The Journal of Experimental Biology 205, 2297–2303 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4174

Review

Maintaining muscle mass during extended disuse: aestivating frogs as a model species

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Accepted 13 May 2002

Summary

Prolonged muscle disuse in vertebrates can lead to a pathological change resulting in muscle wasting and a loss of muscle strength. In this paper, we review muscle disuse atrophy in the vertebrates and examine the factors that influence the magnitude of the atrophic response during extended periods of inactivity, both artificially imposed (e.g. limb immobilisation) and naturally occurring, such as the quiescence associated with dormancy (e.g. hibernation and aestivation). The severity of muscle atrophy is positively correlated with mass-specific metabolic rate, and the metabolic depression that occurs during dormancy would appear to have a protective role, reducing or preventing muscle atrophy despite periods of inactivity lasting 6–9 months. In the light of these findings, the role of reactive oxygen species and antioxidants during muscle disuse is emphasised.

Key words: skeletal muscle, disuse, inactivity, atrophy, dormancy, aestivation, reactive oxygen species.

Introduction

Skeletal muscle is a highly plastic and responsive tissue whose phenotype is modulated by exercise (Musacchia et al., 1988). Usage builds or maintains muscle mass, while periods of inactivity due to disuse typically lead to degenerative changes culminating in the atrophy of muscle fibres. The growth of the muscle depends upon the dynamic balance between synthetic and degradative processes (Schimke, 1975). The synthetic processes and some of the degradative processes are tightly regulated and controlled in a multi-faceted way, exhibiting both local (e.g. intracellular Ca^{2+} levels) and systemic (e.g. endocrine control) components (Vanderburgh et al., 1999). It is the interaction between these physiological processes that underlies the conventional plasticity of muscle. In addition, other factors such as the partially stochastic process of oxidative damage can have a non-specific degradative role during muscle atrophy.

The effect of muscle disuse or inactivity, characterised by a reduction in muscle fibre cross-sectional area and a concomitant loss of muscle strength, appears to be common to the vast majority of vertebrates studied. Research on muscle wasting associated with disuse has focused extensively on mammals, especially studies in which limbs have been artificially immobilised for extended periods with a splint, pin or cast (Booth, 1982; Musacchia et al., 1988; Nordstrom et al., 1995; Soares et al., 1993). A significant amount of the data on muscle disuse atrophy comes from human studies in which muscle wasting occurs as a result of limb immobilisation (stemming from bone fractures), extended bed rest or as a consequence of micro-gravity effects during prolonged space travel (Fitts et al., 2000). Apart from these studies, most investigations have used laboratory-reared mammals (namely mice, rats, guinea pigs, cats and dogs) to look at muscle disuse atrophy (Bebout et al., 1993; Boyes and Johnston, 1979; Maier et al., 1976; Nordstrom et al., 1995; Soares et al., 1993). These animals reflect the fact that, historically, muscle disuse has been biased towards biomedicine.

In more recent years, a number of comparative physiological studies have investigated muscle structure and function in animals that undergo natural periods of muscle disuse, such as are imposed during dormancy (Harlow et al., 2001; Tinker et al., 1998; Wickler et al., 1991). Examples include hibernating mammals and frogs (Harlow et al., 2001; St-Pierre et al., 2000), and, from our laboratory, investigations on muscle morphology and locomotor performance in aestivating (burrowing) frogs (Hudson and Franklin, 2002).

This paper aims to provide an overview of muscle disuse in vertebrates, describing the defining characteristics of muscle atrophy and discussing possible causative factors. Equal emphasis is given to experimentally induced and naturally occurring inactivity. In describing the cues and processes that underlie muscle disuse atrophy, the role played by reactive oxygen species (ROS) is emphasised. In particular, we

highlight the work we are currently conducting on burrowing frogs, which can remain immobile for longer than 9 months yet still maintain muscle mass and function. In taking this comparative approach, we expose some trends and patterns that we believe provide a valuable insight into possible mechanisms that may regulate or inhibit muscle disuse atrophy.

Muscle disuse atrophy: characterisation

Skeletal muscle atrophy is characterised by a suite of structural, biochemical, physiological and functional changes, largely prevalent in the muscle tissue itself but also evident at neuromuscular junctions (Fahim, 1989) and in the microvasculature of the muscle (Oki et al., 1998).

On a gross level, there is a reduction in muscle fibre cross-sectional area. Other structural changes include sarcomere dissolution and endothelial degradation (Oki et al., 1995; Tyml et al., 1990). In addition, there is a reduction in the number of mitochondria (Rifenberick et al., 1973), an increase in the amount of connective tissue (Oki et al., 1995) and apoptotic myonuclear elimination (Smith et al., 2000). At the biochemical level, amounts of muscle protein, α-actin mRNA and cytochrome *c* mRNA are all reduced (Babij and Booth, 1988). Per gram of muscle mass, there is a decreased utilisation of β-hydroxybutyrate, palmitate and glucose, and levels of high-energy phosphates decline (Booth, 1977), as do levels of oxidative enzymes such as citrate synthase (Bebout et al., 1993) and malate dehydrogenase (Rifenberick et al., 1973). In addition, there is a reduction in levels of phosphokinase (Carmeli et al., 1993).

The structural and morphological changes associated with muscle atrophy ultimately impact upon the muscle function and locomotor performance of the animal. Force production by muscle is related to muscle cross-sectional area, so muscle fibre atrophy results in a reduction in maximal force production (Witzmann et al., 1982) and muscle power output. This, in turn, leads to an impairment of locomotor performance. Some of the structural changes associated with prolonged muscle disuse are pathological, and lengthy recovery periods are often required before full muscle and locomotor performance is re-established. For example, Booth and Seider (1979) found that, in the rat, 3 months of immobilisation required 4 months of recovery before muscle performance returned to control levels.

Factors influencing muscle disuse atrophy

Disuse atrophy is most commonly (and correctly) assayed by comparing the mean cross-sectional area of a sample of fibres within a muscle before and after treatment or with those in the contralateral limb. However, it has also been inferred by tracking enzyme profiles, measuring levels of biochemicals and comparing some performance parameter such as isometric force production. Normally, a single study will examine changes at one or two levels of organisation only (e.g Tinker et al., 1998), which can make comparisons among studies difficult. In these cases, a functional impairment is assumed to occur in line with the decrease in cross-sectional area. In this review, we have only made direct comparisons (e.g. of changes in muscle fibre size) when analogous data were available.

A variety of extrinsic and intrinsic factors influence the extent of muscle atrophy during immobilisation/inactivity. Apart from the duration of inactivity, muscle disuse atrophy is influenced by muscle position (whether the limb/muscle is immobilised in a stretched or contracted position), muscle fibre type, age and species.

Muscle length

Muscle length has been shown to have a significant influence on muscle atrophy in mammals. Muscles immobilised in the shortened position suffer significantly greater atrophy than muscles fixed in a stretched position (Tabary et al., 1972). McComas (1996) found that the length of muscle at fixation is critical for sarcomere reabsorption. In the cat, immobilisation of the soleus muscle in the shortened position resulted in 40~% fewer sarcomeres in series than control (active) muscle, whilst in the lengthened position sarcomere number increased by 20 % compared with active muscle (Tabary et al., 1972).

Muscle fibre type

Within an organism, muscle atrophy is greater in slow oxidative (SO) than in fast oxidative glycolytic (FOG) muscle fibres (Booth and Kelso, 1973; Booth and Giannetta, 1973; Maier et al., 1976; Booth, 1977, 1982; McComas, 1996; Musacchia et al., 1988; Witzmann et al., 1982). In the rat (*Rattus rattus*), the soleus muscle is composed of both SO and FOG muscle fibre types. Immobilisation of the hindlimb of the rat resulted in a preferential atrophy of SO muscle fibres over FOG muscle fibres, which culminated functionally in an increased contraction speed of the soleus.

Age

The age of the animal has been reported to influence the rate of muscle atrophy resulting from limb immobilisation, and it appears that muscle wasting decreases with age (Carmeli et al., 1993). In rats immobilised for 4 weeks, 4- to 5-month-old animals showed 49–64 % atrophy of the soleus muscle, whereas 20- to 21-month-old animals suffered only 27–38 % muscle atrophy (Ansved, 1995).

Species differences

There are conspicuous differences among species in the rate of muscle wasting following limb immobilisation. Soares et al. (1993) found that, after 4 days of hindlimb immobilisation, the mouse *Mus musculus* showed 15 % atrophy of the gastrocnemius muscle. Boyes and Johnston (1979) reported that after 3 weeks of immobilisation of the hindlimbs of the rat (Wistar strain) there was 50 % wasting of the vastus intermedius muscle; in the guinea pig, 4 weeks of hindlimb immobilisation incurred 43 % wasting of the gastrocnemius muscle (Maier et al., 1976). Meanwhile, Bebout et al. (1993) discovered that 3 weeks of hindlimb immobilisation of the

Table 1. *Comparing the effect of limb immobilisation on muscle atrophy in mammals*

Reference	Organism	Number of days immobilised	% Atrophy	% Atrophy (normalised to 12 days)
Soares et al. (1993)	Mouse	4	15	45
Boyes and Johnston (1979)	Rat	21	50	28
Maier et al. (1976)	Guinea pig	28	43	18
Nordstrom et al. (1995)	Cat	42	24	7
Bebout et al. (1993)	Dog	21	31	17
Veldhuizen et al. (1993)	Human	28	21	9

Table 2. *The effect of prolonged inactivity on muscle mass in dormant vertebrates and the relationship with mass-specific metabolic rate*

Organism	% Atrophy (normalised to 80 days)	Body-mass-specific metabolic rate ($ml\,g^{-1}\,h^{-1}$)	
		Active	Dormant
Frog (*Cyclorana alboguttata*)	0	0.035	0.015
Black bear (*Ursus americanus*)	0	0.22	0.042
Ground squirrel (*Spermophilus lateralis*)	7	0.75	0.045
Hamster (*Mesocricetus auratus*)	30	1.25	0.07

Data from Altman and Dittmer (1972–1974), Geiser and Ruf (1995), Tinker et al. (1998), Hudson and Franklin (2002) and Wickler et al. (1987, 1991).

gastrocnemius of the guinea pig led to 31 % atrophy. In humans, 4 weeks of knee immobilisation caused 21 % atrophy of the quadriceps muscle (Veldhuizen et al., 1993).

It is clear that comparisons of muscle disuse atrophy data from different species are confounded because studies have used different periods of disuse and indeed different immobilisation methods and different muscles. However, if these differences in immobilisation technique are disregarded and the data normalised to 12 days, there is a significantly greater rate of muscle atrophy in the smaller mammals (Table 1). When normalised to an immobilisation period of 12 days, the rate of muscle atrophy ranges from only 9 % in humans (Veldhuizen et al., 1993), to 17 % in dogs (Bebout et al., 1993), 18 % in guinea pigs (Maier et al., 1976), 28 % in rats (Boyes and Johnston, 1979) and 45 % in mice (Soares et al., 1993) (see Table 1).

Muscle disuse in dormant animals

Table 1 summarises and compares muscle disuse atrophy in mammals that have been artificially restrained or have had limbs immobilised. In the natural world, few organisms maintain constant activity levels through their lives, but instead undergo cyclical periods of activity and rest. Indeed, wide variations in locomotor demands can be considered the norm where seasonal variations in food abundance and climatic conditions define viable arousal times. Some animals routinely experience muscle inactivity on a seasonal basis, and perhaps the most impressive are those organisms that enter dormancy, a condition that imposes a prolonged period of chronic muscle inactivity. The list of organisms that fall into this category is both extensive and taxonomically disparate. It includes hibernators, over-winterers, aestivators and freeze-tolerant organisms. For these animals, the consequences of muscle atrophy resulting from prolonged inactivity and muscle disuse (often many months) would compromise locomotor performance following dormancy.

Studies on muscle structure and function in dormant organisms have focused primarily on hibernating mammals. This work has consistently shown less of an atrophic response than would be predicted from laboratory models (compare Tables 1 and 2). For example, the ground squirrel *Spermophilus lateralis* exhibited only a 15–20 % decrease in gastrocnemius fibre cross-sectional area after 6 months of hibernation (Steffen et al., 1991), and the brown bear *Ursus americanus* displayed no muscle atrophy after 4 months of dormancy (Tinker et al., 1998). Even though some muscle atrophy occurred in the case of *S. lateralis* over 6 months, it was no more severe than that following just 1 or 2 weeks of artificial limb immobilisation in a rat (Musacchia et al., 1988).

Vyskocil and Gutmann (1977) investigated the effect of hibernation on the latency period, rate of tension development, contraction time and half-relaxation time in the golden hamster *Mesocricetus auratus*. After 3 months of inactivity and muscle disuse, they found no change in muscle performance, which indicated that the functional capacity of actin, myosin, the sarcoplasmic reticulum and the myofibrillar organelles were all maintained through hibernation. This is in stark contrast to the findings for artificially immobilised muscles after a few weeks. For example, time to peak tension decreased by 30 % in the guinea pig soleus after 4 weeks of limb immobilisation (Maier et al., 1976), and isometric force development in the soleus muscle decreased by 34 % in the rat after 3 weeks of hindlimb suspension (Anderson et al., 1999). Moreover, hibernation has

little effect on muscle function in the black bear *Ursus americanus*. After 130 days of hibernation, *U. americanus* was found to lose only 23 % of its hindlimb strength (Harlow et al., 2001) in comparison with a predicted 90 % loss for humans.

Other vertebrate classes show even more impressive periods of prolonged inactivity. Several species of arid-zone Australian frogs, such as the green-striped burrowing frog *Cyclorana alboguttata*, survive the lengthy droughts by digging an underground chamber, forming a waterproof cocoon of shed skin and mucus, and conserving energy in a process called aestivation (Flanigan et al., 1993; Withers, 1993). In this capacity, these frogs are inactive and immobile in their burrows often for months and possibly years. However, when the summer rains finally come, a selective advantage is conferred on those frogs capable of compressing their feeding and breeding into a narrow time frame of only a few weeks when water is plentiful. During this short period of opportunity, locomotor performance, which varies with muscle performance, is at an absolute premium.

We have found that the chronic disuse associated with 3 and 9 months of aestivation had no effect on muscle mass, *in vitro* force production and swimming performance in *C. alboguttata* (Hudson and Franklin, 2002; N. J. Hudson and C. E. Franklin, in preparation). The absence of atrophic changes is consistent with the patterns found during periods of disuse in hibernating mammals discussed above, and it is clear that the response of skeletal muscle to disuse during dormancy is quantitatively different from that found in artificial immobilisation studies.

Muscle disuse atrophy: etiology

It is apparent that muscle disuse atrophy is a pervasive biological phenomenon occurring across a range of taxa, yet marked differences in the rate of muscle wasting occur among species. However, skeletal muscle is an ancient, highly conserved tissue, so it is inevitable that the etiology of muscle wasting across the various taxa shows similarities.

The ultimate causation of atrophy is the muscle disuse *per se*, and it is important to note that, in quantifying the actual disuse stimulus, both the period of inactivity (i.e. duration) and the relative reduction in the activity of the muscle at the onset of immobilisation are significant. Thus, within a species, the duration of inactivity has been shown to be positively correlated with the degree of muscle atrophy, although the actual rate of atrophy decreases with time (Booth, 1977). In addition, wasting is greater in muscles used regularly/intensively prior to immobilization than in muscles that are used only intermittently. In frequently used muscles, the disuse stimulus is greater as there is a larger difference between use and disuse (Musacchia et al., 1988; McComas, 1996).

Proximately, there are two key processes that govern the extent of muscle atrophy: (i) the magnitude of the regulated decline in the rate of protein synthesis and (ii) the level of oxidative damage and subsequent unregulated protein degradation.

Decline in rates of protein synthesis

The exact signal transduction pathways that translate changes in contractile activity into regulated declines in rates of protein synthesis have not been elucidated (Rennie, 2001), although several changes operating at different levels of organisation have been described. For example, among the first changes during muscle atrophy is an impairment of sarcolemmal ion transport, which can lead to changes in rates of protein turnover and degradation (Reznick et al., 1995). Within muscle fibres, the number of lysosomes and autophagic vacuoles containing proteolytic enzymes increases, which attack the myofibres (Reznick et al., 1995). Increases in intracellular levels of Ca^{2+} in muscle fibres help to stimulate this autophagic response (Soares et al., 1993). Calpains, Ca^{2+}-activated cysteine proteins, also play a key role in the disassembly of sarcomeric proteins. Furthermore, loss of Ca^{2+} homeostasis is thought to impair mitochondrial respiratory function (Soares et al., 1993).

Overall, there is a sharp decline in the rate of protein synthesis, and concentrations of myogenic regulatory factor transcripts are reduced during disuse (Loughna and Brownson, 1996). Muscle atrophy is associated with reduced rates of transcription (Gunderson and Merlie, 1994) and a progressive loss of muscle protein.

Oxidative damage

Reactive oxygen species (ROS), which leak out of mitochondrial membranes during aerobic respiration, have a significant degenerative effect on muscle fibres, degrading (stochastically) muscle proteins and lipids (Kondo et al., 1991, 1993, 1994). Examples of ROS include superoxide, hydrogen peroxide and hydroxyl radicals. All aerobically respiring tissues are exposed to ROS during routine functioning; however, oxidative stress occurs only when the rate of formation of ROS exceeds the rate of removal. Thus, an accumulation of oxidative damage occurs as a result of either a decline in anti-oxidant defences or a loss of repair function (i.e. the rate of *de novo* protein synthesis) (Ames et al., 1993).

Skeletal muscle is particularly susceptible to oxidative damage. Muscle generally has a large aerobic scope, which means it has to deal with a fluctuating supply of oxidants. It also has surprisingly low levels of antioxidants (Avellini et al., 1999), which compromises defence. Finally, during immobilisation, the negative protein balance that leads to muscle atrophy is primarily a result of a decline in the rate of protein synthesis (which compromises repair) and not an increase in the rate of regulated protein degradation (Tucker et al., 1981). Moreover, it has been established that, as the damage progresses, the muscle fibres release bound transition metals such as myoglobin iron, which catalyse some of these oxidative processes and further accelerate atrophy (Kondo et al., 1993).

It follows that, because ROS represent a fixed proportion of the oxygen processed (Avellini et al., 1999; Adelman et al., 1988), their production must correlate with both the density of mitochondria and the aerobic activity of the muscle. This stochastic relationship is important in understanding the differences in the extent of muscle wasting that occur between

muscle fibre types and the differences observed in muscle disuse atrophy among species.

Slow-twitch fibres (such as typically found in postural muscles) have repeatedly been shown to suffer a more severe disuse response than fast-twitch fibres in experimental systems, although the underlying cause for this disparity is unclear. We believe that this difference can be attributed to the combined effects of the relative size of the disuse stimulus and the extent of oxidative damage between fast- and slow-twitch fibres. As fast-twitch fibres are recruited only intermittently compared with slow-twitch fibres, the relative size of the initial disuse stimulus following immobilisation is proportionately smaller, and this elicits a correspondingly smaller atrophic response. In addition, because ROS are produced during aerobic respiration and leak out from mitochondrial membranes, fast-twitch fibres, with their dependence on anaerobic pathways for energy production and low density of mitochondria, are less afflicted. Consistent with this hypothesis is the finding that, under normal circumstances (i.e. not during immobilisation), slow-twitch fibres suffer more oxidative damage as measured by the production of protein carbonyl (Sen et al., 1997).

With respect to understanding difference in the rates and extent of muscle disuse atrophy among species, we have found that the severity of muscle atrophy is highly correlated with mass-specific metabolic rate (Fig. 1). Mice, which have comparatively higher mass-specific metabolic rates, are subject to accelerated atrophy, whilst animals with lower mass-specific metabolic rates such as humans are intrinsically spared. Thus, organisms with lower mass-specific metabolic rates experience a less dramatic atrophy than their high-metabolic-rate counterparts. We believe there are two reasons for this.

First, low-metabolic-rate organisms are relatively quiescent, which means that prior to immobilisation their muscles are less active. This exposes them to a smaller disuse stimulus, which in turn elicits a smaller atrophic response. Second, because the production of ROS represents a fixed proportion of the total oxygen processed, the magnitude of the ROS insult must vary in line with metabolic rate (Grundy and Storey, 1998) so that an organism such as a human has tissues that are subjected to a smaller ROS insult than comparable tissues in a mouse.

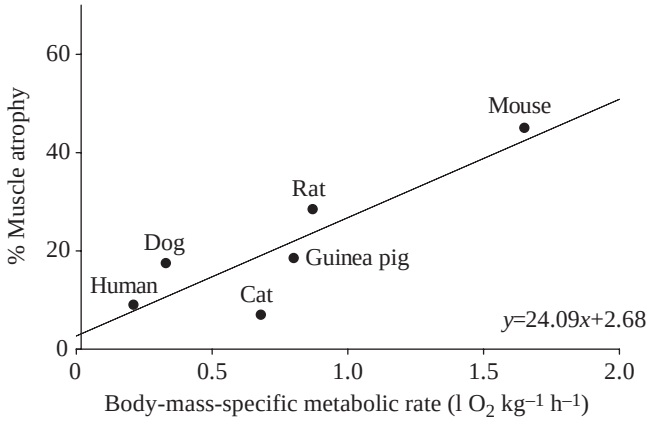

Fig. 1. The relationship between body-mass-specific metabolic rate and percentage muscle disuse atrophy (normalised to 12 days) for mammals with hindlimbs artificially immobilised (r^2=0.76, P<0.01).

The effect of age on muscle disuse atrophy can also be explained using the above criteria. Older animals tend to become progressively more sedentary, and so the initial disuse stimulus must become less dramatic. Furthermore, as vertebrates grow and increase in size with age, there is a corresponding decrease in mass-specific metabolic rate, which would reduce the ROS insult inflicted upon their muscle.

Of all the patterns described above, the effect of muscle length is the only one that cannot adequately be explained by the impact of the disuse stimulus or oxidative damage. The factors influencing muscle atrophy during disuse are summarised in Fig. 2.

Preservation of muscle structure in dormant animals

The mechanisms underpinning the preservation of muscle condition in both hibernating mammals and aestivating frogs warrant examination because lengthy periods of rehabilitation are required to regain muscle condition and locomotor performance after artificial immobilisation in mammals (Booth, 1982). A number of competing hypotheses have been presented in the literature as to how muscle atrophy is avoided in dormant animals. They include the position of the immobilised muscle (Tinker et al., 1998), the recycling of urea in over-wintering bears (Harlow et al., 2001) and the use of ‘shivering thermogenesis’ as an exercise cue in ground squirrels (Wickler et al., 1991). However, although these intrinsic mechanisms may limit muscle atrophy, we feel that there are more parsimonious explanations.

Winter dormancy is characterised by a marked decrease in metabolic rate in all animals concerned (Table 2). The production of ROS represents a fixed proportion of the total oxygen processed, so the reduction in mass-specific metabolic rate that occurs during dormancy automatically reduces the ROS insult and, thereby, limits the extent of oxidative damage. Consequently, the demands placed on both the muscular defence (antioxidants) and repair (*de novo* protein synthesis) systems are also alleviated and the rate of atrophy is reduced accordingly. In comparing the degree of muscle wasting across hibernators/over-winterers, bears showed the smallest response, ground squirrels were intermediate and hamsters atrophied the most. This is surprising given that the differences in their metabolic rates during dormancy are not that dramatic (Table 2).

However, if the pre-dormancy metabolic rates are compared, a pattern emerges that is consistent with the differences in the atrophic response. Thus, the hamster mass-specific metabolic rate during dormancy itself is only 66 % greater than that of the bear. But, prior to dormancy, the mass-specific metabolic rate of the hamster is 468 % greater than that of the bear (Table 2). Thus, part of the reason that the hamster suffers a much greater atrophy than a bear or ground squirrel may be related to the greater activity of its muscle before

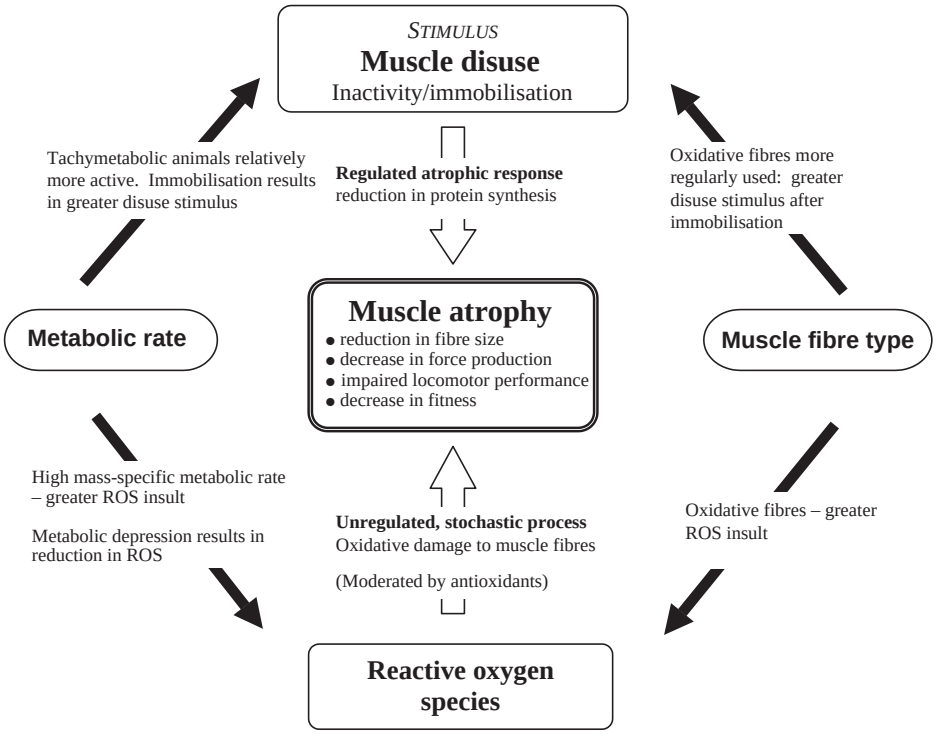

Fig. 2. A schematic diagram showing factors contributing to the rate of muscle atrophy resulting from prolonged inactivity or limb immobilisation. ROS, reactive oxygen species.

immobilisation, leading to a more pronounced disuse stimulus when the inactivity associated with dormancy begins.

Frogs (including our study animal, *C. alboguttata*) are bradymetabolic organisms whose limb skeletal muscle is predominantly composed of fast-twitch fibres (Sperry, 1981) used in brief anaerobic bursts as part of a sit-and-wait strategy of prey capture or for escaping predators. Consequently, during immobilisation/aestivation, they are subject to a relatively small disuse stimulus and also a relatively small ROS insult. In addition, as in the hibernators, the marked depression in metabolic rate that accompanies aestivation (up to a 60 % drop in metabolic rate) further reduces the ROS insult, which must automatically act to preserve skeletal muscle structure during aestivation. However, there is some evidence that more active mechanisms may be in operation in aestivating frogs.

All tissues, including skeletal muscle, are protected against oxidative damage by a range of scavenging antioxidants such as superoxide dismutase. The experimental administration of antioxidants has been shown to decelerate muscle atrophy by 15 % in artificially immobilised rats (Kondo et al., 1991). The endogenous regulation of antioxidant levels therefore represents a potential means of further reducing muscle wasting. Consistent with this hypothesis is the finding that levels of antioxidants are upregulated during extended periods of dormancy in amphibians. In the spadefoot toad *Scaphiosus couchii*, skeletal muscle oxidative damage was found to be less severe during aestivation than during arousal (Grundy and Storey, 1998). Of the six antioxidants analysed, levels of two were elevated during aestivation, whilst those of the other four matched control levels. Given that metabolic rate drops by approximately 80 % during aestivation, maintenance at control levels can be interpreted as a functional upregulation. We believe that the relative increase in antioxidant levels offers a means, in concert with a depression of metabolic rate, of reducing muscle atrophy in aestivating anurans by the amelioration of oxidative damage. The verification of this hypothesis by assessing seasonal fluctuations in levels of skeletal muscle antioxidants in other animals that undergo dormancy, such as hibernating mammals, will be of considerable interest.

This work was funded in part by a Large Australian Research Council grant to C.E.F. N.J.H. was in receipt of a postgraduate scholarship from the Northcote Trust during this study. The authors would like to thank Peter Kraft, Ed Meyer, Toni Priest, Louise Kuchel and other members of the University of Queensland Physiological Ecology Laboratory for stimulating discussion and encouragement.

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