What is new in Musculo-Skeletal Interactions

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Abstract

In this new column of the JMNI, we will give a six-monthly overview about new publications which, to our eyes, are interesting and relevant. Keeping in line with the ‘spirit’ of ISMNI, we take the freedom to discuss with a more holistic approach and most importantly, we encourage feed-back from the readers.

Muscle, Muscle & Bone, Motor Control, Exercise (J. Rittweger)

Muscle

A look at the latest editions of our textbooks of physiology teaches us that the world is simple. α-motoneurones release acetylcholine and thereby make the muscles contract. Diabetes is a disease in which insulin production or effectiveness is insufficient. A couple of recent publication makes perceivable a more holistic concept of the relationship between muscle contraction (i.e. mechanical power) and regulation of glucose uptake (i.e. chemical power delivery) by the organism’s prevailing consumer of glucose, the muscle.

Ariel Zisman and co-authors show that it is mainly the glucose transporter 4 (GLUT4) of the muscles which plays an important role in glucose tolerance¹. It had been known before that the rate-limiting step in muscle glucose use is the transmembrane transporter GLUT4. Transport of glucose by GLUT4 can be stimulated both by plasma insulin and by contraction or exercise. Through the work of Zisman et al, we can appreciate the role of skeletal muscle in the homeostasis of plasma glucose.

The investigators developed a rat model in which GLUT4 was selectively non-functional in skeletal muscle after birth. As a consequence, the animals developed glucose intolerance, hyperglycemia, and hyperinsulinemia, that was resistant to insulin and muscle contraction/exercise. GLUT4, the ‘bottle neck’ in glucose uptake, seems to be influenced independantly by contraction and insulin, via different pathways. A possible mechanism that may explain the contraction coupled increase of glucose uptake is by the co-transmission of adenosin from the motor endplate. Interestingly, adenosin A₂A and A₁ receptors have now been found to be expressed on the muscle cell membrane².

The above results are interesting in several ways with respect to neuro-muscular interactions. Firstly, they increase our knowledge about the mechanisms that let the muscles adapt to their usage. Athletes, for example, have an increased capacity of glucose uptake. Secondly, they again indicate that the inhibited utilization of glucose by muscle is a crucial point in the development of diabetes mellitus (and not hypo-insulinemia alone). In support of this view, it has been known for some time now, that the expression of GLUT4 decreases with age³. And thirdly, they permit us to speculate about mechanisms that cause the muscles’ power output to decline in the elderly: Is it possible that in some of our sedentary patients with unimpaired circulation, respiration and ‘normal’ mechanical power of their muscles have an impaired flow of glucose towards their muscle? Is there a ‘hidden’ group of patients, who suffer from unrecognised muscular dysfunction, but are currently treated as primarily diabetic? The lack of knowledge with respect to these questions is illustrated by a recent publication in which the correlation between fibre type composition and glucose sensitivity is significant, but remains poor (r = 0.33) even if adjusted by adequate, refined statistical tools⁴. New studies are required that compare energy flow, mechanical power production and GLUT4 activity to clarify these issues.

Muscle & Bone

Myostatin is a secreted growth and differentiation factor, which is expressed in skeletal muscle. It is believed to
function as a general ‘negative regulator’ of skeletal muscular growth. This view is based on the observation that in myostatin knock-out mice individual muscles weigh about 2 to 3 times more compared to wild-type littermates. However, body weight is increased by about 30% only suggesting that not all muscles are abnormally heavy. Hamrick et al. have investigated this interesting model with respect to the muscle and bone relationship. It is reported that hindlimb muscle mass of myostatin null mice is approximately double that of weight-matched controls. With respect to bone, however, there was no difference in cross sectional area or moment of resistance in the femoral midshaft or near the third trochanter. The only difference was that the knock-out mice had a higher polar moment of inertia and ‘bone strength index’ near the trochanter. The authors conclude that in this model, muscle gain ‘does not appear to induce systematic increases in diaphyseal strength or axial rigidity’. As pointed out by Charles Turner in an accompanying editorial, there actually was no reason to expect such an increase in diaphyseal strength, because the knock-out mice and controls did not differ either in body weight or lifestyle and therefore most probably put similar loads on their bones. Thus, since no information is given about the forces actually exerted to the bones, the very exciting data of Hamrick et al. do not necessarily conflict with Frost’s theory of a ‘mechanostat’. It would certainly have been worth while to study whether all the muscles, i.e. also the other muscles of the leg were 100% heavier in the myostatin knockout mice. The simple fact that the total body weight was indistinguishable from that of the controls suggests a negative answer. It also would be very interesting to see if the surplus of musculature in the myostatin knockout mice is functional, and what the strain-levels are in their bones.

Motor Control

Motor control and probably most central nervous functions are not hard wired, but rather undergo adaptive changes throughout life. The term plasticity has been coined for this observation. Plastic changes of the cerebral cortex may be clinically assessed within 10 days as demonstrated by Weiss et al. The observation was made in a single patient, who had lost his right middle and ring finger in an accident. When applying the technique of somatosensory evoked magnetic fields (SEF) ten days after this accident, differences between the right and left cortical representation of the hands were found, that could not be explained on basis of natural variance.

Proprioception as the afferent stream of information about body and limb posture is an absolute prerequisite of gait and balance. In two articles, the group of Edith Ribot-Ciscar demonstrated, that ensembles of spindle afferents encode two dimensional proprioception, both in active and in passive movements of the foot. These findings extend our understanding of how proprioception and motor activation are represented in the cortex namely rather in terms of axes of movements than in terms of single muscles. While this view is not new, the demonstration that such type of information coding takes place at a peripheral level probably is.

An interesting paper comes from Earhart and Bastian. In a kinematic study in 15 human subjects, the authors found that in traversing wedges with a single step, the gait pattern changed from 10° to 20° inclination of the wedge, with a transition zone at 15°. Of course, different people have different gait patterns, and that makes quantitative gait analyses difficult in clinical practice. The present scientific work, however, may give us a clue of how to handle inter-individual variability by defining reproducible test protocols.

Gladden et al. have published results from an immunohistochemic study, showing noradrenergic (inhibitory) and serotonergic (facilitatory) varicosities that impinge on γ-motoneurones. γ-motoneurones are believed to adjust the set-point in the monosynaptic spindle reflex, i.e. they control muscle length and shortening velocity. While noradrenergic impingement on the α-motoneurones has been reported for a while, this new finding relates vegetative control and behavioural patterns to the control of muscle function. This may explain altered motor function in excited and agitated patients.

Exercise

The Olympic games of Sydney are just over, but research on sports and exercise continues in revealing new knowledge. In male rats, Harjola and colleagues have found that loss of muscle mass and fibre composition is not distinguishable in hindlimb immobilised male rats, whether orchiectomised or not, orchiectomised and substituted to reach a plasma testosterone concentration of 10-fold the normal value. Yao et al. have developed a new cage that forces the rat to raise on its hindlimbs during feeding. This implies some ‘extra’ exercise compared to the controls crouching in the regular cages which as a consequence led to an increase in muscle mass. If animals were orchiectomised, the bipedal stance feeding partially prevented cancellous bone loss in the proximal tibial metaphysis, and it totally prevented net bone loss in the tibial shaft by inducing periosteal bone formation. Taken together results indicate that anabolic steroids, although highly effective only work if combined with exercise.

Another interesting publication is from Robling, Burr & Turner. The authors subjected the right tibia of female rats to 360 loading cycles on 3 days, partitioning the cycles into 1, 2, 4, and 6 bouts. The observed ‘osteogenic response’, i.e. midshaft endocortical bone formation, was enhanced in the animal groups with fewer repetition cycles per bout. The largest difference was observed when data from exercise groups were pooled and compared to the control group. This is in line with the older work of Umemura et al., who reported that a single trial per day was enough to induce bone formation. These very appealing data may provide an important clue on how to optimise the design of ‘osteogenic’
exercise. Likewise valuable data are presented by Iwamoto and colleagues\textsuperscript{16}. These investigators have compared the effects of conditioning by treadmill running and subsequent deconditioning in female rats. Surprisingly to the authors, those rats that passed 4 weeks of a ‘sedentary lifestyle’ lost all of the benefits that they had gained during 8 weeks of exercise, and they were basically indistinguishable from animals that were sedentary during the entire 12 weeks of observation. One should keep in mind, that the rats of this study were growing, and hence that modelling was prevailing. In contrast, the major problems in human bone loss come from remodelling. Possibly due to this circumstance, Bass et al. have found that retired female gymnasts seem to profit in later years from their so-called ‘peak bone mass’\textsuperscript{19,20}. Nevertheless, Iwamoto’s data might provide interesting news for those who believe, ‘peak bone mass’ has an influence per se on the bone state in the later periods of life.

**Bone Metabolism, Endocrinology, Clinical Care (F. Rauch)**

**Bone Metabolism – New Concepts, More Molecules**

Several authors have recently presented models of the remodeling process, which are surprisingly similar among each other\textsuperscript{21-27}. All agree that remodeling is coordinated by osteocytes, that osteocytes translate mechanical information into biological signals and that these signals are transmitted to the bone surfaces, where they determine the actions of lining cells, osteoblasts and osteoclasts.

Martin’s ‘unifying theory of bone remodeling’ assumes that bone lining cells are inclined to activate remodeling unless they are restrained by an inhibitory signal\textsuperscript{21}. He proposes that osteocytes send out such an inhibitory signal when the bone is mechanically strained. This would explain, why remodeling activity is low under physiologic loading conditions, but why it increases in disuse. The same model could account for the observation that remodeling is also increased when bone is subjected to excessively high strains: The resulting microcracks would sever the lines between osteocytes and lining cells and thus cut off the inhibitory signal.

Huiskes et al. translated a virtually identical model into mathematical equations and conducted computer simulations using finite element analysis\textsuperscript{22}. This simulation correctly reflected the adaptation of trabecular orientation according to the direction of the prevailing strains. When applied to several clinically relevant situations - disuse, strenuous exercise and increased remodeling activity in menopause - the model predicted changes in trabecular bone mass which matched well with the actual findings.

Smit et al. present a finite element analysis of strain distribution in cortical and trabecular remodeling sites\textsuperscript{23}. Assuming a longitudinal load causing a deformation of 1000 microstrain, the model predicted that there was an area of decreased strain at the cutting cone of a nascent osteonal canal, whereas elevated strain levels were present just behind that area. The model of a trabecular remodeling site revealed increased strains at the bottom of a Howship’s lacuna, but decreased strain levels appeared in the direction of loading. Thus, in both the cortical and the trabecular model, low strains were predicted at sites where osteoclasts are found in vivo, whereas high strain levels were calculated for locations of osteoblast action. The authors therefore conclude that their computer model is consistent with the hypothesis that the coupling between osteoclasts and osteoblasts during remodeling is a strain-regulated phenomenon.

Remodeling research was not limited to theories and computer simulations, but also produced experimental data. Verborgt et al. tested the association between microdamage and osteocyte apoptosis\textsuperscript{24}. Ulnae of adult rats were subjected to fatigue loading in vivo to produce matrix damage. Osteocyte apoptosis was assessed by TUNEL staining and by morphologic criteria on histological sections of the bones. Within a day after loading, the number of apoptotic osteocytes was significantly increased in areas containing microcracks. Following Martin’s ‘unifying theory’ outlined above, osteocyte apoptosis should increase remodeling activity. In fact, the authors observed increased osteoclastic resorption in areas with elevated numbers of dead osteocytes.

It is interesting to compare these concepts and analyses of bone remodeling to two recent studies of transgenic mouse models, which - according to an accompanying editorial – also dealt with remodeling\textsuperscript{25-27}. In these studies two members of the same family of transcription factors, Fra1 and Delta-FosB respectively, were over-expressed in osteoblasts (and other cell types). In both cases progressive osteosclerosis of postnatal onset was reported. Histomorphometric analyses of trabecular bone at the distal femoral and proximal tibial metaphyses showed increased bone formation parameters and normal osteoclast indices. Detailed in vivo and in vitro experiments were performed which all supported the hypothesis that the osteosclerosis was due to increased bone formation rather than impaired bone resorption. In both reports it is speculated that the findings might open new avenues for therapeutic interventions.

While these two articles present superb examples of state-of-the-art molecular bone biology, it is also interesting to note what was not studied. Given the remodeling concepts discussed earlier, one might expect that mechanistic analyses would focus on the osteocyte. Yet, experiments were limited to osteoblasts and osteoclasts. It was not clarified which tissue-based mechanism – modeling, remodeling or something else? – was responsible for the increase in trabecular bone mass. Finally, bone function was not considered. No data are presented on whether the transgenes made the bones stronger or weaker, and periosteal bone formation - a key mechanism determining bone strength - was not analyzed. Why these topics were considered to be non-issues is a matter of speculation. These observations highlight the importance of JMNI’s mission: to
promote the flow of ideas between neighboring fields in musculo-skeletal research.

**Endocrinology**

Schoenau et al. investigated the changes in musculo-skeletal interaction during puberty\(^3\). Peripheral QCT was performed at the proximal forearm to study the relationship between the cross-sectional area of the radial shaft cortex and the cross-sectional area of the muscle at the same site. A close correlation between these two parameters was found in a group of 318 subjects from 6 to 22 years of age. Prepubertal boys and girls had a similar relationship between cortical area and muscle area. In contrast, girls had significantly more cortical area relative to muscle area than boys after pubertal stage 3. This was entirely due to smaller marrow cavities in girls, while the relationship between external bone circumference and muscle area was identical in the two sexes. These findings are in accordance with Frost’s hypothesis that estrogen lowers the mechanostat set-point for bone next to marrow\(^3\).

Interestingly, similar observations were made by Wang et al. using different methods\(^3\). They studied 304 healthy children from 6 to 18 years of age. Total body muscle mass was estimated from total body potassium (measured by whole body 40K counting) and dual energy x-ray absorptiometry was used to assess total body calcium. While in boys the ratio between total body potassium and calcium remained constant at all ages, this ratio decreased in pubertal girls. This again suggests that during female puberty more bone is gained relative to muscle mass than during male puberty. These two papers are independent confirmations of earlier reports from Schiessler et al.\(^31\) and Ferretti et al.\(^32\). Thus it can now be considered an established fact that postpubertal girls and young women have more bone relative to their muscle mass than postpubertal boys and men.

Myopathy is a well known feature of osteomalacia due to vitamin D deficiency. A report from Glerup et al. demonstrates that muscles may actually be more sensitive to hypovitaminosis D than bones\(^3\). They studied 55 veiled Arab women living in Denmark who had low serum levels of 25OH vitamin D but normal serum calcium concentrations. Maximal isometric quadriceps force in these women was by a third lower than in a healthy Danish control population. After three months of therapy with a high dose vitamin D regimen, quadriceps force had increased by 14%. During the same time, maximal quadriceps force in single twitch stimulation testing increased by 20%. Baseline muscle function was affected to a similar degree in women with and without biochemical signs of bone involvement, as judged by serum alkaline phosphatase levels. This suggests that muscle weakness precedes bone disease in hypovitaminosis D. The authors hypothesize that the effect of vitamin D therapy on the muscle system could also explain the decreased hip fracture rates in elderly women who are treated with vitamin D.

**Clinical Care and Rehabilitation**

Hip fracture is the most serious complication of osteoporosis, often resulting in severe disability or death. Fox et al. longitudinally followed the changes in areal bone mineral density and muscle mass occurring after hip fracture in 205 elderly women with a mean age of 81 years\(^3\). Dual energy X ray absorptiometry was used to determine total lean body mass as an indicator of muscle mass. Within two months after fracture total lean body mass had decreased by 6%, which in healthy women is about the loss expected to occur within a period of 20 years. After this rapid initial drop, total lean body mass remained about stable until the end of the follow up period (one year after fracture). While the decrease in areal bone mineral density of the whole body, at the femoral neck and the intertrochanteric area was also most rapid in the first two months after the fracture, it continued throughout the study interval. As discussed in the article, one may be surprised about the finding that areal BMD decreased even after the women had returned to an ambulatory state. However from the viewpoint of musculo-skeletal interaction, such a downward adaptation of the bones to the decreased muscle mass (and thus force) does not come unexpected. The authors conclude that interventions which aim at a preservation of muscle and bone after hip fractures need to take place shortly after the fracture.

Loss of bone mass is a serious complication after spinal cord injury, frequently leading to fractures of the distal femur and proximal tibia. This problem was investigated in two studies which arrive at different conclusions. Dauty et al. present bone mineral content data from 31 men aged 18 to 60 years who had sustained a spinal cord injury 1 to 27 years earlier\(^3\). Bone mineral content in sublesional parts of the skeleton was 41% lower than in the corresponding skeletal regions of healthy controls. In contrast, supralesional bone mass was preserved, and the paraplegic subgroup even had a slightly increased bone mineral content of the humerus. Bone mineral content of the lower limbs and the lumbar spine was not associated with the duration of daily passive verticalization and sitting, respectively. The authors apparently had assumed that simple weight bearing would stimulate bone modeling in these patients. Consequently, they arrived at the somewhat surprising conclusion that their data reflected the direct effect of the neurological lesion on bone mass and the absence of influence of mechanical stress.

Bélanger et al. demonstrate that in fact far more vigorous strains than simple weight bearing are necessary to increase bone mass in such patients\(^3\). They describe the effect of functional electrical stimulation in 14 spinal cord injury patients aged 23 to 41 years who were 1 to 23 years post-lesion at the start of therapy. Baseline areal bone mineral density of the mid tibia, proximal tibia and distal femur was 26 to 44% lower than in healthy controls. Subsequently, one hour training sessions were held 5 days per week for 24 weeks. Training consisted of 5 sec on / 5 sec off stimulation cycles of both quadriceps muscles, corresponding to 720
contractions per session. The right quadriceps contracted against gravity, whereas extension of the left leg was resisted by an isokinetic load. At the end of the study period, the distal femur and the proximal tibia had recovered nearly 30% of the difference to the control levels. Not surprisingly, there was no effect of quadriceps stimulation on the tibial midshaft areal bone mineral density. Contracting against resistance or against gravity yielded the same result as far as bone mass was concerned. However, resistance training appeared to make a difference for the muscle. The maximal torque increased by 75% in the unresisted leg, but by 150% in the resisted leg.

References


