

What is new in neuro-musculoskeletal interactions?

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Training the ‘mighty’ mouse

There is a rapidly increasing interest in musculoskeletal interactions, and former antagonists are rapidly converting into JMNI-protagonists. Evidence for this notion comes, amongst others, from the study of genetic mouse models. Among the plethora of such models, the myostatin knockout mouse has become popular as the ‘mighty’ mouse – its muscles are about twice as large as in the wild type. However, regarding bone, the increased muscle mass in the mighty mouse did at first not ‘appear to induce a systematic increase in diaphyseal strength or axial rigidity’¹. At the time when this study was published, Harold Frost felt urged to write an epistle and send it both to his disciples and to the authors. The latter was then partly amalgamated into the first ever ‘What is New’ in the JMNI². The authors have since undertaken further studies into the muscle-bone interactions in the mighty mouse. These studies yielded results that were in direct contrast to the former findings, leading to the author’s recognition that ‘muscle strength is an important determinant of bone density in both the axial and the appendicular skeleton of rodents’³.

In a recent study, the same researchers have now addressed the fundamental criticisms to the study of muscle-bone interactions in genetic models – which is the mechanical usage of muscles. Just growing bigger muscles does not automatically imply that they will also be used more forcefully. Myostatin-deficient mice and wild type controls were exercised for 30 minutes per day in a treadmill⁴. Comparing the two strains, Hamrick et al. demonstrate quite convincingly that treadmill running leads to a loss of muscle mass in the myostatin knockouts, but has the opposite effect in the wild type mice. Moreover, ‘increases in bone strength with exercise are greater in the myostatin-deficient mice than in

wild-type mice, suggesting that the combination of increased muscle mass and physical activity has a greater effect on bone strength than either increased muscle mass or intense exercise alone’⁴. In simple words: just having big muscles is not enough, you also have to use them.

The question arises as to how the conflicting results and interpretations emerged. All studies were in the same model, with virtually identical methods, applied by (very much) the same authors. Noteworthy, the first study (with no effect on bone) was in mice 9-10 months old, which is quite old for a mouse. The more recent work, however, relied upon animals that were 3 months old (i.e., adult animals). This might suggest that strong muscles can help to build strong bones in young animals, but that this does not imply an improvement of bone strength in old age.

Can you hear me?

Falls and subsequent fractures are a major challenge to our societies. Help might come from an artificial neuro-skeletal interaction, which has recently been developed⁵. Apparently, all that is needed to improve static balance is a set of earplugs and an accelerometer⁶. The accelerometer, attached close to the body’s centre of mass, gives feedback via some earplugs as to the instantaneous acceleration of the body. The sound signal is altered differently in response to accelerations in the frontal plane and in the sagittal plane. Surprisingly, just one minute of familiarisation seems to be enough in order to reduce the body sway in patients with bilateral vestibular loss. A closer look reveals that, according to the measures obtained during static balance testing, patients could largely compensate their deficits by the audio-feedback system. This is a promising development for future application in the elderly. If it can really reduce the risk of falls, then we might see quite as many ‘ear-plugged’ older people in the future as we see young kids nowadays – obviously with a different kind of ‘music’ turned on.

Building bodies

To many people, it may seem quite obvious that body builders differ from ‘ordinary’ people. A closer look by Bot-

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tinelli's group has substantiated that notion and obtained results that challenge an old dogma of muscle physiology⁷. As textbooks have it, training can never induce hyperplasia^{8,9}. In other words, according to current belief, increases in muscle mass in adults are only possible through enlargement of the single muscle fibres, but not by growing more fibres. In contrast to that, comparing the thigh muscle volumes (assessed by MRI) and the fibre cross-sections (from vastus lateralis biopsies) of male body builders and control subjects, D'Antona et al. elegantly conclude that the increase in muscle size cannot be solely explained by hypertrophy of the single fibres⁷. Instead, the authors suggest that, indeed, hyperplasia is a cause of the body-builder's phenotype.

The underlying problem is more than an academic question. There is quite some evidence that the age-related changes of skeletal muscle are partly due to fibre hypoplasia¹⁰, i.e., a loss particularly of fast twitch fibres. Importantly, body building enhances the number, the size and the function of these fast twitch fibres. The recent publication might hence show another mechanism by which resistive exercise can help to counteract the effects of age on the neuromuscular system.

However, the presented evidence is quite indirect. Unfortunately, the authors did not assess muscle architecture and therefore had to rely on some breathtaking mathematical manipulations in order to work around that, which might have biased the results by group differences in anthropometric characteristics, such as body height. Therefore, it may be cautious not to scrap your textbooks immediately.

The white spots on our maps of disuse

The study of bone loss turns out to be one of the favourite topics in the JMNI. Indeed, there is an accumulating knowledge about it, but some important white spots remain on our knowledge maps. Two of them have been addressed in a recent publication by Basso et al., namely the role of progenitor cells and the degree to which bone loss is recovered¹¹. In a rat model of 14 days of tail suspension, the authors found that osteoprogenitor cells (i.e., the cells from which osteoblasts arise) reacted in the expected way: their number went down during suspension, the latter effect being in proportion to the reduction in bone volume. A bit surprisingly, however, the number of osteoclasts was not changed, neither was the surface that they covered. Potentially, this could be due to the young age of the animals (6 weeks) and the fact that they were still growing. Interestingly, the decrease in bone volume and osteoprogenitor activity was almost completely recovered after 14 days.

This is an important study, as it clearly shows that cells upstream in the lineage of osteoblasts, and not (only) osteoblasts themselves, are involved in the bone's response to disuse.

Calcium homeostasis without remodelling

A look into the textbooks of medicine suffices to assure us that bones fulfil their role in calcium homeostasis by the

process of remodelling, which encompasses bone resorption by osteoclasts and bone formation by osteoblasts. However, such simplistic view has been repeatedly challenged^{12,13}. The criticisms were, among others, based on the observations that ions are exchanged between bone and the extracellular fluid (ECF) at a greater rate and in greater volume than is explicable by osteoclastic and osteoblastic activities alone, and that the exchange could be observed even in the absence of these cells. Now, for the first time, ion exchange between the ECF and the Bone ECF (BECF) has been measured directly in what may become a classical study¹⁴. Calcium exchange was much faster from holes that were drilled into mouse metatarsals than from the undamaged bone envelope. The steady influx of calcium into the bone was found to be a passive process and dependent on calcium plasma levels. Conversely, by the addition of Na-cyanide and subsequent blocking of cellular metabolism, the flux from the intact envelopes ceased within minutes, but not from the severed surfaces. The authors infer from this that the calcium efflux, which balances the constant influx, is an active process, most likely carried out by the syncytium made up by lining cells, osteoblasts and osteocytes. It can only be hoped that these processes will be understood in detail in the not too distant future, and that the textbooks will be changed as soon as possible.

Microdamage: the tiny huge killer

As the JMNI reader may recall, Harold Frost suspected that the inability to repair microdamage (and thereby material fatigue) is a key mechanism for the emergence of "true" osteoporosis (p. 249 in¹⁵). Well, if this is so, there seems to be good news for the scientific community. So far, microdamage¹⁶ could be assessed only histologically and thereby *ex vivo*. Now, David Burr's group has devised a technique that seems to be sensitive and specific to spot newly formed microdamage¹⁷. The technique is based on positron emission tomography (PET), a method with unlimited applications and expenses. Here, a labelled fluoride was used, which is naturally incorporated into the extracellular bone matrix. Accordingly, a PET signal was picked up in fatigue-loaded rat ulnae, which coincided well with the histological and autoradiographic evidence of microdamage. As the authors emphasize, the fact that rats normally do not have intracortical remodeling is a strength of this study, as the newly emerged microdamage could not be mistaken with already existing ones. For the future, however, one might wish that this technique can be demonstrated not only to detect newly formed microdamage, but also old microdamage that has not been repaired – which is the supposedly important mechanism for osteoporoses in the Frostian sense. Then, whoever can afford it may well decide to have a PET-bone scan in order to check for osteoporosis.

The notion that this might be a good idea is further substantiated by an interesting related article¹⁸. Research in the past couple of years has shown that microdamage actually

comes in two forms, linear microcracks and diffuse microdamage. Linear microcracks are more likely to occur in compression and emerge during a later stage of fatigue loading. Conversely, diffuse microdamage forms in tensile loading, its propagation is thought to be self-limited, and it absorbs more energy than linear microcracks. It can therefore be said that linear microcracks are particularly harmful with regards to fatigue fractures. Now, it has been elegantly demonstrated by fatigue loading experiments that cortical bone samples from old donors accumulate mainly linear microcracks, whereas bone from young donors is more prone to diffuse microdamage¹⁸. This suggests that the material's ability to resist fatigue deteriorates with age.

Muscle perfusion at old age

As some readers may notice in themselves, increased bone fragility is not the only unpleasant thing about ageing. A deterioration of locomotion and the inability to lead a self-sustained life are other hallmarks of it. The underlying causes are a loss of muscle power and the inability to sustain any given power output (=endurance). As a recent study shows, this might very well be an effect of limited blood supply, and it could be explained by an inability to counteract sympathetic vasoconstriction during exercise (=sympatholysis)¹⁹. That particular study investigated the effects of tyramine and phenylepinephrine, i.e., sympathetic vasoconstrictor drugs during rhythmic handgrip exercise. It was found that, unlike in young men, muscle contractions did not hamper the drug-induced vasoconstriction in older men (~65 years). These results are remarkable, as they pinpoint to a mechanism within the muscle. If inappropriate muscle perfusion during exercise really is relevant to the locomotory impairment observed at old age, then the search for the mechanisms involved in sympatholysis (e.g., NO, K⁺, prostaglandins etc.) may reveal a promising target for future pharmacological interventions.

Growing old

There are an increasing number of theories trying to explain why and how we age. One theory that has recently received quite some attention suspects a central mechanism of cellular senescence to lie in the shortening of telomeres.

Telomeres are the caps of our chromosomes. It is believed that they contain no meaningful information themselves, but rather get shorter each time that the cell divides and thereby protect the rest of the chromosomes from erosion. Therefore, in all cells except tumor cells, telomeres could indeed serve as cellular clocks. For the first time, experimental evidence for this theory has now been presented in over 500 female twin pairs between 18 and 76 years of age²⁰. Apparently, in this population, the rate at which telomeres shorten is increased in smokers and obese women. As the authors calculate, smoking one package of cigarettes per day accelerated telomere shortening by 18%. Possibly, the increased

shortening may be due to the oxidative stress that is associated with obesity and with smoking.

All this looks like a sound story. However, there is no evidence as to how telomere shortening *per se* would cause the cellular deficits that we usually observe with ageing. Moreover, in the present study, telomere length declined from 8,000 base pairs at age 20 to about 6,500 base pairs at age 80, thus leaving more than 80% of the telomeres and certainly the rest of the chromosomes untouched. It may therefore be that telomere shortening constitutes more of a theoretical, rather than a practical limit to the lifespan of our cells. The latter notion is underpinned by a recent study that compared the survival in 812 people older than 73 years of age²¹. In that study, there was the expected association found between age and telomere length, but no relation could be established between survival and telomere length. However, this should not be mistaken as a proof that telomere length is not mechanistically involved in the ageing process, as ageing has a number of consequences other than death.

References

1. Hamrick MW, McPherron AC, Lovejoy CO, Hudson J. Femoral morphology and cross-sectional geometry of adult myostatin-deficient mice. *Bone* 2000; 27:343-349.
2. Rittweger J, Rauch F. What is new in neuro-musculoskeletal interactions? *J Musculoskelet Neuronal Interact* 2000; 1:171-176.
3. Montgomery E, Pennington C, Isales CM, Hamrick MW. Muscle-bone interactions in dystrophin-deficient and myostatin-deficient mice. *Anat Rec A Discov Mol Cell Evol Biol* 2005; 286:814-822.
4. Hamrick MW, Samaddar T, Pennington C, McCormick J. Increased muscle mass with myostatin deficiency improves gains in bone strength with exercise. *J Bone Miner Res* 2006; 21:477-483.
5. Chiari L, Dozza M, Cappello A, Horak FB, Macellari V, Giansanti D. Audio-biofeedback for balance improvement: an accelerometry-based system. *IEEE Trans Biomed Eng* 2005; 52:2108-2111.
6. Dozza M, Chiari L, Horak FB. Audio-biofeedback improves balance in patients with bilateral vestibular loss. *Arch Phys Med Rehabil* 2005; 86:1401-1403.
7. D'Antona G, Lanfranconi F, Pellegrino MA, Brocca L, Adami R, Roissi R, Moro G, Miotti D, Canepari M, Bottinelli R. Skeletal muscle hypertrophy and structure and function of skeletal muscle fibres in male body builders. *J Physiol* 2006; 570:611-627.
8. Morpurgo B. Über die Aktivitäts-Hypertrophie der Willkürlichen Muskeln. *Virchows Arch Pathol Anat Physiol* 1879; 150:522-554.
9. Gollnick PD, Timson BF, Moore RL, Riedy M. Muscular enlargement and number of fibers in skeletal muscles of rats. *J Appl Physiol* 1981; 50:936-943.
10. Rauch F, Rittweger J. What is new in neuro-muscu-

- loskeletal interactions? J Musculoskelet Neuronal Interact 2005; 5:393-396.
11. Basso N, Jia Y, Bellows CG, Heersche JN. The effect of reloading on bone volume, osteoblast number, and osteoprogenitor characteristics: studies in hind limb unloaded rats. Bone 2005; 37:370-378.
 12. Talmage RV. Perspectives on calcium homeostasis. Bone 2004; 35:577-578.
 13. Parfitt AM. Misconceptions (3): calcium leaves bone only by resorption and enters only by formation. Bone 2003; 33:259-263.
 14. Marenzana M, Shipley AM, Squitiero P, Kunkel JG, Rubinacci A. Bone as an ion exchange organ: evidence for instantaneous cell-dependent calcium efflux from bone not due to resorption. Bone 2005; 37:545-554.
 15. Frost HM. The Utah Paradigm of Skeletal Physiology. ISMNI, Athens, Greece; 2004.
 16. Frost HM. Presence of microscopic cracks 'in vivo' in bone. Henry Ford Hospital Medical Bulletin 1960; 8:25-35.
 17. Li J, Miller MA, Hutchins GD, Burr DB. Imaging bone microdamage *in vivo* with positron emission tomography. Bone 2005; 37:819-824.
 18. Diab T, Condon KW, Burr DB, Vashishth D. Age-related change in the damage morphology of human cortical bone and its role in bone fragility. Bone 2006; 38:427-431.
 19. Dinunno FA, Masuki S, Joyner MJ. Impaired modulation of sympathetic alpha-adrenergic vasoconstriction in contracting forearm muscle of ageing men. J Physiol 2005; 567:311-321.
 20. Valdes A, Andrew T, Gardner JP, Kimura M, Oelsner E, Cherkas LF, Aviv A, Spector TD. Obesity, cigarette smoking, and telomere length in women. Lancet 2005; 366:662-664.
 21. Bischoff C, Petersen HC, Graakjaer J, Andersen-Ranberg K, Vaupel JW, Bohr VA, Kolvraa S, Christensen K. No association between telomere length and survival among the elderly and oldest old. Epidemiology 2006; 17:190-194.