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# POWER OUTPUT AND THE FREQUENCY OF OSCILLATORY WORK IN MAMMALIAN DIAPHRAGM MUSCLE: THE EFFECTS OF ANIMAL SIZE

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## Summary

Bundles of muscle fibres were isolated from the diaphragm of mouse, rat and rabbit. Mean oscillatory power output was determined during phasic stimulation and imposed sinusoidal length changes. Maximum power output was measured over a range of cycle frequencies. The cycle frequency for maximum power output ($f_{opt}$) decreased with increasing body mass and was described by the equation, $f_{opt}=4.42M^{-0.16}$, where $M$ is body mass. A very similar relationship has been reported between body mass and the frequency of the trot–gallop transition in terrestrial, quadrupedal mammals [Heglund *et al.* (1974), *Science* **186**, 1112–1113), and the significance of this similarity is discussed.

## Introduction

The size of any animal is a major determinant of its morphology, physiology and mechanics (Schmidt-Nielsen, 1984). Within the constraints of a given evolved 'design', different processes should work together to function optimally. Several studies have shown that the mechanical properties of muscles are determined by the size of the animal and the demands of locomotion. Muscle mechanical properties have been investigated in animals with a wide range of stride (Close, 1972), tailbeat (Wardle, 1975; Altringham and Johnston, 1990*b*) and wingbeat frequencies (Molloy *et al.* 1987). One or more of the following limitations apply to much of the work carried out to date. First, the mechanical properties of the muscles are rarely determined under conditions appropriate to locomotion. The experiments demonstrate the existence of scaling-related changes in mechanical properties, but not how well these changes match the changing demands. Second, data on locomotory parameters are not always available for comparison with those from the muscle. Finally, homologous muscles do not always have the same role in different species.

The function of the diaphragm remains essentially the same in all mammals, and we might expect its muscle fibres to have evolved to perform near optimally, within the constraints set by the varied conditions that prevail *in vivo*. In the

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present study we have measured the maximum mechanical power output of small bundles of fibres isolated from diaphragms of mice, rats and rabbits. Fibre bundles were phasically stimulated during imposed sinusoidal length changes, over a wide range of frequencies, which included those occurring *in vivo*. Our aim was to determine the relationship between animal size and the frequency for maximum oscillatory muscle power output, and to compare this to the relationships between size, breathing rate and stride frequency.

## Materials and methods

White rats and mice were killed by cervical dislocation, and rabbits by controlled $CO_2$ anaesthesia. All animals were quickly weighed, and their diaphragms removed and immersed in oxygenated Ringer at 30°C. Small strips of muscle (<1 mm wide) were dissected from the ventral part of the costal diaphragm, together with part of the central tendon and the rib. This part of the diaphragm in the rat has a fibre-type composition similar to that of the muscle as a whole (Metzger *et al.* 1985). Furthermore, no significant differences were found between any one area and the rest of the diaphragm. Small aluminium foil clips were attached to the tendon. Fibres were attached directly to the rib, which was pierced for attachment to the apparatus. All experiments were carried out at 35±0.5°C, in Ringer bubbled with 100 % $O_2$ (composition in mmol $l^{-1}$: NaCl, 144; sodium pyruvate, 10; KCl, 6; $MgCl_2$, 1; $CaCl_2$, 2; $NaH_2PO_4$, 1; $MgSO_4$, 1; Hepes, 10; pH 7.4 at 35°C, by the addition of Tris base, Daut and Elzinga, 1989).

The preparation was quickly transferred to a flow-through chamber containing the Ringer's solution, and one end was attached to a servo motor, the other to an isometric force transducer (AME 801, SensoNor, Horten, Norway), at *in situ* rest length (Altringham and Johnston, 1990*a*). Preliminary experiments established that this corresponded to the length for maximum force generation. The preparation was then left in the chamber for 30 min before experimentation. The fibres were stimulated directly, by means of two parallel platinum wire electrodes, with a 2 ms supramaximal stimulus. Twitch kinetics, fusion frequency and the frequency for maximum force were first determined under isometric conditions. When multiple stimuli were used in oscillatory experiments, the frequency which just gave maximum tetanic force was used.

Preparations were subjected to sinusoidal length changes, symmetrical about *in situ* rest length, and stimulated at a selected phase in each cycle. Initially, eight cycles were performed in each experimental run, with a 10 min rest between runs. This was subsequently reduced to four cycles and a 6 min rest, once it had been established that a steady state was achieved by the fourth cycle. A typical experiment is shown in Fig. 1A, in which a preparation is subjected to sinusoidal length changes at an amplitude of ±6.5 % of resting fibre length. By plotting force against muscle length for each cycle a series of loops is generated (Fig. 1B), the areas of which are the work performed during each cycle. Anti-clockwise components indicate positive work, clockwise components negative work (see

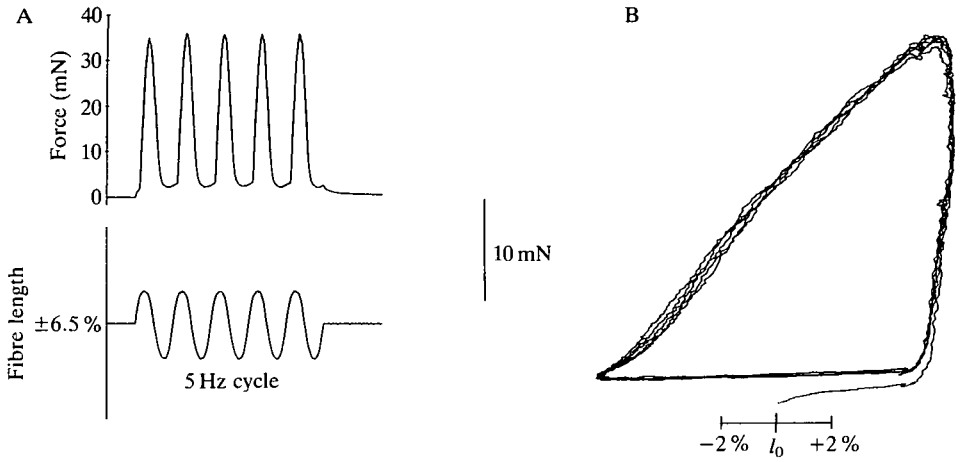


Fig. 1. The performance of oscillatory work by muscle fibre bundles from the rat diaphragm. The preparation was subjected to eight sinusoidal length change cycles of ±6.5 % of resting fibre length at 5 Hz; three supramaximal stimuli (at 100 Hz) were given 40° after the start of each cycle from rest length. (A) Force and muscle length plotted against time; (B) force plotted against muscle length to produce a work loop for each cycle. $l_0$, resting fibre length.

Josephson, 1985; Altringham and Johnston, 1990*a*). Power output is net work per cycle multiplied by frequency. The amplitude of the length change (strain), the cycle frequency, the number of stimuli and the phase shift between the start of stimulation and the start of each length change cycle all interact to determine power output. Optimum strain was first determined at a given frequency using other parameters known from preliminary experiments to be close to their optima. This strain was then used throughout the experiment, and the number and timing of stimuli were optimised at each frequency. An optimum strain of ±6.5 % was found at 2 and 5 Hz in the rat, and Altringham and Johnston (1990*b*) found no change in optimum strain over the frequency range for optimum power output (8–15 Hz) in cod fast fibres. However, the effects of strain amplitude may become significant at frequencies away from the optimum (Josephson, 1989). Power output was expressed as the mean of that for cycles 2–4. As a control, the parameters that gave maximum power output at 5 Hz were repeated every third or fourth run throughout the experiment to monitor any deterioration in the preparation. Where significant deterioration in power output was noted a small correction was made. Deterioration was typically a fall in force of less than 10 %, with no change in kinetics, over the course of the entire experiment. The aim of the present study was to manipulate these parameters to obtain maximum power output at different cycle frequencies, for muscle preparations from different sizes of animal. Further details can be found in Altringham and Johnston (1990*b*). The experiments were set up and controlled through a microcomputer (IBM compatible), and the data collected and analysed on-line, using in-house software.

## Results

### *Isometric experiments*

Maximum isometric tensions (mean±S.E.) were 154.6±19.5 kN m$^{-2}$ ($N$=6) and 158.9±20.8 kN m$^{-2}$ ($N$=6) in the mouse and rat, respectively. This is lower but not significantly different from that measured by Syme and Stevens (1989) for rat diaphragm (188±29.8 kN m$^{-2}$). Extensive connective tissue and the presence of firmly attached debris from dead fibres around the periphery prevented a reliable measurement of absolute tension being made for rabbit. The preparations were in all other respects as stable as those from mouse and rat, yielding reproducible results. In an isometric twitch, the time from the beginning of force generation to 90 % relaxation ($t_{90}$) increased with increasing animal size (significance, $P$<0.01). Values for $t_{90}$ were [mean±S.E.($N$)]: mouse, 55.4±5.1 ms (6); rat, 142.6±7.5 ms (8); rabbit 175±15.4 ms (4). Individual data points can be fitted to the allometric equation, $y=ax^b$, where $a$ represents the intercept, and $b$ the slope:

$$t_{90} = 0.186M^{0.31},$$

and where $M$ is mass ($r^2$=0.78).

Tetanic fusion frequencies were around 100 Hz, 60 Hz and 60 Hz in the mouse, rat and rabbit, respectively. Increasing the frequency to 150 Hz, 100 Hz and 80 Hz, respectively, increased tetanic force by around 10 %.

### *Oscillatory experiments*

Strain amplitude for maximum power output was ±6.5 % (i.e. 13 % peak to peak) in both rat and mouse muscle, in agreement with that found by Syme and Stevens (1989) for rat diaphragm and slightly higher than that reported for fish myotomal muscle (±5–6 %, Altringham and Johnston, 1990*a*,*b*). The amplitude against work curve was very flat around the optimum, and it was assumed that a similar strain would be very close to optimal for the rabbit. At a given frequency, maximum power output was achieved when the active muscle was given a small stretch prior to shortening (Fig. 2). Under these conditions, extra force is recruited by stretching the active muscle (Altringham and Johnston, 1990*b*). Furthermore, starting stimulation before, rather than at, the onset of shortening often allowed more stimuli to be given in each cycle. The optimum number of stimuli decreased with increasing frequency, for example in the rat, from 50 stimuli at 1 Hz to two stimuli at 18 Hz (Fig. 2).

In Fig. 3, maximum power output at each frequency, normalised to the overall maximum, is plotted against cycle frequency. Curves from mouse and rat preparations showed little scatter, and all data for each species were pooled. Substantial variation was observed in the rabbit data, and three representative curves are presented. The lines were drawn by fitting a third-order polynomial to the data, using a least-squares regression. The frequency for maximum power output decreases as animal size increases. This is shown more clearly in Fig. 4, where the logarithm of the optimum frequency from each preparation is plotted against the logarithm of mass. The optimum frequency ($f_{opt}$) is defined as the

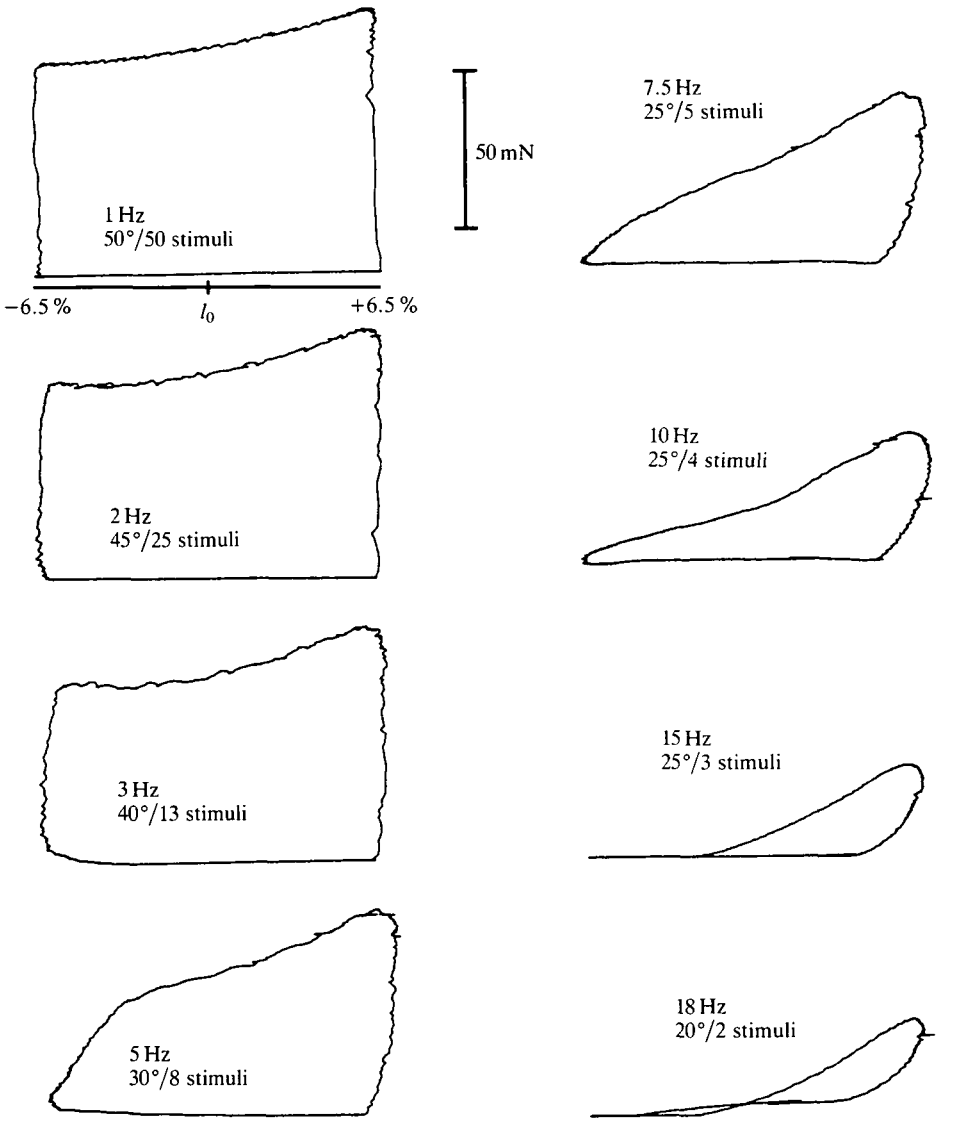


Fig. 2. Superimposed loops using parameters yielding maximum power output at different frequencies, from a rat diaphragm preparation. Parameters for each loop are indicated in the form $x°$ phase/$y$ no. of stimuli. $l_0$, resting fibre length.

frequency giving maximum power output on the fitted polynomial curve. The data can be described by the allometric equation:

$$f_{opt} = 4.42M^{-0.16},$$

(95 % confidence limits of the slope = −0.09, −0.23; $r^2$=0.63; $P$<0.01).

Maximum power output for mouse and rat preparations [mean±S.E. ($N$)] were 49.5±5.2 W kg$^{-1}$ (5) and 43.7±5.7 W kg$^{-1}$ (6), respectively.

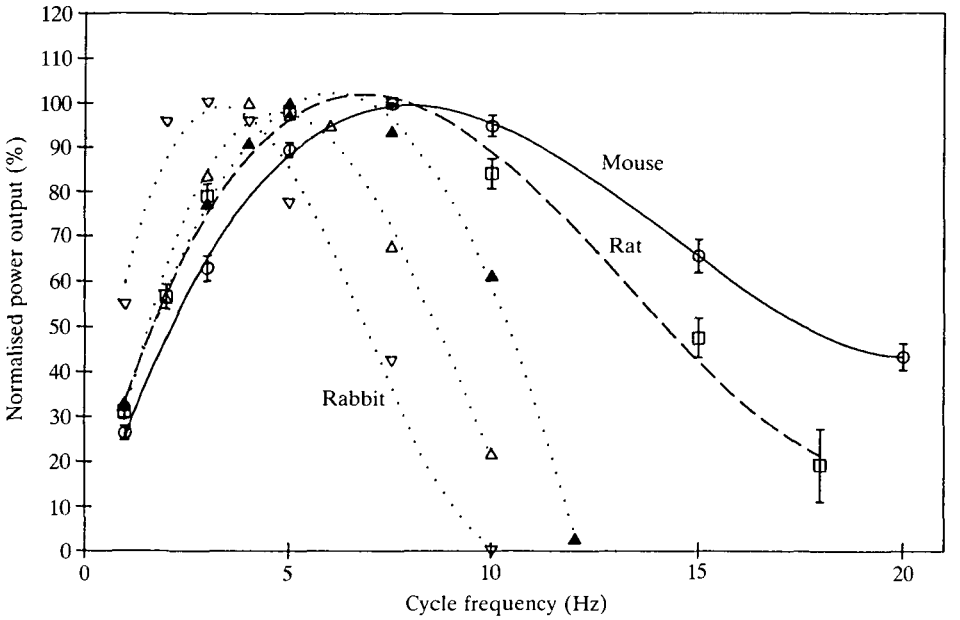


Fig. 3. Maximum power output at each frequency, normalised to the overall maximum, plotted against cycle frequency. The lines were drawn by fitting a third-order polynomial to the data, using a least-squares regression. Error bars show ±S.E. ($N \geqslant 6$). Curves for representative, individual preparations are shown for the rabbit.

## Discussion

Maximum power output values are higher than that observed by Syme and Stevens (1989) (4 Hz at 30°C) for rat diaphragm, but the higher frequencies and the temperature used in the present study probably account for the difference. When allowances are made for temperature differences, our results fall within the range of measurements made on a wide variety of muscles, using the work loop technique (see Fig. 8 in Stevenson and Josephson, 1990).

In all three species, the power output against frequency curves show a clear optimum. In the mouse and rat, the optima are broad, with near maximum power output being obtained over a wide range of frequencies (Fig. 3). In both these species, there was little variation in relative power output at a given frequency between preparations. In the rabbit, power output fell steeply above and below the optimum frequency for a given preparation. However, substantial variation in the optimum frequency was observed from preparation to preparation.

Histochemical studies show a tendency towards greater fibre type heterogeneity in the diaphragm as animal size increases (Davies and Gunn, 1972). In the mouse, the diaphragm consists almost exclusively of aerobic fibres, with more than 50 % of them fast twitch and less than 20 % of them slow twitch fibres. The rat and rabbit contain aerobic and anaerobic fast twitch fibres, in addition to aerobic slow twitch fibres. The low inter-preparation variation in the mouse could be explained by the

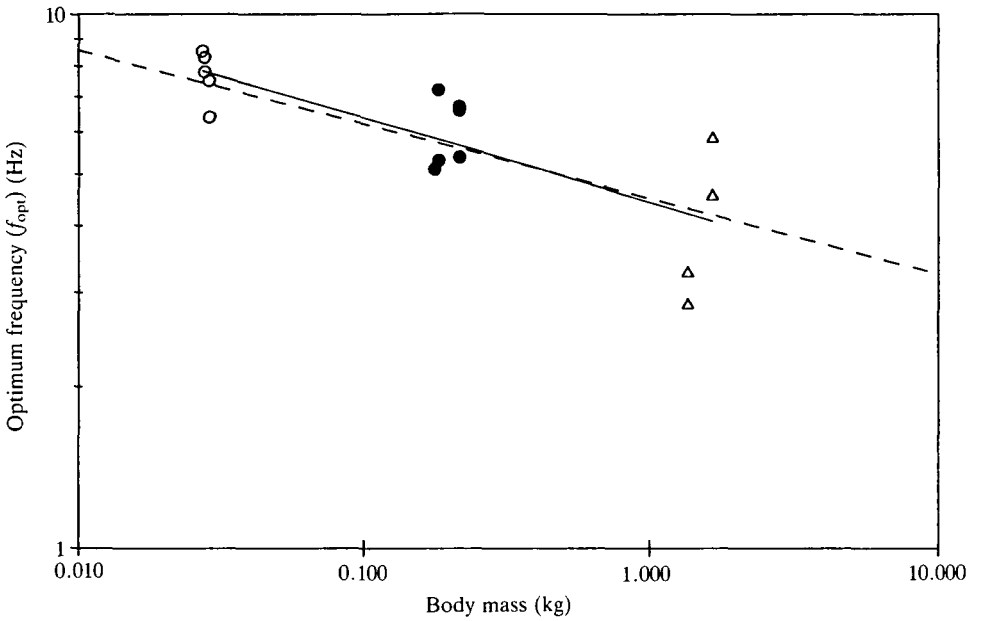


Fig. 4. Double logarithmic plot of body mass against frequency for maximum power output ($f_{opt}$) for all preparations. ○, mouse; ●, rat; △, rabbit. The solid line was fitted by least-squares regression (see text). The dashed line is the relationship between stride frequency at the trot–gallop transition and body size, determined by Heglund *et al.* (1974).

predominance of one fibre type. Mouse diaphragm muscle fibres are small and, since there are many muscle fibres within a preparation, this too may reduce variation due to sampling a heterogeneous tissue. At the other extreme, rabbit fibres are significantly larger than those of both mouse and rat (Davies and Gunn, 1972), and the preparations (similar in size to those used from the mouse) contain fewer fibres of a wider range of histochemical fibre types. This may explain at least part of the inter-preparation variation in mechanical properties (allowing for the fact that histochemical typing is only a guide to the mechanical properties).

If we assume that resting breathing rates are approximately 1–2 Hz in these three species, diaphragm muscle is operating at around 25–50 % of maximum power output under these circumstances. At rest it is likely that only the more slowly contracting fibre types are recruited.

The frequency for optimum power output shows a weak dependence on body mass; $f_{opt} \propto M^{-0.16}$. The isometric twitch time ($t_{90}$) increases with increasing body size. If we assume that the maximum rate at which the muscle can be driven is determined largely by twitch kinetics, then the maximum frequency of oscillatory work may be estimated from the reciprocal of $t_{90}$. On this basis, mouse diaphragm muscle should have a maximum of around 18 Hz, that of the rabbit around 6 Hz. In fact, both can be driven at considerably higher frequencies (Fig. 3) because of the

influence of dynamic muscle properties, e.g. the force–velocity relationship and shortening deactivation (Altringham and Johnston, 1990*b*).

The equation describing the relationship between the frequency for maximum power output and body mass ($f_{opt}=4.42M^{-0.16}$), is remarkably close to that relating stride frequency at the trot–gallop transition to body mass ($f=4.5M^{-0.14}$, where $f$ is stride frequency in Hz) (Heglund *et al.* 1974). The similarity of the slopes indicates that both breathing frequency and stride frequency scale with the same dependence on body mass. The similarity of the intercepts indicates a 1:1 linkage between breathing and locomotory frequencies.

In many terrestrial, quadrupedal mammals, breathing is synchronised 1:1 with body movement during locomotion. The frequency and phase of breathing are matched precisely with those of stride, over a wide range of speeds [Bramble and Carrier, 1983 (jackrabbit, dog, man, horse); Attenburrow, 1982 (horse)]. Baudinette *et al.* (1987) have also shown a 1:1 coupling in the hopping wallaby. Stride frequency and breathing frequency are essentially maximal at the trot–gallop transition: further increases in speed are achieved by increasing stride length (Heglund *et al.* 1974) and depth of breathing (Woakes *et al.* 1987). Thus, assuming evolution towards optimal design, diaphragm muscle should yield maximum power output at the frequency of the trot–gallop transition. A linkage between breathing and locomotory movements has not been investigated in the animals used in the present study. However, $f_{opt}$ in the present study shows the same dependence on body mass as the frequency of the trot–gallop transition. Do the properties of diaphragm muscle predispose terrestrial mammals towards a linkage between breathing and locomotory movements? If so, the stride frequency at the trot–gallop transition, $f_{opt}$ and the breathing rate at the transition, should all be described by the same allometric equation.

What do we know about the scaling of breathing rate? As animal size increases breathing rate decreases, but the exact relationship is equivocal. The relationship usually cited is that given by Stahl (1967), where breathing rate (in Hz)$=0.89M^{-0.26}$. However, the data used to derive this equation were collected from many sources, and the circumstances under which the measurements were made are not always clear, e.g. whether the animal was resting or active, stressed by surgery or under the influence of anaesthetics. We clearly need to look again into the relationship between breathing rate and animal size.

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