

**Coventry University Repository for the Virtual Environment
(CURVE)**

Author name : James, Rob S.

Title: Effect of stimulation frequency on force, net power output, and fatigue in mouse soleus muscle in vitro

Article & version: Author's own post peer reviewed copy

Original citation: James, R.S. , Cox, V.M. and Vassilakos, George (2009) Effect of stimulation frequency on force, net power output, and fatigue in mouse soleus muscle in vitro. *Canadian journal of physiology and pharmacology*, volume 87 (3): 203-210

DOI: 10.1139/Y09-002

This is an NRC Research Press publication

Publication website: <http://www.nrcresearchpress.com/doi/abs/10.1139/Y09-002>

Copyright © and Moral Rights are retained by the author(s) and/ or other copyright owners. A copy can be downloaded for personal non-commercial research or study, without prior permission or charge. This item cannot be reproduced or quoted extensively from without first obtaining permission in writing from the copyright holder(s). The content must not be changed in any way or sold commercially in any format or medium without the formal permission of the copyright holders. This copy may be different to the final published version of the chapter which is available from NRC Research via the publisher's website.

Available in the CURVE Research Collection: February 2012

1 **Article**

2

3 **Effect of stimulation frequency on force, net power output and fatigue in mouse soleus muscle**

4 **in vitro**

5

6 **George Vassilakos, Rob. S. James¹ and Val. M. Cox**

7

8 Department of Biomolecular and Sport Sciences, Coventry University, James Starley Building,

9 Coventry CV1 5FB

10 Telephone 024 7688 8326

11 Fax 024 7688 8778

12 ¹Corresponding author (e-mail: r.james@coventry.ac.uk).

13

14

1 **Abstract:** The effects of electrical stimulation frequency on force, work loop power output and
2 fatigue of mouse soleus muscle were investigated in vitro at 35 °C. Increasing stimulation frequency
3 did not significantly affect maximum isometric tetanic stress (overall mean \pm SD, 205 ± 16.6
4 kN m^{-2} between 70 and 160 Hz) but did significantly increase the rate of force generation. The
5 maximal net power output during work loops significantly increased with stimulation frequency,
6 $18.2 \pm 3.7 \text{ W kg}^{-1}$, $22.5 \pm 3.3 \text{ W kg}^{-1}$, 26.8 ± 3.7 and $28.6 \pm 3.4 \text{ W kg}^{-1}$ at 70, 100, 130 and 160 Hz
7 respectively. The stimulation frequency used affected the pattern of fatigue during work loop
8 studies. At stimulation frequencies of 100 Hz and 130 Hz there were periods of mean net negative
9 work during the fatigue tests due to a slowing of relaxation rate. In contrast mean net work
10 remained positive throughout the fatigue test when stimulation frequencies of 70 Hz and 160 Hz
11 were used. The highest cumulative work during the fatigue test was performed at 70 and 160 Hz,
12 followed by 130 Hz, then 100 Hz. Therefore, stimulation frequency affects power output and the
13 pattern of fatigue in mouse soleus muscle.

14

15 *Key words:* fatigue, force, mouse, power output, skeletal muscle, soleus, stimulation frequency,
16 work loop.

17

1 **Introduction**

2 During locomotion many muscles undergo cyclical length changes with phasic activation to
3 produce force and in some situations net power output (Dickinson et al. 2000; Kargo and Rome
4 2002). To maximize net power output during cyclical length changes the muscle must not only be
5 able to shorten rapidly while generating high force, but must also maintain force during shortening,
6 relax rapidly before subsequent lengthening and then activate rapidly near its maximal length ready
7 for high force generation during shortening (James et al. 1996; Josephson 1999; Caiozzo 2002). The
8 work loop technique has been used in vitro to subject muscle to cyclical length changes and phasic
9 stimulation to more closely simulate the type of activities that occur in vivo (Josephson 1985;
10 Josephson 1999; Caiozzo 2002). Previous studies have demonstrated the benefits of using the work
11 loop technique to investigate the cumulative mechanical effects of fatigue (decreased generation
12 and maintenance of force, increased relaxation time and decreased maximal velocity of shortening)
13 that lead to decreases in the net power output produced by mammalian muscle during cyclical
14 length changes (Askew et al. 1997; James et al. 2004).

15 Most in vitro studies of muscle performance have used maximal activation (including a relatively
16 high stimulation frequency). However, during many movements, muscles are used in vivo in a
17 submaximal manner, thus not all fibres are used for the specific task performed, and the recruitment
18 order of fibres is altered according to the activity undertaken (Wakeling 2005). The mean firing rate
19 during activity of soleus muscles in freely walking adult laboratory rats ranges from 12 to 45 Hz,
20 much lower than the approximately 100 Hz rate of stimulation required for maximal tetanic force
21 (Hennig and Lømo 1985; Gorassini et al. 2000). Therefore, it is unlikely that in vitro usage of high
22 stimulation frequencies will necessarily aid attempts to simulate in vivo action of muscles.

23 Previous research has demonstrated that under isometric conditions increased stimulation
24 frequency leads to increased tetanic force, until a plateau is reached, under both in vitro and in vivo
25 conditions (Buller and Lewis 1965; Rack and Westbury 1969; de Haan 1998; Mela et al. 2001).
26 Increased stimulation frequency under isometric conditions also increases the rate of force

1 generation, such that the maximal rate of force generation occurs at a higher stimulation frequency
2 than that eliciting maximal force (Buller and Lewis 1965; Rack and Westbury 1969; Bevan et al.
3 1992; de Haan 1998). Increased stimulation frequency also causes increased force and power to be
4 produced during shortening in isovelocity studies (de Haan 1998). Few studies have investigated the
5 effects of stimulation frequency on fatigue. The rate of fatigue elicited during isometric studies does
6 seem to be dependent on the stimulation frequency used (Bigland-Ritchie et al. 1979; Garland et al.
7 1988; Russ and Lovering 2006) but findings differ on the nature of this relationship.

8 The aim of the current study was to determine the effects of different stimulation frequencies on
9 force generation, work output and pattern of fatigue in mouse soleus muscles subjected to repeated
10 work loop cycles in vitro.

11

1 **Materials and Methods**

2 **Dissection**

3 Female white mice (strain CD1, Charles Rivers, Trant, UK) aged between 8 and 10 weeks old
4 (body mass 29.5 ± 1.1 g, $n = 34$ including the two muscles removed from analyses; see end of work
5 loop studies section) were used in the experimental procedure. Animals were cared for in
6 accordance with the Guide to the Care and Use of Experimental Animals (1993, available from the
7 Canadian Council on Animal Care) and the use of animals was reviewed and approved by the
8 Coventry University Ethics Committee. The animals were killed by cervical dislocation in
9 accordance with the British Home Office Animals (Scientific Procedures) Act 1986, schedule 1.

10 Soleus muscle was isolated from the right hind-limb and pinned out at approximately its resting
11 length at room temperature (19 to 21 °C), in oxygenated (95% O₂; 5% CO₂) Krebs-Henseleit
12 solution (composition, values in mM: NaCl 118; KCl 4.75; MgSO₄ 1.18; NaHCO₃ 24.8; KH₂PO₄
13 1.18; glucose 10; CaCl₂ 2.54; pH 7.55 at room temperature prior to oxygenation. For each soleus
14 muscle preparation, a small piece of bone was left attached at the end of both the proximal and
15 distal tendons. Aluminium foil clips were wrapped around the tendons at either end of the muscle.

16

17 **Isometric studies**

18 The soleus muscle preparation was attached via the aluminium foil clips to a force transducer
19 (UF1, Pioden Control Ltd, Barry Way, UK) at one end and a displacement transducer (V201, Ling
20 Dynamic System, Newport, UK) at the other end. For position detection a Linear Variable
21 Displacement Transducer (DFG 5.0, Solartron Metrology, Leicester, UK) was used. The
22 preparation was placed in a flow-through chamber containing circulating oxygenated Krebs-
23 Henseleit solution that was maintained at 35 °C via a water bath.

24 The muscle preparation was stimulated via parallel wire platinum electrodes (these were not in
25 contact with the muscle or nerve branch), while length was held constant, with 1.5 ms pulse width
26 stimuli to produce a series of isometric twitches. The stimulus amplitude (17 to 21 V) and muscle

1 length were optimized to yield the maximum isometric twitch force. The muscle length that
2 corresponded to maximal isometric twitch force was measured via a microscope fitted with an
3 eyepiece graticule and was defined as L_0 ($L_0 = 8.30 \pm 0.83$ mm; $n = 32$). Mean muscle fibre length
4 was calculated as 85% of L_0 (James et al. 1995). Isometric tetanic force responses were elicited by
5 subjecting the muscle to a 320 ms burst of electrical stimulation. In 8 muscles a number of
6 stimulation frequencies (70, 100, 130 and 160 Hz) were used to determine the maximal isometric
7 tetanic force. A recovery period of 5 minutes was allowed between each tetanus, to ensure full force
8 recovery of the soleus muscle preparation (James et al. 1995). In all other muscles the stimulation
9 frequency of 130 Hz was used to generate isometric tetanic force.

10

11 **Work loop studies**

12 The soleus muscle preparation was then subjected to sinusoidal length changes, using the work
13 loop technique (Josephson 1985) to determine net power output. The stimulation amplitude that
14 generated maximal twitch was used. Each soleus muscle preparation was subjected to four
15 sinusoidal length change cycles using a total strain of 0.10 symmetrically around L_0 (lengthened
16 from resting length to 5% longer than resting length, then shortened to 5% shorter than resting
17 length, following a length change pattern of a sine wave) at a cycle frequency of 5 Hz. A strain of
18 0.10 has previously been estimated to be within both the range of strains that produce maximal
19 power output in vitro at 5 Hz cycle frequency and within the range of strains used by soleus muscle
20 during locomotion (James et al. 1995).

21 All muscles were electrically stimulated using a burst duration of 65 ms and a phase of -10 ms
22 (stimulation started 10 ms prior to the muscle reaching maximal length and continued until 45 ms
23 prior to shortest length) that was shown previously by James et al. 1995 to yield maximal power
24 output at this cycle frequency. Muscle stimulation and length changes were controlled and
25 monitored using custom written software (Testpoint, CEC, Norton, USA) via a D/A board
26 (KPCI3108, Keithley Instruments, Cleveland, USA). A plot of force against length produced a

1 “work loop” that represented the net work done by the muscle during the length change cycle
2 (Josephson 1985). The net work produced in the second work loop cycle was not significantly
3 different ($p > 0.05$) from that found in cycle 3 or 4, therefore loop 2 was used as indicative of the
4 ability of the muscle to produce work. In 8 muscles the effect of stimulation frequency on work
5 loop power output was investigated by comparing stimulation frequencies of 70 Hz, 100 Hz,
6 130 Hz and 160 Hz, without changing any other strain or stimulation parameters. A set of control
7 work loops were performed (using 130Hz stimulation frequency, 65 ms burst duration, stimulation
8 phase of -10 ms, strain of 0.10, cycle frequency of 5 Hz) every fourth set of work loops to monitor
9 any changes in the ability of the muscle to generate power over the time course of the experiment.
10 The ability of each muscle preparation to generate power declined slightly over the time course of
11 each experiment remaining higher than 90% of the maximal ability. The decrease in power output
12 was due to a decrease in force production, therefore, all work loop power outputs were corrected
13 assuming a linear decline between each control set of work loops (James et al. 1995).

14 Fatigue tests were performed at each of the stimulation frequencies under investigation (70 Hz,
15 100 Hz, 130 Hz and 160 Hz). Only one fatigue test was carried out per preparation (8 muscles were
16 used for each stimulation frequency of fatigue test), at the end of each experiment, to ensure that
17 metabolite levels were the same at the start of each fatigue test. A fatigue test consisted of
18 subjecting the muscle to repeated cycles of work loops (with each loop subjected to a 5 Hz cycle
19 frequency, strain of 0.10, 65 ms stimulus burst duration and stimulation phase of -10 ms) until 300
20 loops had been generated. Following this treatment the muscle preparation was allowed to recover
21 for 30 minutes before the power output was measured over a series of four control loops. In any
22 case where muscle power output had not recovered to a satisfactory level (to $>85\%$ of the initial
23 power output within this 30 minute recovery period) the preparation was discarded. On this basis,
24 all results from two of the soleus muscle preparations (from the initial 34) were discarded. Changes
25 in muscle power output were monitored, during each fatigue test, as a function of time and as a
26 function of cumulative work.

27

1 **Muscle mass measurement and dimension calculations**

2 At the end of each experiment, the tendons were removed from the muscle preparation, and
3 excess Krebs-Henseleit solution was removed by blotting the muscle with tissue paper. Wet muscle
4 mass was determined using an electronic balance (mass = 9.20 ± 1.14 mg; $n = 32$). Muscle power
5 output and cumulative work were normalized to muscle mass. Muscle stress was calculated as force
6 divided by mean muscle cross-sectional area (CSA). CSA was calculated from fibre length, muscle
7 mass and an assumed muscle density of 1060 kg m^{-3} (Mendez and Keys 1960).

8

9 **Statistical analysis**

10 Values for mouse body mass, muscle mass, muscle fibre length, time to half peak tetanus,
11 maximum isometric stress, maximum net power output (prior to fatigue test) and time required for
12 net work loop power output to decline to 50% of initial power output during the fatigue test were
13 analysed via single factor analysis of variance using SPSS statistical software (Version 13, SPSS
14 Inc., Chicago, USA) using stimulation frequency as the independent variable. Tukey post hoc tests
15 were used when $p < 0.05$ was obtained for the ANOVA. Data are expressed as mean \pm SD.

16

1 **Results**

2 **Morphological measurements**

3 There were no significant differences between work loop fatigue treatment groups in measures
4 of body mass, muscle mass or muscle fibre length (ANOVA $p > 0.24$ and $n = 8$ in each case).
5 Therefore, morphology was not considered in further statistical analyses.

7 **Isometric properties**

8 Increased stimulation frequency caused time to half-peak tetanus (THPT) to significantly
9 decrease (Fig. 1; ANOVA $p < 0.001$), approximately 23%, between 70 and 130 Hz stimulation
10 frequencies (Tukey $p < 0.001$; Note that Tukey $p = 0.04$ between 70 and 100 Hz, $p = 0.18$ between
11 100 and 130 Hz), with little change from 130 to 160 Hz (Tukey $p = 0.84$). The mean maximum
12 isometric twitch stress for soleus muscle was $31.7 \pm 3.2 \text{ kN m}^{-2}$ ($n = 32$). The mean maximum
13 isometric tetanic stress for soleus muscle was $205 \pm 16 \text{ kN m}^{-2}$ ($n = 32$ at 130 Hz stimulation
14 frequency). There was no significant effect of stimulation frequency on maximal isometric stress
15 (Fig. 2; ANOVA $p = 0.73$).

17 **Effect of stimulation frequency on power output during work loop studies prior to fatigue**

18 In work loop experiments stimulation frequency had a significant effect on net work loop power
19 output prior to fatigue (ANOVA $p < 0.001$; Tukey $p < 0.001$ between 70 and 130 Hz and between
20 70 and 160 Hz, $p = 0.01$ between 100 and 160 Hz), increasing power output with increasing
21 stimulation frequency (Fig. 3). Power output increased by 57% when stimulation frequency was
22 increased from 70 to 160 Hz.

24 **Effect of stimulation frequency on fatigue during work loop studies**

25 Peak power output was achieved within the first 5 work loop cycles of the fatigue test in all
26 preparations at all stimulation frequencies (70 Hz, 100 Hz, 130 Hz and 160 Hz) under investigation

1 (Fig. 4). The time required for net work loop power output to decline to 50% of initial power output
2 was significantly dependent on the stimulation frequency used, with times of 14.4 ± 3.8 s at 70 Hz,
3 10.6 ± 2.3 s at 100 Hz, 8.9 ± 1.2 s at 130 Hz and 9.8 ± 1.9 s at 160 Hz (Fig. 4; ANOVA $p = 0.001$;
4 Tukey $p < 0.03$ between 70 Hz and all other stimulation frequencies).

5 At 70 Hz and 160 Hz stimulation frequency, the mean cumulative work continued to increase
6 throughout the fatigue test (Fig. 5). However, at stimulation frequencies of 100 Hz and 130 Hz
7 cumulative work increased to a maximum value, then decreased as net power output became
8 negative (Figs. 4, 5).

9

10 **Effect of fatigue on work loop shape**

11 The typical changes in work loop shape induced by fatigue at different stimulation frequencies
12 (70, 100, 130 and 160 Hz) during the fatigue test (work loops after 0.4 s, 5 s and 20 s) are shown in
13 figure 6. As the muscle fatigued, the net peak force that could be generated in the work loop was of
14 lower magnitude than in the previous work loop shown (e.g. higher force in the work loop after 0.2
15 s than after 5 s of the fatigue test). With further progression of fatigue (e.g. the work loop after 5 s
16 compared with after 20 s of the fatigue test), the force produced during shortening tended to decline
17 at a higher rate. In some instances (e.g. the work loop after 20 s during 100 and 130 Hz stimulation
18 frequency fatigue tests), the soleus muscle failed to relax completely during shortening, resulting in
19 an increase in force produced during lengthening and a subsequent increase in the work required to
20 re-lengthen the muscle. The work loop produced after 20 s in 100 and 130 Hz stimulation
21 frequencies typically resulted in a negative component in the work loop and in some cases in net
22 negative work (Figs. 4, 5, 6).

23

1 **Discussion**

2 **Maximal mechanical properties of in vitro soleus muscle**

3 Soleus muscle produced a maximal isometric tetanic stress of $205 \pm 17 \text{ kN m}^{-2}$ that is similar to
4 the values of 212 kN m^{-2} and 224 kN m^{-2} previously found for mice at the same experimental
5 temperature of $35 \text{ }^\circ\text{C}$ (James et al. 1995; Askew et al. 1997). The maximal power output determined
6 using the work loop technique in this study was $28.6 \pm 3.4 \text{ W kg}^{-1}$ at a cycle frequency of 5 Hz,
7 which is approximately 15% lower than in previous studies (James et al. 1995, 34 W kg^{-1} ; Askew et
8 al. 1997, 33.2 W kg^{-1}). This difference could be due to differences in strain or age of mice used
9 resulting in muscle fibre type differences, and/or could be due to the shorter soleus muscle length
10 and the lower stress in the present study (10% lower than the stress in Askew et al. 1997).

11

12 **Effect of stimulation frequency on isometric stress**

13 The maximum isometric stress produced by soleus muscle was independent of the stimulation
14 frequencies used in this experiment (Fig. 2) indicating, that in mouse soleus muscle at $35 \text{ }^\circ\text{C}$, the
15 physiological maximal active state can be reached, at any stimulation frequency between 70 and
16 160 Hz. However, as in previous studies, as stimulation frequency was increased the rate of force
17 generation also increased (Buller and Lewis 1965; Rack and Westbury 1969; Bevan et al. 1992; de
18 Haan 1998).

19

20 **Effect of stimulation frequency on work loop power output**

21 The soleus muscle maximum power output, prior to fatigue, significantly increased with
22 increasing stimulation frequency (Fig. 3). Cycle frequency (5 Hz) and maximal strain (0.10) were
23 the same throughout the experiment, so any changes in power output (power output = [mean force \times
24 length change] \div time taken) were related to changes in force. A number of previous studies have
25 modelled the changes in force output observed during work loop experiments (James et al. 1996;
26 Askew and Marsh 1998; Curtin et al. 1998; Woledge 1998; Caiozzo and Baldwin 1997; Lichtwark

1 and Wilson 2005). The key findings of these studies are that the force produced at any instant
2 during a work loop can be predicted from a combination of the force-velocity relationship, length-
3 force relationship, activation state of the muscle and series elastic element stiffness. Figure 1
4 demonstrates that the rate of muscle force generation during a tetanus increases with increasing
5 stimulation frequency, probably as a result of increased rate of calcium release. Therefore, our
6 experimental manipulation of stimulation frequency is likely to have affected power output by
7 altering the rate of activation of the muscle. The aforementioned modelling papers indicate that any
8 increase in the rate of force generation during an isometric tetanus should translate into an increase
9 in the rate of force increase during work loop studies.

10

11 **Effect of stimulation frequency on muscle fatigue**

12 All the changes induced by fatigue at the stimulation frequencies used were reversible, a key
13 factor in ensuring that changes were due to peripheral fatigue and not due to muscle damage (James
14 et al. 2004; Askew et al. 1997). The soleus muscle recovered rapidly to attain almost pre-fatigue
15 performance within 30 minutes of the end of the fatigue test.

16 The total cumulative work achieved was initially greater at higher stimulation frequency due to
17 higher stimulation frequencies resulting in higher net power output per loop (Figs. 4, 5). However,
18 as the fatigue test progressed the work produced decreased more rapidly at 100 Hz and 130 Hz
19 stimulation frequencies, with corresponding changes in work loop shape. During the fatigue test
20 peak work loop force decreased at all stimulation frequencies (Fig. 6). However, at both 100 Hz and
21 130 Hz stimulation frequencies the work loop obtained after 20 s of the fatigue test had a large
22 “negative work” component which can be attributed to the prolongation of relaxation that increases
23 the work required to extend the muscle to its initial length (lengthening work). Increases in
24 lengthening work could be associated with a range of intracellular factors that affect the rate of
25 relaxation, via their effect on $[Ca^{2+}]_i$ (Westerblad and Allen 1994). Prolongation of relaxation has
26 been observed in other studies where a large number of cycles of work loops have been performed

1 (Stevens and Syme 1993; Askew et al. 1997; James et al. 2004). In the present study 160 Hz
2 stimulation frequency resulted in higher peak work loop force and higher force to be maintained
3 during shortening when compared with other stimulation frequencies (Fig. 6). The reasons
4 underlying improved fatigue resistance at 160 Hz stimulation frequency remain unclear.

5

6 **Relevance of the present study to in vivo conditions**

7 During locomotion in the mouse, the soleus muscle is believed to be active during both
8 shortening and near isometric phases of the movement (James et al. 1995). Therefore, the
9 experimental conditions used in this study are a simplification of in vivo conditions (James et al.
10 1995). Variation in muscle length change waveform complexity can have large effects on muscle
11 force and power-output (Askew and Marsh 1998). Therefore, further studies need to be performed
12 to determine in vivo strain waveforms for mouse muscle and to subject in vitro muscle to these
13 strain waveforms to simulate in vivo function.

14 During locomotion the activation patterns imposed on mouse soleus will likely be far more
15 complex than those used in the present study, but further studies need to be performed using
16 electromyography to determine the in vivo muscle activation patterns for running mice. However,
17 we can use the in vitro data in the present study to make some predictions about how muscle
18 activation might alter either to maximise performance during sprinting or to enhance endurance
19 during prolonged in vivo activities. During activities such as sprinting, where maximal acceleration
20 is important, it is likely that higher motor unit firing rates would be used in vivo to maximise the
21 rate of force generation. In contrast, during endurance type activities lower motor unit firing rates
22 are likely to be used to increase fatigue resistance. Previous work on walking rats has demonstrated
23 that mean firing rates in soleus muscle are far lower than those required to produce maximal tetanic
24 force (Hennig and Lømo 1985; Gorassini et al. 2000). However, little is known about firing rates in
25 running rodents, so it is difficult to determine how relevant the higher stimulation frequencies used
26 in the present study are in relation to locomotory firing rates. In the present study stimulation of

1 soleus muscle at submaximal levels (e.g. 70 Hz) delayed the onset of fatigue. Lower stimulation
2 frequencies than those generating maximal force are likely to be regularly used in vivo to limit the
3 decrease in power output due to slowing of relaxation rate during the fatigue test in the present
4 study. Under in vivo conditions the burst duration, frequency and intensity of 'stimulation' could be
5 modulated (Wakeling 2005), possibly with changes made in response to the alteration in force
6 output of the muscle during fatigue. In vitro studies have also demonstrated that isometric peak
7 force generation is higher and fatigue resistance is greater when the inter-pulse interval of
8 stimulation is relatively short at the start of stimulation compared to when a constant stimulation
9 frequency is used (Bevan et al. 1992; van Lunteren and Moyer 2004). In vitro experiments on locust
10 flight muscle have demonstrated that a reduction in the stimulus burst duration during work loops
11 decreases the initial power output, but increases efficiency and allows power output to be
12 maintained for a longer period of time (Josephson and Stevenson 1991). Such a reduction in
13 stimulus burst duration would probably have reduced the negative components of the work loop
14 cycles observed later in the fatigue test in the present study. Rotation of stimulation pulses round
15 the motor units in a muscle (asynchronous stimulation) can also result in a higher force than when
16 synchronous stimulation of the whole muscle at the same stimulation frequency is used, especially
17 when length is lower than L_0 and lower stimulation frequencies are used (Rack and Westbury 1969;
18 Sandercock 2006). Supramaximal firing of soleus muscle in vivo, is unlikely to be a common
19 phenomenon, since for maximal economy, the muscle should be stimulated at a frequency not
20 greater than that required for full activation.

21 In summary, there was no effect of electrical stimulation frequency on the maximum isometric
22 tetanic stress produced by soleus muscle in the present study. The net power output, generated
23 during work loop studies, significantly increased as stimulation frequency was increased from
24 70 Hz to 160 Hz. The stimulation frequency used affected the pattern of fatigue during work loop
25 studies. At stimulation frequencies of 100 Hz and 130 Hz there were periods of mean net negative
26 work during the fatigue test, probably due to a slowing of relaxation rate. In contrast mean net work

1 remained positive throughout the fatigue test when stimulation frequencies of 70 Hz and 160 Hz
2 were used. Therefore, electrical stimulation frequency has a direct effect on both power output and
3 the overall pattern of fatigue in mouse soleus muscle.

4

5 **Acknowledgements**

6 The authors thank Mark Bodycote for technical assistance and an anonymous referee for
7 thorough consideration of the submitted manuscript.

8

1 **References**

- 2 Askew, G.N., and Marsh, R.L. 1998. Optimal shortening velocity (V/V_{\max}) of skeletal muscle
3 cyclical contractions: Length-force effects and velocity-dependent activation and deactivation.
4 J. Exp. Biol. **201**: 1527-1546.
- 5 Askew, G.N., Young, I.S., and Altringham, J.D. 1997. Fatigue of mouse soleus muscle, using the
6 work loop technique. J. Exp. Biol. **200**: 2907-2912.
- 7 Bevan, L., Laouris, Y., Reinking, R.M., and Stuart, D.G. 1992. The effect of the stimulation pattern
8 on the fatigue of single motor units in adult cats. J. Physiol. **449**: 85-108.
- 9 Bigland-Ritchie, B., Jones, D.A., and Woods, J.J. 1979. Excitation frequency and muscle fatigue:
10 Electrical responses during human voluntary and stimulated contractions. Exp. Neurol. **64**:
11 414-427.
- 12 Buller, A.J., and Lewis, D.M. 1965. The rate of tension development in isometric tetanic
13 contractions of mammalian fast and slow skeletal muscle. J. Physiol. **176**: 337-354.
- 14 Caiozzo, V.J. 2002. Plasticity of skeletal muscle phenotype: Mechanical consequences. Muscle
15 Nerve **26**: 740-768.
- 16 Caiozzo, V.J., and Baldwin, K.M. 1997. Determinants of work produced by skeletal muscle:
17 potential limitations of activation and relaxation. Am. J. Physiol. **273**: C1049-C1056.
- 18 Curtin, N.A., Gardner-Medwin, A.R., and Woledge, R.C. 1998. Predictions of the time-course of
19 force and power output by dogfish white muscle fibres during brief tetani. J. Exp. Biol. **201**:
20 103-114.
- 21 Dickinson, M., Farley, C., Full, R., Koehl, M., Kram, R., and Lehman, S. 2000. How animals move:
22 an integrative review. Science **288**: 100-106.
- 23 de Haan, A. 1998. The influence of stimulation frequency on force-velocity characteristics of in situ
24 rat medial gastrocnemius muscle. Exp. Physiol. **83**: 77-84.
- 25 Garland, S.J., Garner, S.H., and McComas, J. 1988. Relationship between numbers and frequencies
26 of stimuli in human muscle fatigue. J. Appl. Physiol. **65**: 89-93.

1 Gorassini, M., Eken, T., Bennett, D.J., Kiehn, O., and Hultborn, H. 2000. Activity of hindlimb
2 motor units during locomotion in the conscious rat. *J. Neurophysiol.* **83**: 2002-2011.

3 Hennig, R., and Lømo, T. 1985. Firing patterns of motor units in normal rats. *Nature* **314**: 164-166.

4 James, R.S., Altringham, J.D., and Goldspink, D.F. 1995. The mechanical properties of fast and
5 slow skeletal muscles of the mouse in relation to their locomotory function. *J. Exp. Biol.* **198**:
6 491-502.

7 James, R.S., Young, I.S., Cox, V.M., Goldspink, D.F. and Altringham, J.D. 1996. Isometric and
8 isotonic muscle properties as determinants of work loop muscle power output. *Eur. J.*
9 *Physiol.* **432**: 767-774.

10 James, R.S., Wilson, R.S., and Askew, G.N. 2004. Effects of caffeine on mouse skeletal muscle
11 power output during recovery from fatigue. *J. Appl. Physiol.* **96**: 545-552

12 Josephson, R.K. 1985. Mechanical power output from striated muscle during cyclic contraction. *J.*
13 *Exp. Biol.* **114**: 493-512.

14 Josephson, R.K. 1999. Dissecting muscle power output. *J. Exp. Biol.* **20**: 3369-3375.

15 Josephson, R.K., and Stevenson, R.D. 1991. The efficiency of a flight muscle from the locust
16 *Schistocerca Americana*. *J. Physiol.* **442**: 413-429.

17 Kargo, W.J., and Rome, L.C. 2002. Functional morphology of proximal hindlimb muscles in the
18 frog *Rana pipiens*. *J. Exp. Biol.* **205**: 1987-2004.

19 Lichtwark, G.A., and Wilson, A.M. 2005. A modified Hill muscle model that predicts muscle
20 power output and efficiency during sinusoidal length changes. *J. Exp. Biol.* **208**: 2831-2843.

21 van Lunteren, E., and Moyer, M. 2004. Combination of variable frequency train stimulation and K⁺
22 channel blockade to augment skeletal muscle force. *IEEE Trans. Neur. Syst. Rehab. Eng.* **12**:
23 288-294.

24 Mela, P., Veltink, P.H., and Huijing, P.A. 2001. The influence of stimulation frequency and ankle
25 joint angle on the moment exerted by human dorsiflexor muscles. *J. Electromyogr. Kinesiol.*
26 **11**: 53-63.

- 1 Mendez, J., and Keys, A. 1960. Density and composition of mammalian muscle. *Metab.* **9**: 184-188.
- 2 Rack, P.M.H., and Westbury, D.R. 1969. The effects of length and stimulus rate on tension in the
3 isometric cat soleus muscle. *J. Physiol.* **204**: 443-460.
- 4 Russ, D.W., and Lovering, R.M. 2006. Influence of activation frequency on cellular signalling
5 pathways during fatiguing contractions in rat skeletal muscle. *Exp. Physiol.* **91**: 957-966.
- 6 Sandercock, T.G. 2006. Extra force from asynchronous stimulation of cat soleus muscle results
7 from minimizing the stretch of the common elastic elements. *J. Neurophysiol.* **96**: 1401-1405.
- 8 Stevens, E.D., and Syme, D.A. 1993. Effect of stimulus duty cycle and cycle frequency on power
9 output during fatigue in rat diaphragm muscle doing oscillatory work. *Can. J. Physiol.*
10 *Pharmacol.* **71**: 910-916.
- 11 Wakeling, J.M. 2005. Motor unit recruitment during vertebrate locomotion. *Anim. Biol.* **55**: 41-58.
- 12 Westerblad, H., and Allen, D.G. 1994. The role of sarcoplasmic reticulum in relaxation of mouse
13 muscle; effects of 2,5-di(*tert*-butyl)-1,4-benzohydroquinone
14 *J. Physiol.* **474**: 291-301.
- 15 Woledge, R.C. 1998. Muscle energetics during unfused tetanic contractions. Modelling the effects
16 of series elasticity. *Adv. Exp. Med. Biol.* **453**: 537-543.
- 17

1 **Figure captions**

2

3 **Fig. 1.** Effect of stimulation frequency (70, 100, 130 and 160 Hz) on time to half peak tetanus
4 (THPT) of mouse soleus muscle prior to fatigue at 35 °C. Data represent mean \pm SD ($n = 8$).

5

6 **Fig. 2.** Effect of stimulation frequency (70, 100, 130 and 160 Hz) on maximum isometric tetanic
7 stress of mouse soleus muscle prior to fatigue at 35 °C. Data represent mean \pm SD of muscle force
8 values normalised to muscle cross-sectional area ($n = 8$)

9

10 **Fig. 3.** Effect of stimulation frequency (70, 100, 130 and 160 Hz) on net work loop power output
11 for mouse soleus muscle prior to fatigue at 35 °C. Data represent mean \pm SD of power values
12 normalised to muscle mass ($n = 8$)

13

14 **Fig. 4.** Effect of stimulation frequency on fatigue of mouse soleus muscle power output during
15 work loop studies at 35 °C. Data represent mean \pm SD of power values normalised to muscle mass
16 ($n = 8$) over 20 s of the fatigue tests. For clarity not all standard deviation bars are shown.

17

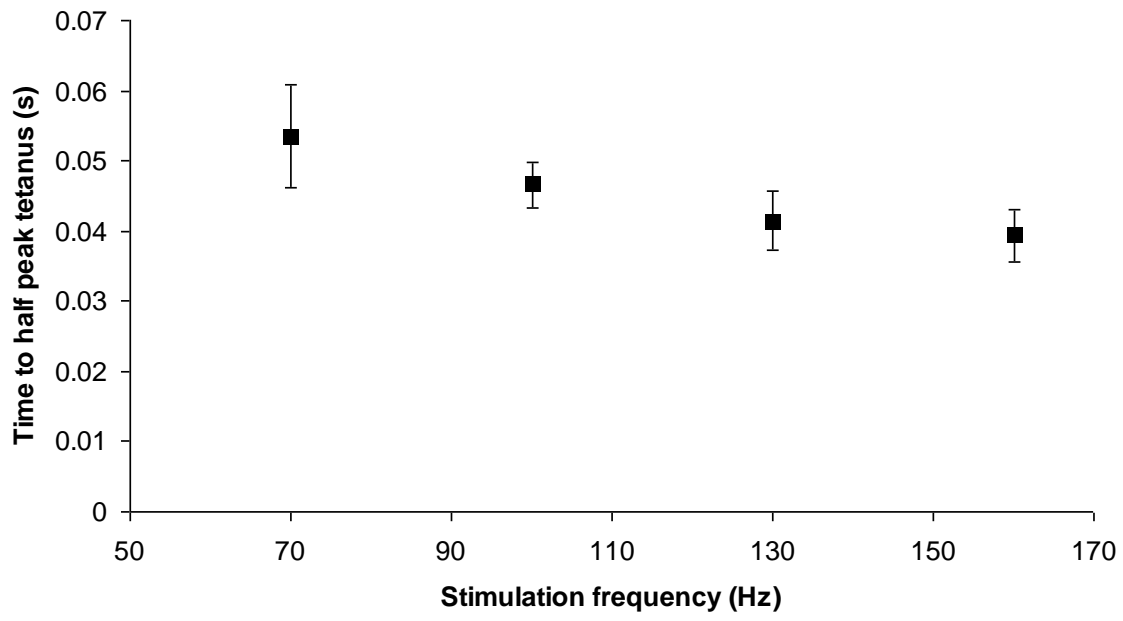
18 **Fig. 5.** Effect of stimulation frequency on the relationship between mouse soleus net muscle power
19 output and cumulative work produced during work loop studies at 35 °C. Values represent mean \pm
20 SD of power values normalised to muscle mass ($n = 8$) over 20 s of the fatigue tests. For clarity not
21 all standard deviation bars are shown.

22

23 **Fig. 6.** Typical changes in work loop shape at different stimulation frequencies (70 Hz, 100 Hz, 130
24 Hz and 160 Hz) over 20 s of the fatigue tests of mouse soleus muscle at 35 °C. For each work loop
25 stress is plotted against strain.

26

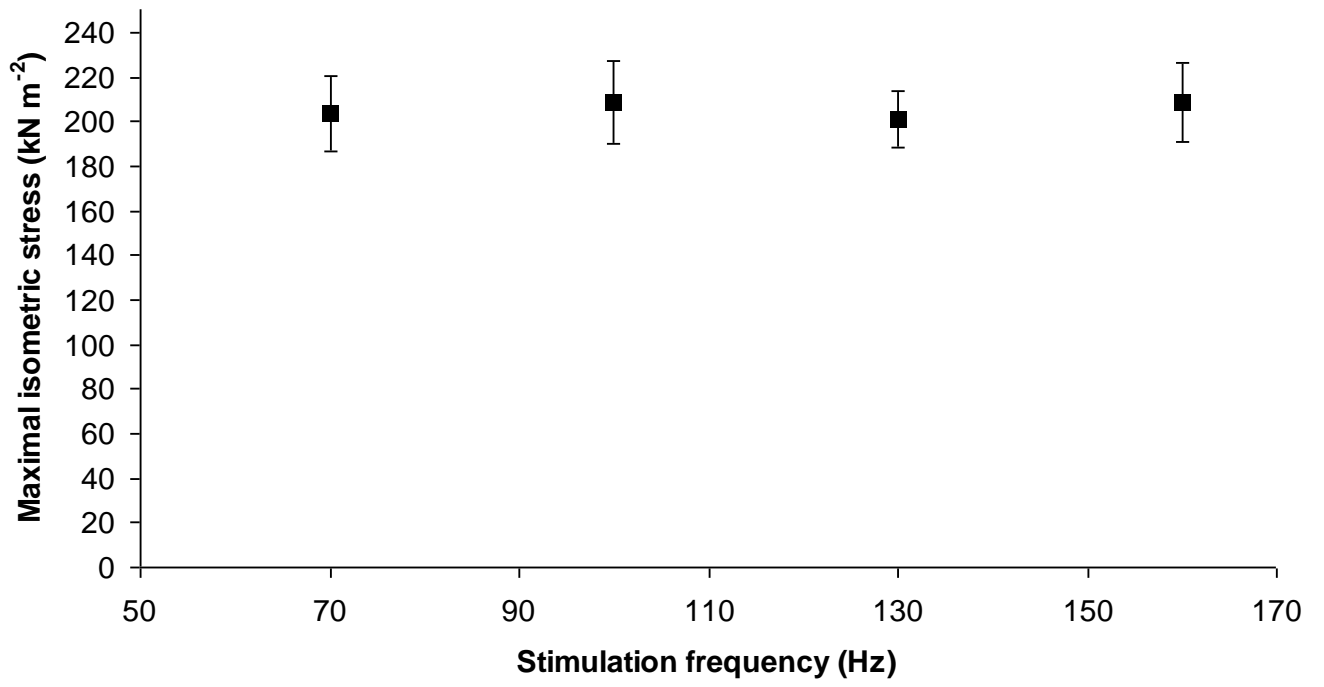
1 **Figure 1.**



2

3

1 **Figure 2.**

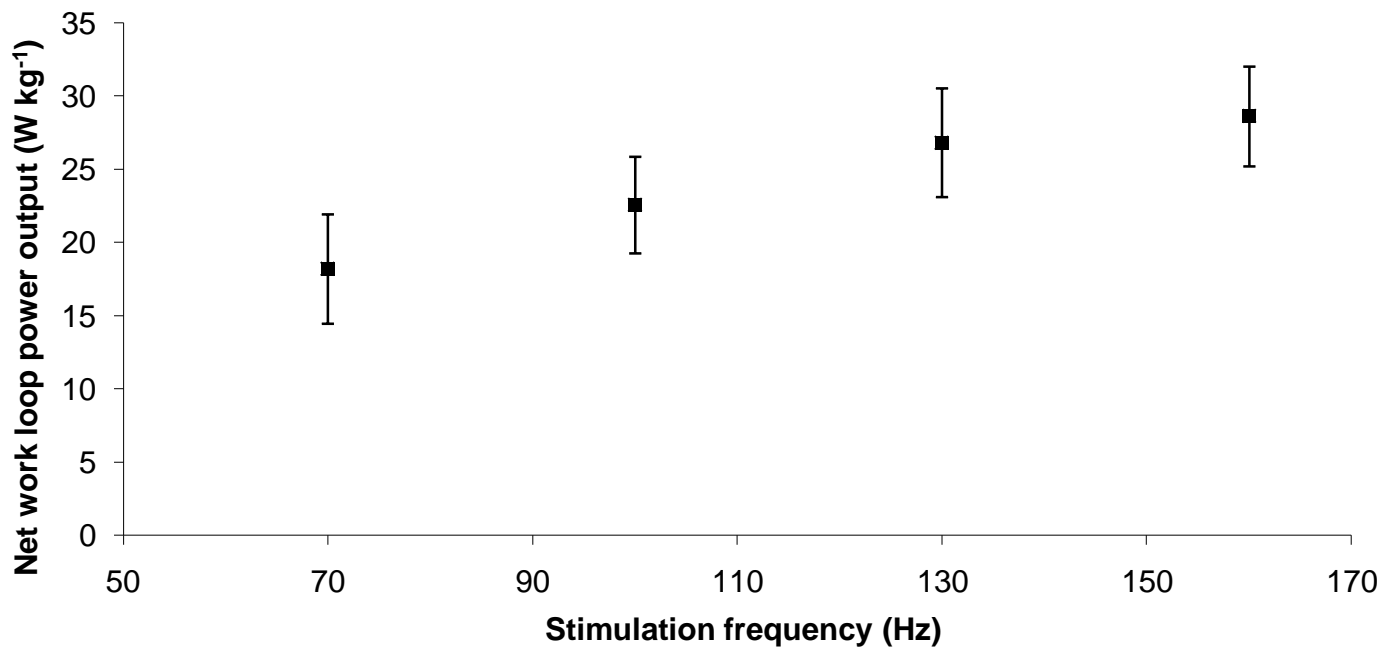


2

3

4

1 **Figure 3.**

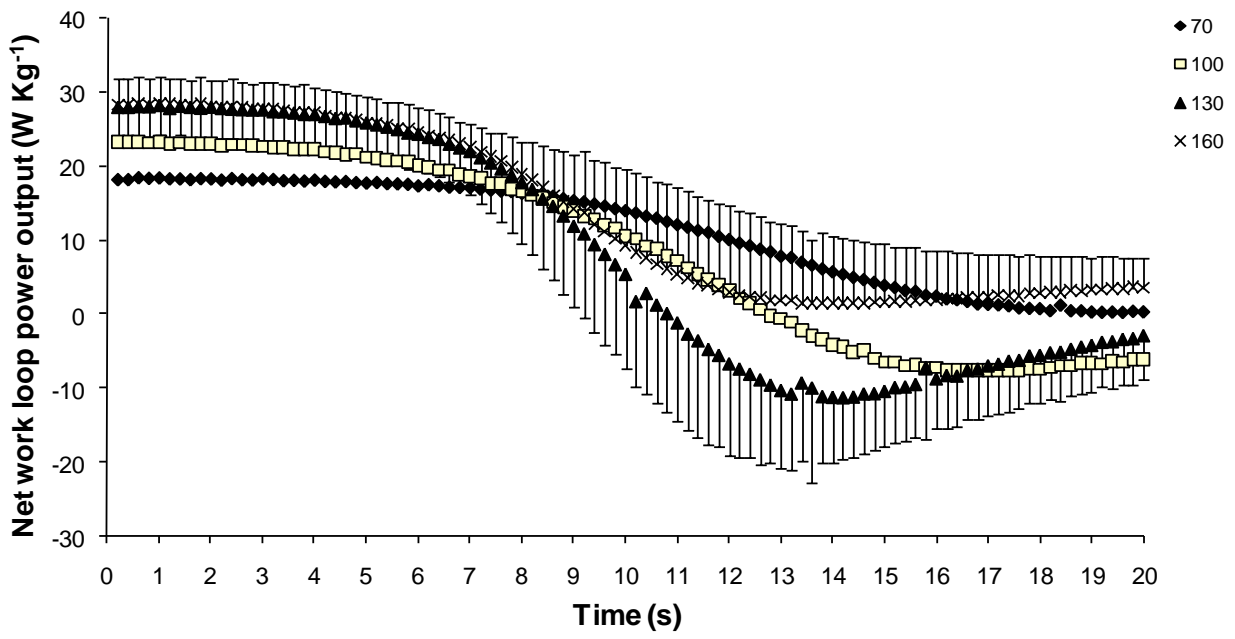


2
3

4

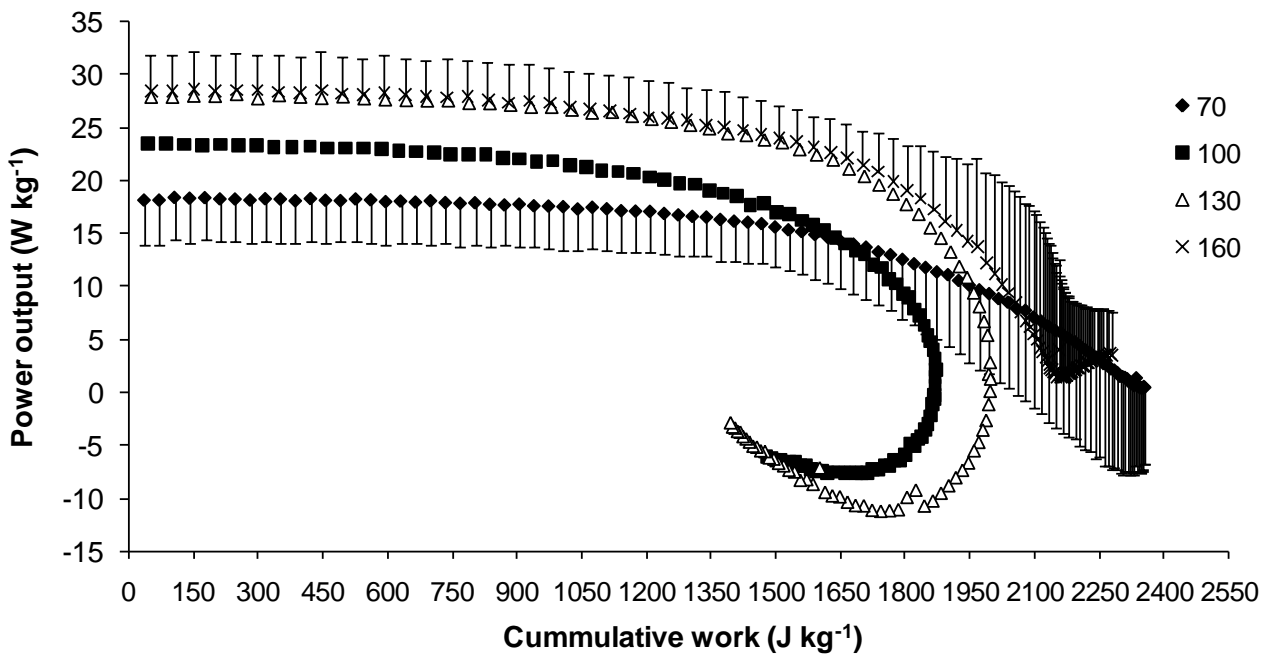
5

1 **Figure 4.**



2

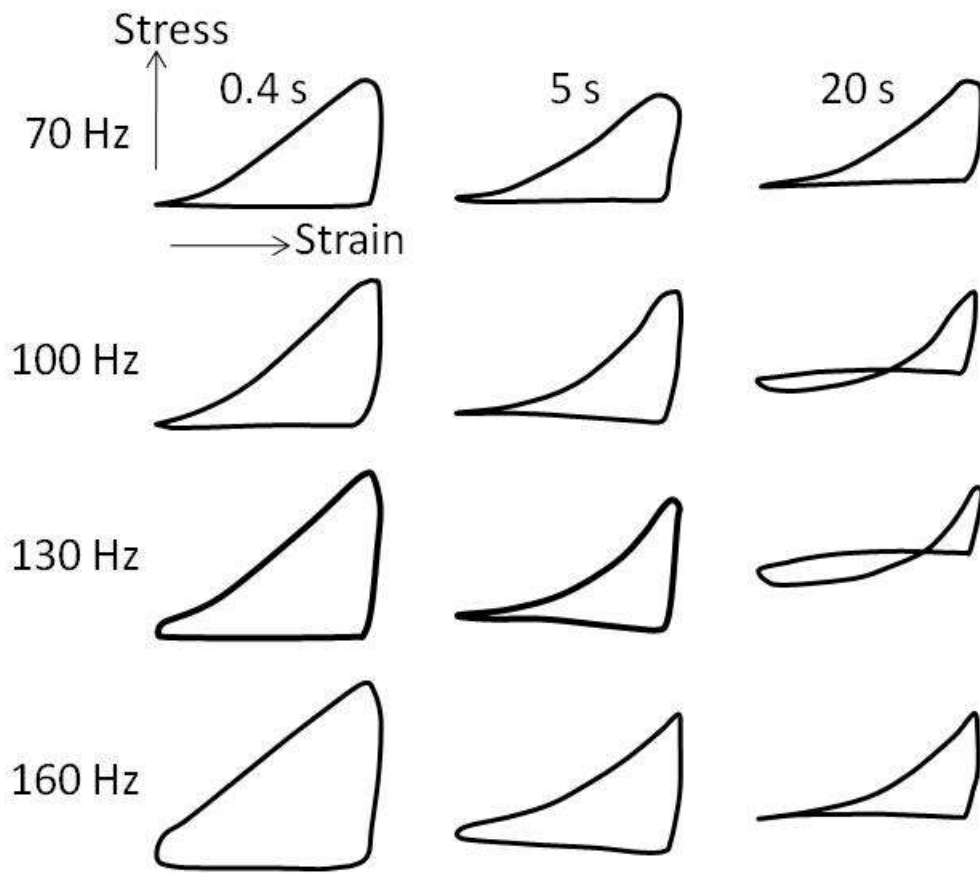
1 **Figure 5.**



2

3

1 **Figure 6.**



2