Potassium Homeostasis During Exercise In Domestic Species: the role of the sodium-potassium pump in skeletal muscle

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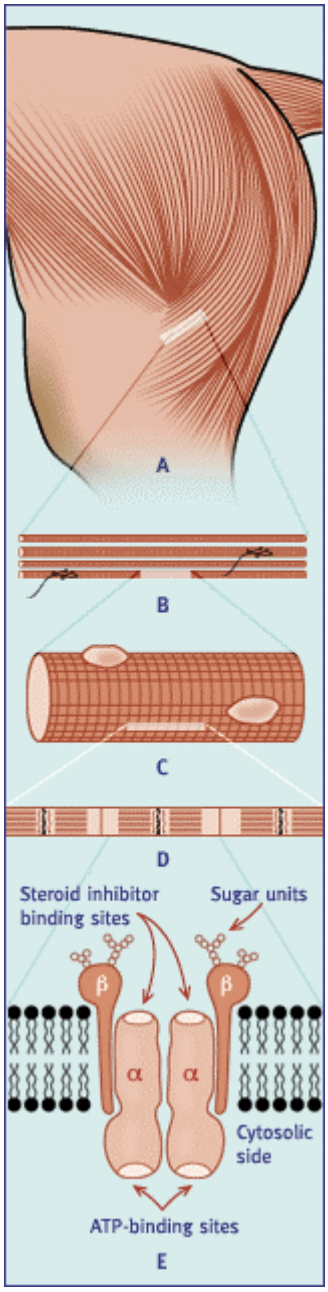
Introduction



In 1997, Jens Christian Skou was awarded the Nobel Prize in Chemistry for his discovery and elegant description, some 40 years earlier, of the sodium-potassium (Na^+,K^+) pump in crab nerve fibres [37]. It is now widely accepted that this cation transport system is essential for cell function, and that it plays a central role in the Na^+,K^+ homeostasis of virtually all animals [4,6]. Since its identification, the Na^+,K^+ pump has been the subject of numerous investigations, including ones on the mechanism for controlling ion transport through the pump, the detailed molecular structure of the pump and its regulation. Regulation of plasma K^+, both long-term (by kidney Na^+,K^+-ATPase), and acute (by skeletal muscle Na^+,K^+-ATPase, during exercise), have been questions frequently addressed by physiologists. Most of this work has been performed using small rodents and man [4,5,6], however, the following review will discuss the up- and down regulation of the Na^+,K^+ pump concentration in the skeletal muscle of domestic animal species, including cats, dogs, horses and cattle.

The Na^+,K^+ pump in skeletal muscle

To maintain the high concentration of K^+ and the low concentration of Na^+ that exists in animal cells, relative to the external environment, a specific transport system is required. Skou's discovery of an enzyme that hydrolyses ATP only when Na^+ and K^+ are present (in addition to Mg^{2+} required by all ATP enzymes), was the beginning of our understanding of the Na^+K^+ pump [37]. The enzyme, known as Na^+,K^+-ATPase, forms an integral part of the Na^+,K^+ pump and the splitting of ATP provides the energy required to drive the active transport of the cations.

Fig. 1 A - D Schematic representation of the component parts of a skeletal (striated) muscle. (A) whole muscle. (B) a small part of the muscle magnified to show the individual muscle cells or fibres. (C) a part of the muscle fibre magnified. (D) an individual myofibril. A group of myofibrils together forms a muscle fibre or cell. The Na+,K+ pump is located within the plasma membrane of these muscle cells. E - Diagrammatic representation of the subunit structure and orientation of the Na+,K+ pump in the plasma membrane.

The pump is located within the sarcolemma of skeletal muscle cells (Figure 1, A-D) and is a so-called αβ heterodimer, consising of two α and two β subunit proteins that have a fixed orientation within the lipid bilayer of the cell membrane (Figure 1; Part E). It is the larger α subunit that has the three receptor sites for binding sodium ions on the portion of the protein protruding to the interior of the cell, the two potassium ion receptor sites found on the exterior and, adjacent or near to the sodium ion binding sites, it has the ATPase activity, hence the terms 'pump' and 'enzyme' are often used interchangeably. Like many enzymes, Na^+,K^+-ATPase exists in various isoforms, giving rise to tissue-specific expression and differential regulation of the molecule. Skeletal muscle expresses α1, α2, β1 and β2 isoforms and thus has the possibility of four αβ combinations [41]. The role of the Na^+,K^+ pump in the restoration of membrane potential after exitation [4,5,6], and its confirmational changes during a transport cycle, in which sodium ions are transported out and potassium ions in to the cell, are illustrated in Figure 2.

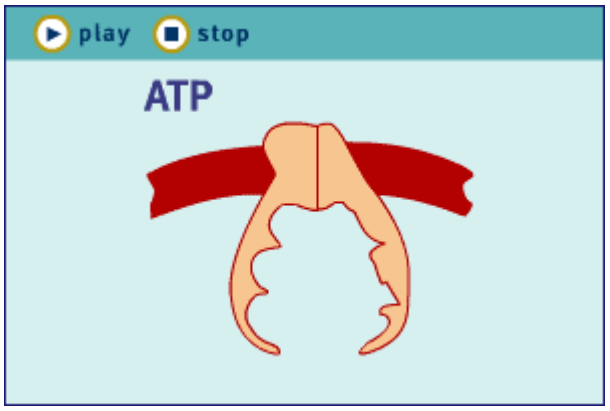


Fig.2 Animation showing a transport cycle of the Na+,K+ pump. During the cycle, the Na+,K+ pump is first phosphorylated and subsequently de-phosphorylated. Binding of intracellular Na+ ions to the pump initiate phosphorylation of the enzyme, while the binding of extracellular K+ ions trigger the dephosphorylation reaction. After a complete cycle, three Na+ ions have been extruded from and two K+ ions have entered into the cell. (Adapted from an illustration by Kjell Lundin, featured in a special publication to mark the award of the 1997 Nobel Prize in Chemistry to Professor Jens Christian Skou).

Thus, as a result of repetitive action potentials, exercise induces a loss of K^+ from the muscle cells into the extracellular space, giving rise to an increase in plasma K^+ [4,5,27]. In man, hyperkalemia occurs during both dynamic and static exercise and is believed to play a role in the development of muscular fatigue [27,36]. While the long-term control of plasma K^+ concentrations depends ultimately on kidney function, achieved by increasing the concentration of Na^+,K^+ pumps in the cell membrane (for example, by thyroid hormones or training), it is skeletal muscle that plays the dominant role in its acute adjustment, by increasing the activity of the Na^+,K^+ pump (for example, by adrenaline; Figure 3), or by increasing the concentration of Na^+,K^+ pumps in the cell membrane (for example, by thyroid hormones or training). These muscles represent the body's largest pool of K^+ and Na^+,K^+ pumps, and therefore provide an enormous capacity for rapid Na^+,K^+ exchange [4,5,6].

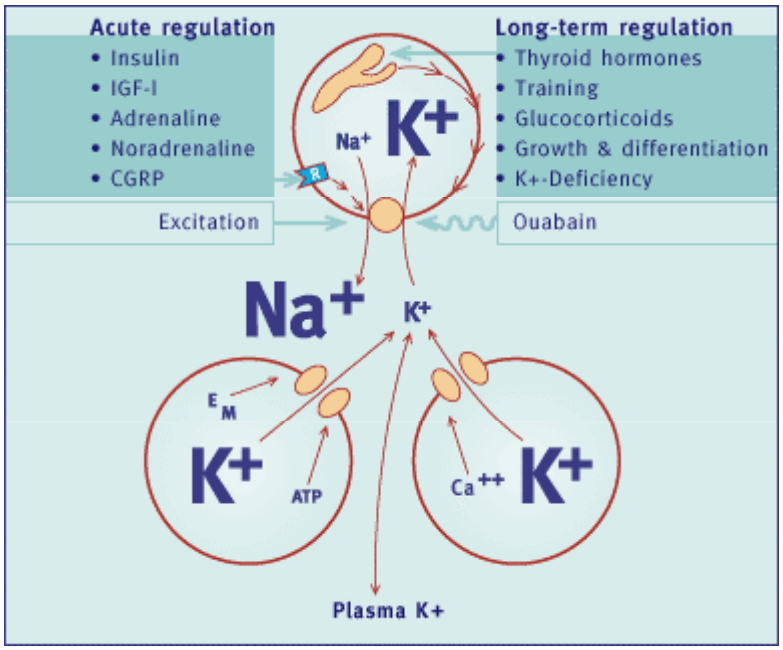


Fig. 3 Diagram of regulatory factors controlling the activity and the concentration of Na+,K+ pumps in a skeletal muscle cell. Left: factors eliciting acute stimulation of the Na+,K+ pump (not discussed in text). Right: factors known to influence the concentration of Na+,K+ pumps by stimulation or inhibition of their synthesis and insertion into the plasma membrane. The first two factors (thyroid hormones and training) are discussed in this review. The lower two muscle cells indicate that K+ leaves the cell during excitation through ATP or Ca2+-dependent K+ channels.
Abbreviations: IGF-1 - insulin-like growth factor I; CGRP - calcitonin generelated peptide. (Modified from Reference 4).

An increase in the capacity for active Na^+,K^+ transport in skeletal muscle should, however, lead to a 'blunted', or 'dampened', rise in plasma K^+ during exercise (Figure 4), and hence to an improvement in muscle endurance. Indeed, this does occur in man after sprint training [17,29]. Furthermore, a correlation exists between maximum O_2 uptake, running distance and Na^+,K^+ pump concentration in skeletal muscle [14]. It has also been reported that a large increase in the capacity for active Na^+,K^+ transport occurs in the skeletal muscle of patients suffering from hyperthyroidism [4,7,22], despite the condition being associated normally with increased fatigability and reduced endurance [13,19].

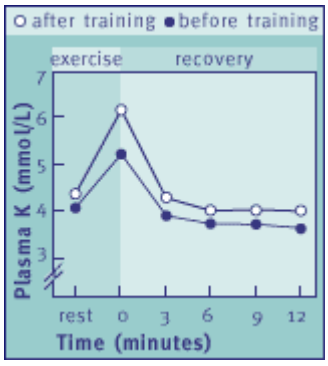


Fig. 4 Changes in plasma K+ concentrations in 4-year old horses (n=3) during an exercise test performed before and after a 10-day training period. The rise in plasma K+ during exercise is significantly "blunted" after training.
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Questions

In 1997, the author began investigating the regulation of the Na^+,K^+ pump and K^+ homeostasis during exercise in cats, dogs, horses and cattle. Three questions were addressed:

- Is thyroid hormone a major determinant in the concentration of Na^+,K^+ pumps in skeletal muscle of domestic animal species, as it is in man?
- Does a reduction in Na^+,K^+ pump concentration lead to hyperkalemia during exercise? And,
- Does training lead to an upregulation in the Na^+,K^+ pump concenttration in skeletal muscle?

Analysis of the concentration of Na^+,K^+ pumps in skeletal muscle

Quantitative analysis of membrane bound enzymes, such as Na^+,K^+-ATPase, is often performed on plasma membrane fractions that have been isolated from cell homogenates using differential centrifugation. However, this procedure requires large amounts of tissue, making it impractical for both medical and veterinary clinical studies, it may result in a loss of > 95% of Na^+,K^+ pumps and recovery rates between preparations vary enormously [18]. Thus, the development of techniques to measure the concentration of Na^+,K^+ pumps in small samples of intact skeletal muscle has proved invaluable to physiological studies [30].

Cardiac glycosides, such as digoxin and ouabain, bind specifically to the outer surface of the Na^+,K^+ pump, a stoichiometric process: one molecule of cardiac glycoside binds to one Na^+,K^+ pump molecule. The concentration of Na^+,K^+ pumps is measured using radioactively-labelled [^{3}H] ouabain, provided the isozyme of the Na^+,K^+ pump in a specific tissue has a high affinity for the molecule. Analysis of mRNA coding for the Na^+,K^+ pump in skeletal muscle has revealed that most of it does, indeed, code for an isozyme with a high affinity for ouabain [41].

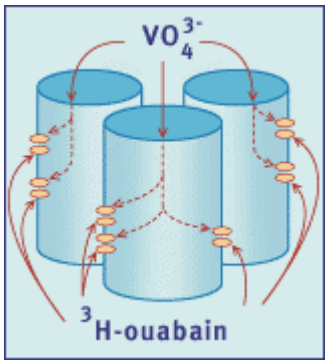


Fig.5 Schematic representation of the vanadate-facilitated binding of [3H]ouabain to a muscle biopsy. The open ends of the muscle cells allow vanadate ions access to the phosphorylation site of the Na+,K+ pump, on the inner surface of the sarcolemma. This binding results in a configuration of the Na+,K+ pump capable of binding [3H]ouabain to its outer surface (Taken from Reference 6).

Binding of [^{3}H]ouabain to the Na^+,K^+ pump is facilitated by the presence of the phosphate analogue vanadate (Figure 5). Using this anion, a simple and rapid assay has been developed for the measurement of Na^+,K^+ pump concentration in muscle samples weighing as little as 5 mg [30]. The recovery of such small samples has the considerable advantage of enabling multiple biopsies to be taken from a tissue and therefore duplicate, triplicate or quadruplicate measurements may be made.

Thyroid hormones

For about 30 years, it has been known that Na^+,K^+-ATPase activity in skeletal muscle and other tissues increases as a function of thyroid status; hyperthyroidism gives rise to an increase in pump activity, while hypothyroidism results in its decreased activity [20]. The increase in Na^+,K^+ transport associated with hyperthyroidism was once thought to

account for the calorigenic action of the thyroid hormones, however, only 5-10% of the total heat produced in skeletal muscle of eu-, hypo- and hyperthyroid animals can be attributed to active Na^+,K^+ transport [7].

Na^+,K^+-ATPase in rat muscle

Thyroid hormones largely determine the concentration of Na^+,K^+ pumps in skeletal muscle through a general endocrine effect [5,7], which is in stark contrast to observations made during training (see later). In rat skeletal muscle, the Na^+,K^+-ATPase concentration is approximately the same whether it consists predominantly of slow (eg. soleus) or fast (eg. gastrocnemius) fibres [21]. However, gastrocnemius muscle recovered from hyperthyroid rats contained five times the concentration of Na^+,K^+ pumps compared to equivalent samples recovered from hypothyroid animals. This difference rose to as much as ten times when soleus muscle samples were compared. These findings suggest that muscles show a greater response to an alteration in thyroid status when they consist predominantly of slow fibres.

Fatigability and Na^+,K^+ pump capacity

Contrary to expectations, the soleus muscle of hyperthyroid rats shows a greater susceptibility to fatigue and less endurance [13,19] than its increased capacity for active Na^+,K^+-transport suggests [12,19,21]. However, when this phenomenon is considered in relation to the increased influx of Na^+ through specialised channels, it is likely that muscle endurance is determined by the leak-to-pump ratio of Na^+, not by the Na^+,K^+ pump concentration alone. Furthermore, studies in which the time course of the effects of thyroid hormone on Na^+ influx and K^+ efflux was compared with that on Na^+,K^+-ATPase activity in skeletal muscle, have shown that the rise in the unidirectional flux of cations preceded the rise in Na^+,K^+ pump concentration [12,19]. Thus, increased permeability of the sarcolemma to cations after thyroid hormone treatment may be the driving force for the synthesis of Na^+,K^+ pumps.

Na^+,K^+-ATPase in cats

The concentration of Na^+,K^+ pumps in the skeletal muscles of hypo- and hyperthyroid dogs and cats has also been determined. Hypothyroidism is the most frequent thyroid disorder encountered in dogs, while hyperthyroidism is observed more often in cats [33]. Studies in both these species retrieved samples from the sternothyroid muscle due to its easy accessibility during surgical thyroidectomy

Total thyroxine (T_4) concentrations were approximately 400% higher, and Na^+,K^+ pump concentrations around 75% higher, in hyperthyroid compared to euthyroid cats (Schaafsma et al, unpublished data). In both groups of cats, the apparent dissociation constant for ouabain was of the same order of magnitude as that measured in rats with comparable thyroid status [21]. An intriguing observation made recently on a cat that was treated for 10 days with the anti-thyroid drug Strumazol (company, town and country), showed a high concentration of [^{3}H]ouabain binding sites had been maintained while the total plasma T_4 returned to normal (Schaafsma et al, unpublished data).

Na^+,K^+-ATPase and K^+ homeostasis in dogs

Recently, the concentration of Na^+,K^+ pumps was measured in the sternothyroid muscle of Beagle dogs, before and after thyroidectomy [34]. In euthyroid Beagles the Na^+,K^+ pump concentration was almost twice that recorded in euthyroid cats, but fell by 40% after thyroidectomy. The decrease in [^{3}H]ouabain binding capacity was not due to the Na^+,K^+ pump's reduced affinity for ouabain.

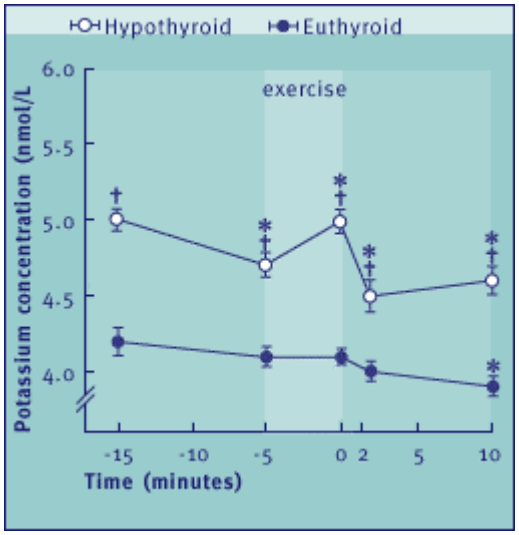


Fig.6 Plasma K+ levels in age-matched euthyroid and hypothyroid Beagle dogs, before and after exercise. Exercise consisted of a single 5 min run at a speed of 4.5 km/h. Each point on the graph represents the mean of 6, or in some cases 7, observations. The bars denote the standard error (SE) for each mean value;
* P<0.05 or better within groups; † P<0.05 or better between groups.

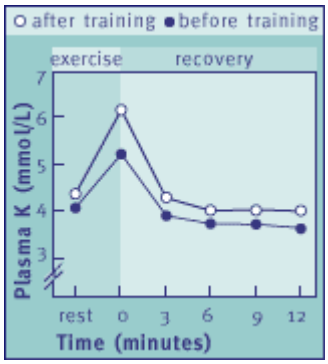
Total plasma T_4 concentrations were about 20 nmol/l in euthyroid and <2 nmol/l in hypothyroid dogs. The resting plasma K^+ concentration was significantly higher in hypothyroid compared to euthyroid dogs and remained higher throughout the experiment, including the work and recovery phases of the exercise test (Figure 6). In addition, hypothyroid dogs showed a significant exercise-induced hyperkalemia. The most likely explanation for this was a decrease in the muscle's capacity to pump K^+ back into the tissue, since neither muscle damage nor kidney failure was apparent [34].

Food restriction

Apart from thyroid disorders, thyroid hormone levels may change dramatically as a result of other diseases or food restriction [10] and may lead to a change in Na^+,K^+-ATPase concentration in skeletal muscle. For example, rats receiving one third to half their normal food supply, for 3 consecutive weeks, revealed a 50% reduction in total plasma triiodothyronine (T_3) in association with a 25% reduction in Na^+,K^+-ATPase concentration. This effect proved to be reversible; after just one week of being fed normal (full) rations, the rats' plasma T_3 and Na^+,K^+-ATPase concentrations had returned to normal [4,5]. Similar observations could not be reported in a group of Shetland ponies subjected to severe, long-term (2.5 years) food restriction; they showed a reduction in total and free T_3, (30 and 50%, respectively), a proportional loss of body weight, but only a modest (14%) decrease in Na^+,K^+-ATPase concentration in skeletal muscle [38]. This raised the questions whether skeletal muscle Na^+,K^+-ATPase isoforms are identical between species and to what extent thyroid hormone regulates specific isoforms [46].

Training and immobilisation

Depending on its intensity, exercise is accompanied by a rise in plasma K^+ concentrations [27,36], most probably originating from the working muscles. It is believed that inadequate sarcolemmal Na^+,K^+ -ATPase activity and a failure to restore Na^+,K^+ gradients across the sarcolemma during excitation are responsible [4,5,6,27]. Exercise-induced hyperkalemia is reduced by training in man [17,29], dogs [25], cattle [16] and horses [28] and is most likely due to an increase in skeletal muscle Na^+,K^+ pump concentration; an observation made in many species including rats [23], guinea pigs [26], man [14,17,29], horses [28,40] and cattle [44]. Alternatively, the early release of K^+ from cells may occur in association with H^+ exchange [45]; in other words, training induces a reduction in the K^+/H^+ exchange, if the blunted rise in plasma K^+ witnessed during exercise is to be explained (Figure 4).



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Training and immobilisation in rodents

Studies in which the concentration of Na^+,K^+-ATPase was measured in the skeletal muscles of different species (rats, guinea pigs, horses, cattle and man) before and after training, showed a relative effect of between 15 and 50%. It remains to be seen, however, whether this difference is related to the muscle type, to the relative size of the animals concerned, or to the duration and type of training.

One study looked at the combined effect of immobility and training on Na^+K^+-ATPase concentration in the fast, gastrocnemius, and slow, soleus, muscles of guinea pigs [26]. Within one to three weeks, the gastrocnemius muscle Na^+,K^+-ATPase concentration had decreased to a maximum of 25% its original value. However, during a fourth week of immobilisation these levels returned spontaneously to their normal value. After three weeks of daily running exercise on a treadmill, the Na^+,K^+-ATPase concentration increased by 50% in fast muscle but only by 15% in slow muscle. In rats, six weeks of swimming was found to induce a comparable (40%) increase in [^{3}H]ouabain binding site concentration in slow (soleus) and fast (extensor digitorum longus) muscles [23].

Training studies in man

Studies in man investigating the effects of training on Na^+,K^+-ATPase concentration in skeletal muscle, often involve the collection of biopsies from the easily accessible vastus lateralis muscle, which consists of mixed types of fibre. Invariably, these studies use bicycle training as the preferred form of exercise. It not only works the relevant muscle group sufficiently, but it is also easily standardised in a laboratory setting. Two simultaneous studies showed an increase of 14% [17] and 16% [29] in the concentration of [^{3}H]ouabain binding sites in the vastus lateralis muscle of male subjects, aged 18 to 20 years. The first of these studies demonstrated this effect after only six, two-hour daily training sessions [17]. In the second study, in which subjects performed short bouts of sprint work three times a week, biopsies were not taken until seven weeks after the start of training [29]. Thus, although the rise in Na^+,K^+-ATPase concentration was similar after endurance and sprint training, a longer period of sprint training was required to attain this effect. Due to the characteristic mixed fibre type of the vastus lateralis muscle, the increase in Na^+,K^+-ATPase concentration cannot be ascribed to one type of muscle fibre and, because biopsies were taken only at the end of the seven-week sprint training period, neither can it be established at what time point changes in Na^+,K^+-ATPase concentration occurred first.

Training studies in young and adult horses

Young horses, sprint-trained from birth until five months of age [42], showed an increase in [3H]ouabain binding capacity in gluteus medius and semitendinosus muscles of 30% and 20%, respectively [40]. Adult horses also revealed a 36% rise in Na^+,K^+-ATPase concentration in the gluteus medius muscle [20]. In adult horses, this rise was associated with a significant reduction in the plasma K^+ concentration during an exercise test [28].

Measurements of Na^+,K^+-ATPase concentrations in the gluteus medius muscle of young and adult horses affected by periodic hyperkalemic paralysis have been compared with those of age-matched control horses [31]. It was concluded that the cell membrane events underlying the periodic episodes of paralysis in hyperkalemic horses could not be attributed to changes in the Na^+,K^+ pump in either the Na^+,K^+ number or affinity. In addition, the decrease in Na^+,K^+-ATPase concentration measured in skeletal muscle showed an age-dependent decrease. This is true also for rat muscle, in which the concentration of Na^+,K^+ pumps rises five-fold from birth to four weeks of age and then falls due to an increase in the diameter of mature muscle cells [4,5]. Finally, when Na^+,K^+ pump concentrations were compared in gluteus muscle samples taken from horses of similar age but of different breeds, including the Quarter horse, Thoroughbred and Dutch warmblood, they were found to be similar [28,31,40].

Cattle

When endurance-trained Hereford calves were exercised at a maximum sustainable rate, they showed a rise in peak arterial plasma K^+ concentrations due to an increased maximum work capacity [16]. However, when they were exercised at a similar work load before and after physical conditioning, the rise was significantly reduced [16]. Young male and female Mozambican Angoni cattle, subjected to two hours of draught work every day for two weeks, showed increases in the concentration of Na^+,K^+-ATPase in semitendinosus muscle of 16 and 30%, respectively. When plasma K^+ concentrations were measured regularly during the daily two-hour training periods, the rise in concentration was lower at the end of the two weeks than it was after only eight days of training. This difference was not significant however [44].

Persistence of the training effect

How long does the training-induced rise in Na^+,K^+-ATPase concentration persist when intensive training is discontinued? Rats, subjected to six weeks of swim training, revealed a large rise in [^{3}H]ouabain binding site concentration, in both soleus (slow) and extensor digitorum longus (fast) muscles [23], which was almost completely reversed within three weeks of training being stopped. However, when a five month training period for young horses was followed by a six month period of rest, the concentration of [^{3}H]ouabain binding sites in semitendinosus muscle remained the same and in gluteus medius muscle was reduced by 10% [39]. Whether this discrepancy is due to species differences or the type of exercise performed is difficult to conclude, but the topic warrants further studies.

Is the training effect due to a general or a specific effect?

In trained rats, swimming induced up-regulation of the Na^+,K^+-ATPase in all hind limb and spinal muscles, but not in the diaphragm [23]. This result provides evidence against the existence of a non-specific endocrine factor, such as thyroid hormone, resposible for eliciting the training effect on the concentration of Na^+,K^+-ATPase [5]. A recent study in young foals has confirmed this idea by demonstrating that the training-induced rise in Na^+,K^+-ATPase was apparent in the gluteus and semitendinosus muscles of the hind limb,

but not in the masseter muscle of the jaw [39]. Considered together, these observations suggest that the factor eliciting an up-regulation in the Na^+,K^+ pump numbers during training is located in the muscle itself.

Perspectives for future research

In addition to being essential for locomotion, skeletal muscle from some animals is consumed as meat by man. A muscle's movement and meat quality are determined by the growth and composition of its composite fibres, as well as by the maintenance of ion gradients. The physiological and morphological properties of adult skeletal muscle are the combined result of genetic predisposition, diet, hormonal influences and the workload that the muscle has been exposed to.

During development skeletal muscles not only hypertrophy but also adapt to their required mechanical functions, such as rapid short-lasting movements (fast muscles) or prolonged actions (slow muscles). With respect to meat quality, slow muscles have better water holding capacity but lower colour stability than fast muscles [24].

A muscle's functional adaptation during development is evident through changes in the cation transport activity [8] and in the myosin heavy chain isoform expression during the postnatal period [9]. Both parameters are strongly affected by thyroid hormones and by exercise [5,32,35]. These effects are not easy to investigate independently since standardising exercise regimes is difficult and maintaining animals with relatively long growth periods is costly.

Muscle cell culture

The use of tissue culture techniques to study the adaptive behaviour and growth of muscle cells has obvious advantages [1]. Foetal myoblasts and adult muscle satellite cells are readily isolated and grown in vitro (Figure 7). After an initial phase of proliferation they fuse to form myotubes and then differentiate to become spontaneously contracting myofibres. During further growth, the satellite cells divide and their nuclei are added to the fibres, mimicking the processes of muscle growth and regeneration after injury.

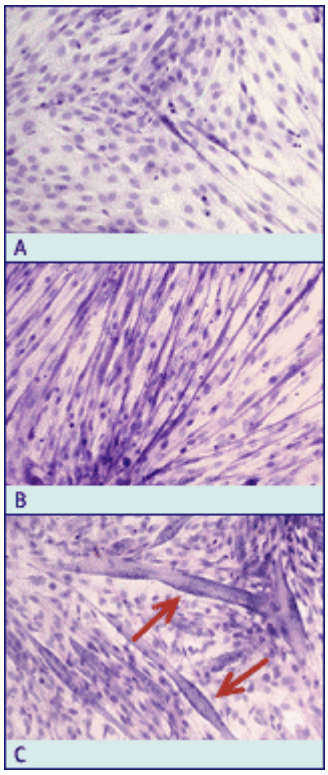

Fig. 7 7 Myotube formation of skeletal muscle cells grown in culture. Cell line C2C12 is used to demonstrate this phenomenon. Heamatoxylin-eosin staining of bouin-fixed muscle cell cultures during several stages of differentiation. After reaching confluence in medium containing 12% fetal calf serum, differentiation is induced by growing the cells in medium containing 2% horse serum. Small myotubes are observed after 3 days in culture (A). By day 7, the number and size of myotubes increases rapidly (B). During the second week of culture, large multinucleated myotubes are formed (C), and spontaneous contraction can be observed. Note the peripheral localisation of the nuclei (arrows). Magnification: x 200 (Photomicrographs provided courtesy of Dr. Karim Sultan).

Future Research

We are currently developing an in vitro model to test the hypothesis that exercise, hormones and growth factors together determine the variations found in growth and fibre composition of skeletal muscle. Two fundamental questions are under consideration. First, do slow and fast muscle fibres respond differently to physical and hormonal stimulation? And second, when during embryonic and post-natal development are the growth and composition of skeletal muscle fibres most affected by these stimuli?

Concluding remarks

The concentration of Na^+,K^+ pumps in the skeletal muscle of cats, dogs, horses and cattle is regulated by mechanisms similar to those described in rodents and man. We already know that hyperthyroidism and physical training increase the number of Na^+,K^+ pumps in skeletal muscle and that hypothyroidism and immobility reduce their number. We know, too, that the rise in Na^+,K^+ pump concentration after training is associated with a blunted rise in plasma K^+ during exercise. However, the question remains whether the mechanism responsible for the up-regulation of the Na^+,K^+ pump during hyperthyroidism is the same as that during training.

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