed using strain patterns that more closely match those occurring *in vivo*.

*Concluding remarks*

In conclusion, the maximum efficiency of papillary muscles appears likely to be the same in both sinusoidal and afterloaded isotonic contraction protocols, provided that comparable definitions of work output are used. If the definition of work output incorporates the energy cost of re-lengthening the parallel elastic elements of the muscle, then the maximum efficiency of rat papillary muscles in both protocols is between 10 and 15%.

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