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Editorial Comment

What is new in musculoskeletal interactions? Muscle oxygenation, myonuclear domain, acupuncture, titin and phosphate

H. Degens[1], J. Rittweger[1,2]

[1]Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, U.K.;
[2]Institute of Aerospace Medicine, German Aerospace Center, Cologne, Germany

Is "just more" enough? Functional anatomy of the muscle cell

(H. Degens)

Researchers, just as bank managers, often start with the idea that more is better. For instance, if someone has the ambition to participate successfully in weightlifting, we prescribe resistance exercise in the expectation that the muscle will grow and as a result becomes better in generating force and power. If, on the other hand, we want someone to be able to do the Hawaiian triathlon we subject such a person to endurance exercise to boost his endurance capacity via, among other factors, the increase in the number of mitochondria and capillaries. Overall, we can say that indeed the improved performance of weightlifters and endurance athletes after their specific training programmes are the result of an increase in muscle mass, or number of mitochondria and capillaries, respectively. It thus seems that the question is settled and a larger fibre size or more mitochondria and capillaries are, as a matter of fact, enough to explain improvements in muscle function and exercise capacity.

Muscle oxygenation and mitochondrial distribution

Given the importance of capillaries and mitochondria for endurance and the size of the fibre for power generation, why does the body then not simply stockpile mitochondria in, and capillaries around, huge muscle fibres? The obvious answer is that the construction and maintenance of structures like myofibrils, mitochondria and capillaries comes at a premium, and if you do not use a structure it is better to reduce its size and hence maintenance costs (and *en passant* the freed building blocks can be used elsewhere). That this does occur is painfully evident after prolonged hospitalisation where the loss of muscle mass and mitochondria can be so severe that the walking ability of patients becomes impaired.

There might also be another reason for not having enormous fibres stockpiled with mitochondria. A recent paper suggests that there is a trade-off between fibre size and mitochondrial content, due to diffusion limitations of oxygen[1]. If this relation holds true, it means that to obtain a highly oxidative muscle, it must consist of small fibres, each with a large number of mitochondria. If, however, weightlifting is required, mitochondrial number can, for argument sake, be zero and the fibres infinitely large. It thus suggests that the oxidative capacity limits the maximal size a fibre can attain. Of course, one might suggest that the oxygen diffusion limitation can be overcome by positioning the mitochondria close to the sarcolemma and in this way minimise the diffusion distances for oxygen. Yet, this poses another problem, as in that case ATP has to diffuse over relatively long distances from the subsarcolemmal mitochondria to the myofilaments in the core of the muscle fibres. In a recent publication, these reaction-diffusion constraints were modelled, and it was suggested that most fibres are not diffusion limited[2]. The paper only considered the density of mitochondria, but not their distribution. That, however, had been the topic of a previous paper by the same group[3] and it suggested that the distribution of mitochondria may impose intracellular diffusion limitations for ATP and metabolites. It thus appears that not only the mitochondrial density but also the distribution of mitochondria plays an important role in adequate maximal aerobic muscle fibre function. Clearly, "just more" is not enough. The qualitative distribution of mitochondria within the muscle fibres may also be crucial.

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Corresponding author: Dr. Hans Degens, School of Healthcare Science, Institute for Biomedical Research into Human Movement and Health, Manchester Metropolitan University, John Dalton Building; Oxford Road, Manchester M1 5GD, United Kingdom
E-mail: H.Degens@mmu.ac.uk

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Muscle capillary supply

When considering the impact of mitochondrial number and distribution on fibre size and function one has also to take into account the capillary supply to the fibre - At the end of the day, it is the capillary network that supplies the oxygen to the mitochondria! It is therefore not surprising that the capillary density in an oxidative muscle or oxidative muscle region is higher than that in a glycolytic muscle or muscle region. It is to be expected that the higher the total number of mitochondria in a muscle fibre (mitochondrial density x fibre size), the higher the number of capillaries supplying that fibre will be. However, no such relationship seems to exist at the fibre level. Size, rather than oxidative capacity, appears to be a more important determinant of capillary supply to a fibre[4]. Even in whole muscle, chronic electrical stimulation of glycolytic fibres can induce angiogenesis without a concomitant increase in oxidative capacity[5]. These observations reflect that simply viewing the capillary bed as a source of oxygen is inadequate, and other functions such as supply of substrates and hormones, dissipation of heat and removal of metabolites should not be neglected. In a recent review the argument is made that 'quality not quantity' of tissue capillary supply is important[6]. It is shown in this review that the heterogeneity of capillary spacing is qualitatively similar, irrespective of whether all anatomically present or only perfused capillaries are considered, between muscles of different fibre type composition. Moreover, the angiogenesis during maturational muscle growth or hypertrophy occurs at such locations that the heterogeneity of capillary spacing is maintained. The positioning of capillaries is, however, limited to the periphery of the muscle fibres and a larger variation in the size of the fibres (e.g. during ageing) within a muscle may be associated with a larger heterogeneity of capillary spacing[7] and impair tissue oxygenation. Again, as for the mitochondria, "just more" is not enough, but also the qualitative distribution of capillaries in a muscle has to be considered.

Myonuclei

In the previous sections it was shown that fibre size was at least partly limited by its mitochondrial content and capillary supply. However, one could also imagine that for the maintenance of a large fibre more proteins have to be produced (and broken down) per unit time than for the maintenance of a small fibre. In other words, the absolute (but not the volume specific!) protein turnover is likely to be larger in large than small fibres. To make this possible, the large fibre must have a larger translational and transcriptional capacity. Translation occurs in the ribosomes, consisting of ribosomal RNA (rRNA), and requires transfer RNA (tRNA) and messenger RNA (mRNA), all transcribed from the nuclear genome. A demand for a higher capacity for transcription in the larger fibres can be realised by either an enhanced transcriptional activity per myonucleus (and/) or a larger number of myonuclei within the fibre. The latter appears to be the case, and a myonucleus seems to have a given cytoplasmic volume or domain associated with it, dubbed the "myonuclear domain"[1,8].

Also during hypertrophy and atrophy it is generally thought that this relationship is maintained. However, recent data have cast doubt on this concept. For instance it was found that hypertrophy in response to overload precedes accretion of new myonuclei[9]. During the first 3 weeks postnatally maturational muscle growth in mice is accompanied with a proportionally smaller increase in the number in myonuclei, followed by a period of muscle growth without any further addition of myonuclei[10]. In analogy with the increase in myonuclear domain size during muscle fibre growth, denervation-induced atrophy was not accompanied by loss of myonuclei, and thus resulted in a decreased myonuclear domain size[11]. Should we cast the whole idea of a constant myonuclear domain size over board then? This is probably too radical, as in general the relationship between the number of myonuclei and fibre size holds true, and, this we must not forget, is modulated by the oxidative capacity of the fibre probably because of their higher protein turnover[1].

Another suggestion is that any myonucleus is normally not working at maximal capacity and has some ability to enhance its transcriptional activity and support a larger domain. However, when a threshold is reached, further fibre growth can only be realised by the addition of new myonuclei, where it might be added that for each transcript this threshold may occur earlier or later. During atrophy the destruction of nuclei is an energy consuming process and preserving myonuclei during atrophy may facilitate subsequent re-growth during reloading. In line with this notion, it has been observed in mice that myonuclei acquired during hypertrophy are not lost for at least 3 months during subsequent denervation-induced atrophy[12]. The authors suggest that this provides the muscle with a sort of 'memory' and explains the attenuated atrophy during disuse and a more rapid regain of muscle mass when subjected again to a hypertrophic stimulus. If this also applies to human muscle, it emphasises the need to start rehabilitation as soon as possible after the initiation of disuse atrophy and/or precede hospitalisation, if possible, with a resistance exercise regime.

Although the oxidative capacity and the nuclei not normally working flat-out undoubtedly explain some of the observed deviations from the nuclear domain and fibre size relationship, it does not explain everything. Given the importance of mitochondrial- and capillary distributions for fibre and muscle function discussed above, could it be that the spatial distribution of the myonuclei within a muscle fibre is also important?

Modelling studies have indicated that the distribution of myonuclei is not random but such that the transport distance between nuclei are minimized[13]. A recent publication shows that while during ageing the nuclear domain size in human muscle fibres largely stays constant, the variability in the size of the domain in type I fibres increases[8]. It is interesting to note that it is particularly type I fibres that show dysfunction during ageing[14]. The authors suggest that the increased variability in nuclear domain size might impair protein turnover in areas of the fibre where the nuclear domain is large, increasing the chance of post-translational modifications of the proteins in that domain and hence cause protein dysfunction. More work needs to be done, but it suggests that also in the case of myonuclei "just more" is not enough, and also the qualitative distribution of the myonuclei within a muscle fibre has to be considered.

Muscle-bone interactions – From acupuncture to phosphate

(J. Rittweger)

Acupuncture – more than just needles and pins?

Are you eager to boost your muscle function? Then you may be interested to use acupuncture – at least this is what could be suggested from some recent research[15]. Currently, acupuncture is mostly used to relieve pain and discomfort. However, as demonstrated in this study, it may also help to enhance voluntary muscle contractions. More specifically, isometric knee extensor strength was increased after acupuncture had been performed at the stomach 36, the spleen 6, the conception vessel 6 and the earpoint 55 points: No such benefits were observed when the needles were placed astray (excluding that the effect was carried by the pricking) or when a 'placebo laser' acupuncture was used. Admittedly, the effects of true acupuncture were moderate (8% increase), it is unclear what mechanisms may be responsible for them, and whether one should explain them in a 'western' or in an 'eastern' set of coordinates. Thinking the western style, however, one might be puzzled by the fact that electromyography failed to show any effect of acupuncture, and that drop jump performance was likewise unaffected – which begs the question whether pain or discomfort, both of which are common problems in 'strength' testing, could not be a reasonable means of explanation.

On eccentric contractions – news from the dark side of muscle physiology

Muscles can contract in different modes: during a concentric contraction, the opposing force is smaller than the maximal force generated by the muscle, resulting in a shortening of the muscle. During an isometric contraction, both opposing forces match each other, and there is consequently no movement. During an eccentric contraction, finally, the muscle is lengthened despite its contraction, just because the opposing force exceeds the force generated by the muscle. Importantly, the force any muscle can develop is smallest during concentric and greatest during eccentric contractions. One should think, therefore, that eccentric contractions are the eyeball of muscle physiologists. Yet the area is under-investigated, and alas, attempts to explain the greater eccentric forces with the usual models of actin-myosin interaction have generally been unsatisfactory. Now, some whole-hearted researchers propose that during stretching the actin-myosin interaction gets boosted by titin in order to develop these high forces[16]. Titin is one of the largest proteins in our body, and it spans the entire sarcomere (=contractile unit) in skeletal muscle. Titin has to date been thought of as a merely passive mediator of stiffness. However, as the authors demonstrate convincingly, titin-related stiffness during stretch can be enhanced by actin-myosin interaction, and the actin-myosin contractile state seems to exert a "memory" effect: the greater the actin-myosin related force at the onset of the stretch, the greater the "amplification" by titin. This novel finding clearly has potential for clinical spin-off. Hence, researchers with an interest in muscle bone interactions might well put the terms "titin" and "eccentric" on their list of automated Pubmed searches – without risking to be too eccentric themselves.

Muscle bone hypothesis – revisited

A propos muscle-bone interaction: a recent study reports that bone and muscle mass are lost in conjunction after stroke[17], confirming the suggestion that skeletal integrity requires healthy muscles. Moreover, a recent publication by Swift et al.[18] could turn into a cornerstone paper for muscle-bone fans. In their laborious and instructive experiment, the authors have tested whether forceful muscle contractions can rescue bone in the classical hind limb unloaded rat model[19]. In this model, rats are suspended by their tail, and thus their hind limbs are lacking ground contact. They can still contract their hind limb muscles, but probably with only little force generation, as they cannot use body mass as a resistor. In the elegant study by Swift et al., however, forceful muscle contractions were evoked by electrical stimulation in a custom-built dynamometer, whilst the rats were anesthetized. Two different contraction regimens were tested; one with eccentric contraction only, and another with a combination of isometric and eccentric contractions. Lo and behold, both contraction regimens were able to not only prevent the loss of tibia bone mineral content (BMC), but even increased it above the values seen in control. So, does this study confirm the much-debated "muscle-bone" hypothesis, namely that bones adapt to muscular forces? Well, not entirely, as the increase in BMC and bone strength in this study was occurring without any increase in peak isometric torque during electrical stimulation[18]. Unfortunately, peak eccentric torque values, which must be expected to be greater than isometric torque values, have not been reported. The jury is therefore hung to further study this highly interesting model.

Can exercise lead to accelerated ageing?

A number of culprits are commonly held responsible for the negative effects of age upon muscle – besides the aforementioned distribution of mitochondria and myonuclei within and capillaries around fibres, 'telomere length' ranks high on the usual lists of suspects. The idea behind the latter is that telomeres, i.e. the caps that shield the ends of chromosomes, get shorter with each cell division and run out of length towards the end of our life. This would then, somehow, engender the notorious ageing effects. Past research had focussed on telomere length in blood cells[20], which is probably not very informative about the ageing effects in muscle. This is now changed with a recent publication that has studied telomere length in cells from the vastus lateralis muscle from both runners and sedentary people[21]. In keeping with previous research, and probably to the dislike of the researchers, the telomeres did not become shorter with age. Quite upsettingly, however, telomere length was negatively related to the years spent in running. So, does this mean that exercise shortens the life span? Not necessarily. If age shortens telomeres, then one would expect runners to have shorter telomeres than sedentary people. However,

such difference was not found, which actually makes it hard to understand why an age effect was found at all within the runners. And more importantly, even if the proposed relationship could be established, it would still remain unclear what the implications of a somewhat reduced telomere length are for muscular function. Hence, there is no need to feel bad about a little run once in a while – at least not yet.

Phosphate – friend or foe?

Bone researchers agree that sitting on your couch and drinking coca cola is bad for your bones – the couch because of the lack of exercise, and the coke because of its high phosphate content. Actually, phosphate is not always all that bad, as has now been demonstrated in a rat model for vitamin D deficiency[22]. Muscle pain and muscle weakness are well-known grievances in that clinical disorder, but it is unknown what the mechanisms are behind these symptoms. The potential effects of vitamin D on muscle are widely appreciated[23], but recent data suggest that the lack of vitamin D itself or low calcium levels are probably not the primary cause (Testerink, Degens et al., submitted). In their study Schubert & de Luca now elegantly demonstrate that muscle function can be rescued by phosphate substitution even in the presence of vitamin D deficiency[22]. Whether correction of hypophosphataemia would also rescue rickets-related muscle symptoms, be it by means of milk, coke or pills, should now be established in clinical studies.

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