Skeletal muscle may provide a totally implantable power source for cardiac assistance devices. Continuous power from electrically conditioned human latissimus dorsi muscle has been obtained in the clinical cardiomyoplasty procedure (Chachques et al., 1997). Maximum sustained power output during repetitive work-producing contractions is required for cardiac assistance (Jarvis, 1993). Previous work in our laboratory has focused on the biomechanical optimization of skeletal muscle cycle work and power (Reichenbach and Farrar, 1994; Reichenbach et al., 1995, 1999). Biomechanical power optimization and the concomitant operating conditions should be constrained by the muscle metabolic factors that limit sustained power. This constraint may be identified on the basis of relative relationships; therefore, an absolute measurement technique is not necessarily required.

Metabolic utilization is reflected in the muscle heat generation during contraction and is dependent on a number of variables, including the specific contraction variables, species and muscle fiber type (Constable et al., 1997). Although myothermal relationships and the biochemical basis of skeletal muscle contraction have been extensively studied in in vitro preparations of amphibian muscle fibers at reduced temperatures, the energetic characteristics of work performance by mammalian muscle are unclear (Barclay et al., 1993a; Curtin and Woledge, 1993). The large in situ mammalian muscles, short contraction durations and cyclic operating conditions inherent in cardiac assistance applications may not be directly comparable with previous work.

The purpose of the present study was (i) to develop an in vivo measurement technique to provide an estimate of metabolic energy utilization in whole mammalian muscle during repetitive contractions; (ii) to identify experimentally the significant contraction variables involved in metabolic energy utilization and their relationships; and (iii) to develop a model to predict metabolic energy utilization over a range of contraction conditions.

Materials and methods

Surgical preparation

Seven New Zealand white rabbits were anesthetized with Ketamine (60 mg kg$^{-1}$) and Xylazine (5 mg kg$^{-1}$), and a tracheostomy was performed. The animals were supported on a volume-controlled mechanical ventilator and monitored with a pulse oximeter, and anesthesia was maintained with intermittent intravenous boluses of sodium pentobarbital. All
animals were handled in accordance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (NIH publication 85-23, revised 1985). The left soleus muscle was exposed, and the tendinous insertion to the calcaneus was isolated. The slow-twitch rabbit soleus muscle was chosen since electrically conditioned skeletal muscle utilized in cardiac assistance applications consists primarily of slow-twitch fiber types (Salmons and Henriksson, 1981). Three or four thermistors were individually inserted into the mid-portion of the soleus. The sciatic nerve was identified, and the branch to the soleus muscle was isolated. A bipolar nerve cuff electrode was placed around the soleus nerve branch and connected to a programmable myostimulator (Teleometrics, model 7220NS). The skin incision was closed, leaving the muscle in situ with the calcaneus Achilles tendon attachment exiting the leg through a small distal incision. The distal femur and left leg were immobilized and fixed in position. The distal leg and foot were insulated, and the area was maintained at a stable resting temperature with radiant heat and tubing from a circulating water bath.

Instrumentation and experimental test apparatus

Thermistors (Thermometrics, Edison NJ, USA; GC11) were fast-responding (time constant approximately 20 ms) and relatively small (30 mm diameter). Resistance and sensitivity at 37 °C were approximately 14 kΩ and −3.7 % °C⁻¹, respectively (520 Ω °C⁻¹). The thermistors were connected to a modified full bridge circuit of low excitation voltage (approximately 350 mV to bridge). The absence of self-heating error was verified in preliminary experiments by varying the excitation voltage across at least a fourfold range. The system resulted in an approximate temperature resolution of 0.001 °C.

The muscle was connected to a loading system similar to that of Reichenbach and Farrar (1994). A nylon-coated stainless-steel cable was attached to the Achilles tendon. The muscle was connected to a load cell (A.L. Design, Inc., Buffalo, NY, USA) to measure muscle tension, to a rotational potentiometer to measure muscle displacement and to a loading system incorporating a low-friction seal-less pneumatic cylinder. Isotonic afterload was controlled with a pneumatic regulator. All thermistor, force and displacement signals were amplified via differential transducer amplifiers (Gould, Valley View, OH, USA) and digitized at 200 Hz with a PC-based analog-to-digital converter (Metabyte, Taunton, MA, USA) and software developed in our laboratory.

Thermistor temperature measurements

The thermistor technique was not applicable to investigation of temperature changes within individual contractions over the range of contraction durations investigated. The amplitude of the initial temperature changes within individual contractions varied between thermistors, preventing accurate investigation of temperature changes during stimulation. Recording temperature change over time through the onset of in vivo muscle activation can be complicated (Saugen and Vollestad, 1995). Relative motion between the thermistor and the muscle tissue may result in motion artifacts due to temperature gradients or non-homogeneities (Saugen and Vollestad, 1995). The thermistor measurements immediately prior to and after a contraction, when the muscle position and tension were stable, were not affected by relative thermistor motion and, therefore, enabled consistent recording of muscle temperatures between contractions.

Blood flow in an in situ preparation has the potential to affect the heat production estimate. In preliminary studies, selected isometric data runs were repeated with the femoral artery occluded to determine the potential influences of perfusion. The relative temperature measurements between contractions did not differ between these runs, suggesting that heat transfer due to perfusion was not a large component of the observed responses. Similar results have been obtained in human vastus lateralis muscle (Saugen and Vollestad, 1995).

Experimental protocol

The muscle was stimulated with bursts of 50 Hz pulse trains at a contraction rate of 40 beats min⁻¹ and with stimulation durations of 100–400 ms. Stimulation voltage and pulse width were adjusted to ensure maximal activation and were typically 700 mV and 0.25 ms respectively. A series of 4–6 contractions was elicited at each test condition. The contribution of recovery heat during a relatively short series of cyclic contractions in mouse muscle has been reported to be less than 5 % of that produced during contractions (Barclay et al., 1994). Therefore, no attempt was made to correct for recovery metabolic heat over the short series of contractions.

The relative thermistor response was evaluated over a range of isometric and isotonic contraction conditions. Series of contractions were elicited in three cases to examine the effects of muscle tension, contraction duration and work output. (i) Isometric tension: active tension was varied by changes in muscle length (five positions). Contraction duration was 200 ms. (ii) Contraction duration: muscle length was fixed (isometric) near the length for maximal tension generation (L₀) and contraction series of 100, 200, 300 and 400 ms duration were elicited. (iii) Isotonic work: the muscle was loaded isotonically and was free to shorten. The initial muscle length (stretch limit) was set near L₀. Contraction series were elicited against a range of 4–5 isotonic afterload tensions. Contraction duration was 200 ms.

Data reduction

The digitized data files were analyzed with TecPlot (Amtec, Bellvue, WA, USA). The muscle position, shortening distance, contraction duration, average passive and active tension (peak minus passive preload) and thermistor signal amplitude immediately prior to each contraction were determined for each contraction series. Shortening distance and work were assumed to be zero for isometric contractions. Net isotonic cycle work was calculated as the product of the average afterload tension during contraction (minus the resting tension) and the
was given by: thermistor response data. The normalized thermistor response relationship was utilized to normalize the values ranging from 0 to approximately 0.25.

Work was non-dimensionalized by normalization to the dimensionalized by normalization to the peak active force. The active tension was non-dimensionalized using the lengths corresponding to zero and peak tension of the polynomial fit, respectively. The active tension was non-dimensionalized by normalization to the optimum length is 0). The negative phases of the thermistor response are shown as examples of motion artifact during muscle contraction. Isotonic work loops are shown in Fig. 1B, where the muscle shortened and worked against a fixed pneumatic load. The net and normalized isotonic work per contraction for this example were 35.6 mJ and 0.15, respectively.

Thermistor response versus contraction duration
The normalized thermistor response versus contraction duration for the isometric contraction duration data runs for all experiments are shown in Fig. 2. The response predicted by the modified linear regression model (Table 1) for isometric contractions and a normalized active tension of 0.9 is also shown (solid line) for comparison with the original data. The thermistor response versus contraction duration relationship demonstrated a linear response for every

| Table 1. Modified linear regression model results for predicting the thermistor response using a \( F_t \) term |
|---------------------------------|---|---|---|
| Term | \( \Delta T \) | \( a \) | \( b \) |
| Value | \(-1.00^*\) | \(5.97^*\) | \(2.12^*\) |
| \( \pm \text{s.e.m.} \) | \(\pm 0.064\) | \(\pm 0.328\) | \(\pm 0.553\) |

\( \Delta T = C + at_{c}F + bW \).

\( r^2 = 0.60; F = 169^*; \text{total s.e.m.} = 0.421. \)

\* \( P < 0.001 \).

\( \Delta T \) is the normalized thermistor response, \( C \) is a constant, \( a \) and \( b \) are coefficients, \( t_{c} \) is the contraction duration (ms), \( F \) is the normalized active force and \( W \) is the normalized net isotonic work.
individual thermistor ($r^2=0.90\pm0.14; \text{mean} \pm \text{s.d.}, \text{range} \ 0.57-0.99$). The individual linear regression of each thermistor (slope $m_t$, y-intercept $b_t$) was used to determine the intercept/slope ($b_t/m_t$) ratio. The $b_t/m_t$ ratio for all thermistors was $0.12\pm0.11$ (mean $\pm$ s.d.).

**Thermistor response versus active tension**

The normalized thermistor response *versus* active tension for the isometric tension data runs for all experiments are shown in Fig. 3. The response predicted by the modified linear regression model (Table 1) for isometric contractions of 200 ms duration is also shown (solid line) for comparison with the original data. The thermistor response *versus* active tension relationship demonstrated a linear response for each individual thermistor ($r^2=0.70\pm0.21; \text{mean} \pm \text{s.d.}, \text{range} \ 0.41-0.99$). The linear regression fits for three individual thermistors from the same experiment are shown in Fig. 3 (dashed lines) to demonstrate the linearity within individual
thermistors. The linear regression relationship fitted for each individual thermistor was extrapolated to zero force to determine the thermistor response intercept, \( b_f \). The zero-force thermistor response was 30.9±23.2 % (mean ± S.D.) of the maximum thermistor response. Maximum thermistor response for these runs was defined by the regression fit at the peak active force.

Multiple linear regression model

The multiple linear regression model for predicting the thermistor response, \( \Delta T \), is shown in Table 2. The final, best-fit model variables included the contraction duration \( t_c \), the active tension \( F \), the net isotonic work \( W \) and a constant \( C \).

None of the potentially outlying data points was identified as influential (Cook’s distance >1.0).

Alternatively, the contractile component of the thermistor response may be represented by a time-dependent force term (\( F t_c \)), as shown in Table 1. Inclusion of this term into the regression procedure resulted in the individual force and duration terms being not significant. This model (Table 1) resulted in a significant fit and is compared with the observed thermistor response and the residuals in Fig. 4.

Work was significant (\( P<0.001 \)) entered into both models as a linear term. Replacement of work in the model (Table 1) with interaction terms between work and distance, work and force, and work and work, however, resulted in essentially similar

Table 2. Linear regression model results for predicting the thermistor response

<table>
<thead>
<tr>
<th>Term</th>
<th>Value ± S.E.M.</th>
<th>( C )</th>
<th>( a )</th>
<th>( b )</th>
<th>( d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta T ):( C + a t_c + b F + d W )</td>
<td></td>
<td>-2.07* ±0.119</td>
<td>5.02* ±0.407</td>
<td>1.30* ±0.116</td>
<td>2.27* ±0.580</td>
</tr>
</tbody>
</table>

\( \Delta T \) is the normalized thermistor response, \( C \) is a constant, \( a, b \) and \( d \) are coefficients, \( t_c \), is the contraction duration (ms), \( F \) is the normalized active force and \( W \) is the normalized net isotonic work.

Fig. 3. Normalized thermistor response \textit{versus} active tension for the isometric active tension runs at a contraction duration of 200 ms for all experiments. The response predicted by the modified linear regression model (see Table 1) for isometric contractions of 200 ms duration is also shown (solid line) for comparison with the original data. Linear regression fits for three individual thermistors (Tm1, 2, 3) from the same experiment are shown (dashed lines and shaded symbols) as examples of the linear response typically observed for individual thermistor placements.

Fig. 4. Comparison of the predicted normalized thermistor response based on the model (see Table 1) \textit{versus} the experimentally observed normalized thermistor response. The broken line is the line of equivalence. The inset shows the residual values \textit{versus} the predicted normalized thermistor response. Dashed lines represent ±2 S.E.M.
significance ($P<0.001$) and no substantial improvement in predictive value ($r^2=0.61, 0.60$ and $0.61$, respectively).

A discrete variable representing each individual thermistor placement was added to the model to examine the variability due to individual thermistor sensitivity, in situ placement and inter-animal variability not corrected for by the normalization. This variable was statistically significant ($P<0.05$) and increased the coefficient of determination $r^2$ from 0.60 to 0.72, but did not substantially change the coefficients.

Discussion

Thermistor response: expected temperature changes

Utilizing a relative temperature measure for in vivo experiments that involve small temperature changes and short contraction durations simplifies the instrumentation requirements and eliminates the need to determine absolute temperature precisely; however, it also requires the assumption that heat transfer is not a function of the contraction conditions. The relationship between absolute temperature and thermistor response is dependent on the individual thermistor coefficient, absolute temperature and bridge and amplifier tolerances. Precise determination of the absolute temperature changes was not required for this application, although these values were estimated for comparison with expected values based on results from other studies.

Temperature increases of 0.010–0.020 °C have been observed during the first second of tetanic stimulation of frog muscle at 15 °C (Curtin et al., 1983), although this value may have been greater at physiological temperatures (Curtin et al., 1983). Rates of temperature increase of 0.010–0.020 °C s$^{-1}$ have also been observed in in vivo human vastus lateralis muscle during long (≥8 s) isometric contractions (Saugen and Vollestad, 1995), although this value is expected to be lower in the soleus muscle and to be higher with complete muscle activation.

Temperature increases for the present study were estimated from the observed thermistor responses using the thermistor resistance/temperature ratio (approximately 520 Ω °C$^{-1}$) and bridge circuit and amplifier gain. Typical responses of 0.020–0.040 °C s$^{-1}$ were calculated for the thermistor response versus contraction duration data sets, which corresponds to a rate approximately twice those given above. These values ignore the heat generated after the end of stimulation while tension is non-zero (Lou et al., 1997), which may slightly overestimate the expected temperature change. The initial energy production during an isometric contraction begins at a much higher initial rate before falling to a steady state (Woledge et al., 1985; Lou et al., 1997). The magnitude of the initial rate may be more than twice that of the steady-state value in frog muscle (Woledge et al., 1985). Therefore, the absolute temperature changes for the relatively short contraction durations (≤400 ms) utilized in the present study are consistent with the magnitude of expected temperature changes on the basis of other studies.

Thermistor response variation

The absolute (pre-normalized) thermistor response for individual placements varied within and among experiments by a factor of approximately 2 despite stable recordings and our best efforts to reduce the variability. Considerable variations in $\Delta T/dt$ (up to 180%) have been observed previously between in vivo temperature recording sites (Saugen and Vollestad, 1995). Muscle temperature during an isometric tetanus is not uniform, but varies from region to region (Kobayashi et al., 1998), and temperature gradients or non-uniform heat generation through the muscle can complicate temperature measurements (Woledge et al., 1985). Thermistor insertion trauma may also have resulted in areas of muscle damage. We did not quantify the impact of these factors on thermistor variability. Normalization of the thermistor responses reduced this variation and allowed combined analysis of all thermistor placements.

Relationship between thermistor response and contraction duration

The strongest observed relationship among the variables examined was the expected linear relationship between relative thermistor response and contraction duration. This response is consistent with previous studies (Mommaerts, 1969; Curtin et al., 1983; Woledge et al., 1985). As expected, the normalization resulted in high correlations among the duration data from multiple experiments.

The pre-normalized data confirm that metabolic utilization, represented by the thermistor response, is a function of contraction duration and that the dependence is not just proportional (there is a positive $y$-intercept). A positive $y$-intercept is consistent with a greater rate of output at the beginning of an isometric contraction before a stable level is attained (Woledge et al., 1985; Lou et al., 1997). The linear relationship for contraction durations between 100 and 400 ms observed in the present study suggests that the rate of energy output reached a stable level prior to 100 ms. The positive $h/m_t$ ratio for the metabolic utilization versus duration relationship has an influence on the sustained power optimization of cyclically contracting skeletal muscle (Gustafson and Reichenbach, 1998).

Relationship between thermistor response and force production

The observed linear relationship between thermistor response and tension development is consistent with previous studies (Gibbs and Gibson, 1972; Saugen and Vollestad, 1995; Lou et al., 1997). As shown in Fig. 3, the correlation was greater for individual thermistor responses than for all thermistors combined. This effect may be a result of the normalization not completely removing the variability between all thermistor measurements.

Extrapolation of the relative heat output and relative force relationship to zero force has been utilized to determine the energetic cost of activation for an isometric tetanus (Lou et al., 1997; Barclay et al., 1993b). The activation heat as a
percentage of the heat produced during an isometric tetanus has been shown to be 27% in frog muscle (Barclay et al., 1993b), 34% in dogfish (Scyliorhinus canicula) white muscle (Lou et al., 1997) and approximately 24% in rat soleus muscle (Gibbs and Gibson, 1972). The relative zero-force thermistor response of 31% determined in the present study is comparable with the values in these studies.

Regression model form and terms

The model was intended to identify the contributing variables, to predict metabolic utilization under specific contraction conditions and to provide a relationship that could be utilized in the prediction of optimal sustained power conditions. It was not intended to include or elucidate the underlying chemical energetics. The thermistor responses predicted by the model (Table 1) generally fitted the observed data (as shown in Figs 2–4). The initial model (Table 2) resulted from the backward stepwise regression procedure and demonstrated an acceptable correlation with the data. Replacement of the individual force $F$ and duration $t$ terms with a single contractile term $F_t$ (Table 1) resulted in similar correlation over the ranges of variables investigated. The single contractile term, $F_t$, is an interaction term between $F$ and $t$ and was therefore excluded prior to either $F$ or $t$ as an individual variable during the backward stepwise regression procedure. However, one advantage of this form is that the rate of energy production is dependent on active tension (Mommaerts, 1969).

The energy expenditure, $E$, of contracting skeletal has been represented in the form $E=A+f(F,t)+W+SH$, where $A$ is the activation component, $f(F,t)$ is the maintenance energy to sustain tension, $W$ is the net work and $SH$ is the shortening energy (Mommaerts, 1969). These terms and form are comparable with the model presented in Table 1. The constant should not be directly interpreted as an activation term because of the normalization procedure. Although work is represented as a linear term, a nonlinear representation cannot be ruled out. The regression analysis did not identify shortening distance as a significant variable. Shortening heat may be represented as a function of shortening distance (Homsher et al., 1973); however, a recent study has suggested that shortening heat for isotonic contractions may be considered to be a constant rather than being associated with the distance shortened (Holroyd et al., 1996). The inclusion of a shortening term along with the individual thermistor into the model as discrete variables did not, however, result in a significant constant shortening term.

The relative contributions of the individual model terms are of the same order of magnitude, and each term is therefore required for a reasonable representation of relative metabolic utilization. Typical contractions expected during power output for cardiac assistance applications have values of 0.2 s, 0.6 and 0.25 for the contraction duration, normalized force and normalized work terms, respectively (Reichenbach and Farrar, 1994). Inserting these values into the models results in relative contributions of 43, 33 and 24% for the duration, force and work terms (Table 2) and 58 and 42% for the contractile ($F_t$) and work terms (Table 1), respectively.

The present study has developed the required thermistor-based methodology and determined the appropriate predictive variables to model relative metabolic utilization. The focus was on *in situ* mammalian skeletal muscle contracting under repetitive isometric and isotonic conditions within the range utilized in skeletal-muscle-powered cardiac assistance applications. Multiple linear regression analysis identified contraction duration, active tension and work output as significant variables related to metabolic utilization during cyclic contractions. A comparison of the results with values in the literature supports the utility of the thermistor-based instrumentation approach and suggests that the model we describe is appropriate. The methodology and empirically based metabolic model are directly applicable to constrain future biomechanical power optimization investigations with cyclically contracting skeletal muscle.

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References


