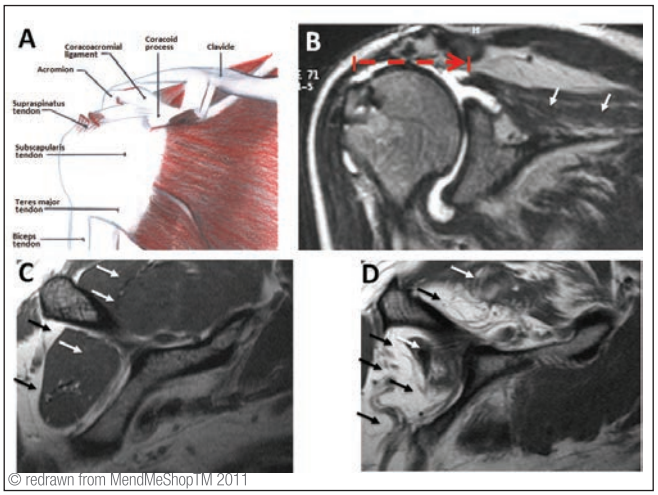
Writing for **Portal, Dr Martin Flück,** of Balgrist University Hospital, discusses the Center for Applied Biotechnology and Molecular Medicine's research into tackling the adiposity pandemic with muscle inactivity

From sports bench to bedside

The adiposity pandemic is a major threat to industrialised societies. There is now support that this metabolic disorder involves the conversion of muscle to fat tissue with reduced load-bearing contractions of muscle cells. Research in the laboratory for muscle plasticity at the Balgrist University Hospital, Switzerland, tracks down the underlying mechanism and asks how this may be translated into approaches tackling fatty atrophy in the orthopaedic clinics and frailty of the bedridden patient. The research is inspired by paradigms of exercise-induced muscle plasticity, which implicates skeletal muscle functioning in setting human strength and fitness, and whole body energy metabolism.

Fatty atrophy

Musculoskeletal tissues show a pronounced dependence on mechanical loading. This mechano-regulation is amply illustrated in clinical situations, abolishing weight bearing of skeletal muscle such as prolonged bed rest, muscle injury, muscle dystrophy or tears of tendons. Consequently, a typical loss of fibre material (atrophy) can be observed. In the situation of tears of tendons this leads to a muscle retraction, which is followed by atrophy and the conversion of muscle into fat tissue (i.e. fatty atrophy, Fig. 1) with severely impaired function.



© redrawn from MendMeShop!M 2011

In Fig. 1, A is a drawing of the human rotator cuff with the indication of a rupture of the supraspinatus tendon. B and C show coronary sagittal images of an MRI scan of the shoulder at different depths of a patient at two time points after a full tear of the supraspinatus muscle tendon. The rate of retraction respective to the site of supraspinatus tendon attachment is indicated with a stippled, red arrow in panel A. Fat tissue (indicated by black arrows) appears in white above the darker contrast of muscle and bony tissue (white arrows).

Dr Martin Flück

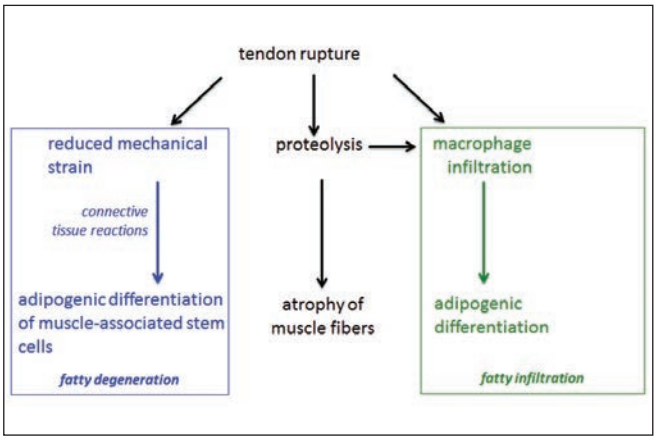
Fig. 1 Fatty degeneration with tears of rotator cuff muscle

Fig. 2 Sketches summarising the proposed mechanisms of fatty atrophy with tears of muscle tendons

Full or partial tears of tendons are a relatively frequent condition in orthopaedic clinics. In Switzerland alone, the costs associated with tendon tears of rotator cuff muscles of the shoulder amount to 240 million Swiss francs (~€198.5m) per year. The recommended treatment aims to re-establish the attachment of tendon stumps to their original site of insertion. This is, however, not always achieved, because the reattachment fails, especially if fat content grades above 50% fat per muscle volume. Fat content of affected muscle is therefore considered a main indicator for a successful intervention and is routinely estimated pre-operatively with magnetic resonance imaging. The figures and dropout rates indicate considerable room for improvement in current practice. Knowledge on the course of the pathology and underlying mechanism is seen as a valid strategy to put forward on this end.

Mechanism implications

The aetiology of muscle degeneration with tendon rupture includes abrupt overload of the musculotendinous junction, but there is also a considerable contribution of chronological ageing. This association is especially indicated for the rotator cuff muscles of the shoulder. For instance, partial or full tears of rotator cuff



tendons are seen in two out of five subjects above 60 years of age.

The cellular mechanisms underpinning the increase in fat content in skeletal muscle with tendon tears involve two postulated mechanisms: the degeneration of muscle cells into adipocytes and the infiltration of fat cell precursors into muscle at the expense of myofibrillar material (Fig. 2). The quantitative contribution of fatty degeneration and infiltration and their respective time course is not understood.

From bedside to bench

The laboratory of muscle plasticity addresses the muscular processes that underpin current problems in orthopaedic surgery. The goal is to identify mechanisms of the pathology and point to targets for future treatments.

A focus of the current research is to track down the mechanism being implicated in muscle-to-fat conversion and the associated deconditioning of the mechanical capacity of skeletal muscle. Towards this end, the laboratory applies an exploratory approach to map the course of molecular changes induced by the detachment of the tendon in relation to endpoints of muscle degeneration. The studies are complemented with investigations in human models to understand the regulation of fat content in muscle by mechanical factors.

In our studies, we follow the hypothesis that the increase in muscle fat content with tendon rupture involves a critical reduction in mechanical strain to the connective tissue of muscle fibres. This contention is suggested from the appearance of fat cell precursors at the surface of muscle fibres, the force generating capacity of which is blunted by the pharmacological inhibition of cytoskeletal contractility. The observations support the concept that spontaneous adipogenic differentiation of muscle-associated stem cells in the connective tissue sheet around muscle fibres contributes to the increased fat cell count in detached muscle. This contention is supported from our investigations in human and animal models of simulated or real microgravity, which demonstrate the rapid and connected down-regulation of adhesive structures at the periphery of muscle fibres (Fig. 3).

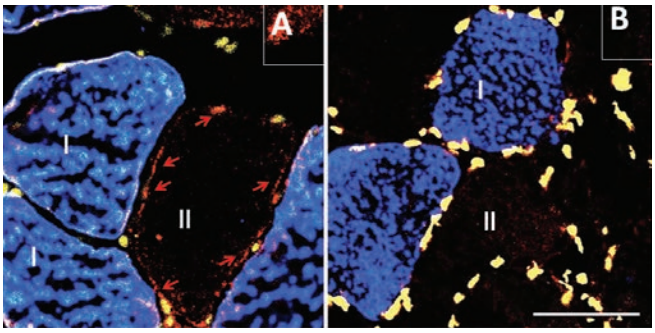


Fig. 3 Down-regulation of costamere components with muscle unloading. Bar indicates 100 micrometres. Adapted from Li *et al.* (2013)

In Figure. 3, A and B show an assembly of confocal microscopic images visualising expression of the regulator of costamere turnover, focal adhesion kinase (FAK, red colour, indicated by arrows). FAK expression mainly localises to the sarcolemma but decreases in type II muscle fibres (designated 'II') after 34 days of bed rest (B) relative to the levels at baseline (A). Type I muscle fibres (designated 'I') appear in blue, and nuclei are stained yellow.

Costameres bridge the myofibrils of the muscle fibre interior via the cytoskeleton to the extracellular matrix of adjacent fibres. Our studies in human models of unloading emphasise a double role of costameres in mechano-regulation of muscle micro-architecture and mechano-sensation.

CABMM studies

Mechanistic studies into muscle degeneration rely on the invasive collection of tissue samples. Our studies are therefore bound to experimental situations where ethical justifications can be granted to conduct experimental manipulations. In order to circumvent this bottleneck, we apply a tenotomy model for rupture of rotator cuff muscle, *M. infraspinatus,* in Swiss Alpine sheep (Fig. 4A/B). This large animal model has, despite shortcomings in relation to clinical and anatomical aspects, the advantage that a considerable degree of muscle-to-fat conversion can be induced in rotator cuff muscle (Fig. 4C/D). Importantly, tenotomy of the infraspinatus tendon and subsequent reattachment to the proximal humerus is useful to address biomechanical, histologic and biochemical processes of rotator cuff degeneration and repair at a relative ease of handling and housing in a species that is accepted by the society as a research animal.

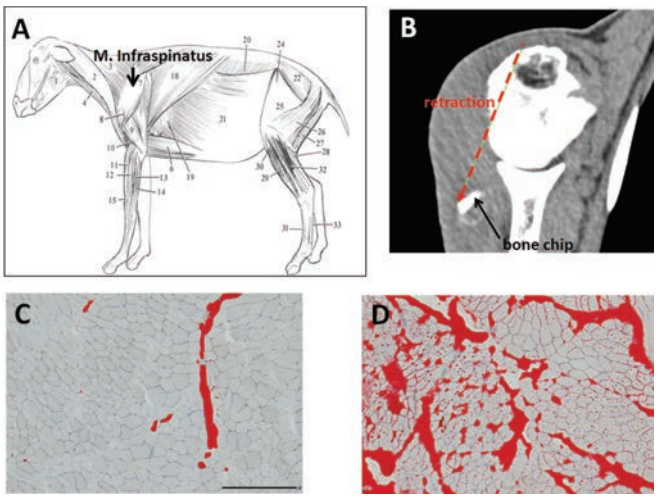


Fig. 4 Experimental model for tears in rotator cuff muscle in sheep. Bar indicates 500 micrometres

A in Fig. 4 depicts the location of the infraspinatus muscle, which is targeted for tendon release via osteotomy of the greater tuberosity. B shows a computed tomography image visualising the retraction of infraspinatus muscle (based on the detached bone chip) after tenotomy.

C and D show microscopic images visualising the increased fat content of infraspinatus muscle in one sheep after 16 weeks of tenotomy (D) compared to baseline levels (C). Fat was detected by Oil Red O staining (red) of cross-sections from the bioptic samples. Single muscle fibres can be identified based on the contrast provided by the sarcolemma.

Towards this end, the Laboratory of Muscle Plasticity actively collaborates through Professor Brigitte von Rechenberg with the Competence Center for Applied Biology and Molecular Medicine (CABMM). The results gleaned though experiments in sheep are then contrasted with findings from clinical trials of rotator cuff disease in humans to test whether an association exists between the identified molecular markers and endpoints of the pathology.

Fat accumulation and inactivity

Intriguingly, the conversion of muscle to fat tissue that is associated with reduced load bearing of muscle after tendon rupture resembles the aspect of adiposity with inactivity-induced metabolic disorders. There is now strong suggestion that inactivity may be the main culprit in the obesity epidemic. Physical exercise is an evolutionary selected countermeasure to postpone or counteract muscle deconditioning which manifests in pronounced difference in fat content of recruited muscle groups (Fig. 5). The observations indicate a possibly common mechanism between the phenomenon of fatty infiltration and adiposity. In this regard, a negative impact of increasing age or inactivity on satellite cell-mediated repair of muscle fibres is a possible common, yet unproven, denominator of the increase in the fat over muscle with rotator cuff tendon rupture and obesity.

Fig. 5 displays magnetic resonance images showing the cross-sectional area of the thigh at a standardised, anatomical position in a trained and untrained subject of the same age. The increase in adipose tissue at the expense of the quadriceps and hamstring muscle area can be readily identified from the indications. The area corresponding to *Vastus lateralis* muscle is circled.

Lessons from sport

The accumulation of adipocytes in skeletal muscle after tendon rupture is characterised by a reduction in the capacity to produce and maintain force over time. These two features of force production per time, i.e. strength and metabolic fitness, are the main traits that characterise performance (Fig. 6). At the extremes of the operation range of locomotion, they become critical to maintain power output. *Vice versa,* it now becomes apparent that the maintenance of either trait (at a relative level) in the adult individual relies on the impact of physiological cues. These relationships are well illustrated in sports by the conditioning of muscle-based performance with repeated muscle work (i.e. training). These adaptations include the qualitative and quantitative remodelling of muscle

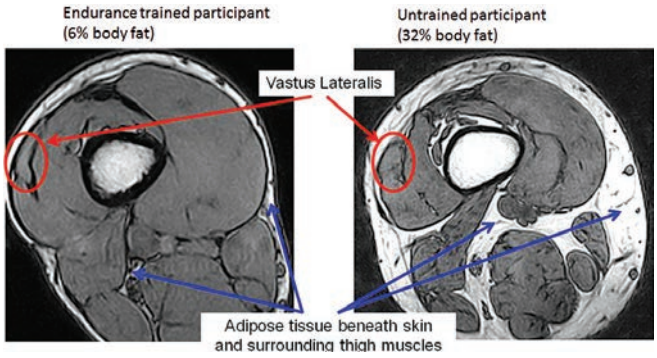


Fig. 5 Increased extramyocellular fat with relative inactivity. Picture courtesy of Vaughan (2012)

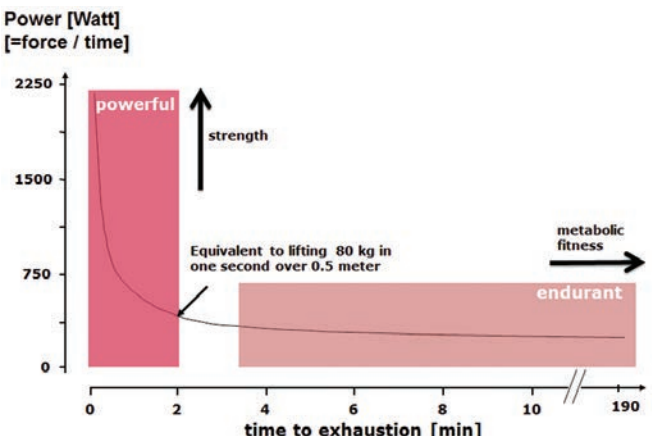
organelles that underlie the production of muscle force through contraction and the provision of metabolic energy to fuel contraction.

In Fig. 6, a composite figure indicating the operation range of human performance based on measured values of a skilled cyclist for the capacity to produce a maximal amount of force over time (i.e. power) on a cycle ergometer for different periods of time before exhaustion sets in is shown.

The deconditioning of muscle performance after the detachment of its tendon thus essentially reflects the shortfall in weight bearing muscle contractions. Feed forward control of strength and fitness with training thus represents a probate means for the rehabilitation of muscle being deconditioned as a result of the prolonged inactivity or detachment of tendon. We therefore enrich our research with human experimentation into the physiological feed forward mechanisms that steer muscle remodelling (i.e. plasticity) with resistance and endurance type training and contrast to the effects of muscle unloading.

Regarding the adaptations of muscle tissue, an important qualitative distinction can be made regarding the conditioning of the traits that set

Fig. 6 Power spectrum of human performance



mechanical and metabolic characteristics of muscle contraction. Whereas strength is conditioned by high load-low volume type of exercise, i.e. resistance training, fatigue resistance is improved with high volume, low load type paradigms, i.e. endurance training. The typical adjustments with endurance training involve – aside from important cardiovascular adaptations – quantitative increases in the organelles that set aerobic metabolic pathways (i.e. mitochondria and capillaries) and store metabolic resources in muscle fibres, i.e. intramyocellular lipids and glycogen. By contrast the content of extracellular lipids/adipose tissue associated with muscle fibres is reduced after years of systematic endurance training, while the contractile apparatus is largely unaffected. This can be visualised by magnetic resonance imaging (Fig. 5). The adaptations of exercised muscle to endurance type thus contrast quantitatively to those seen after resistance training, which produces a typical increase in the total volume and volume density of myofibrils at the expense of mitochondria.

Systems biological approach

With the entry of high throughput methodologies in the daily routine of research in the Life Sciences, it has become evident that the adaptation of muscle tissue involves the interplay of many processes. We have shown before that skeletal muscle is an important part of the network, which explains improvements in metabolic fitness in humans (Fig. 7). Collaterally, we identify that the load-modulated processes that govern biomechanical function of muscle fate in response to physiological clues are integrated through the musculoskeletal system.

In our current studies, we adopt a systems biological approach to track down the pathways involved in the conversion of muscle into adipose tissue. The set-up combines the expertise of high throughput methodologies of next-generation sequencing and metabolomics with those of the clinician/radiologist and biomechanics.

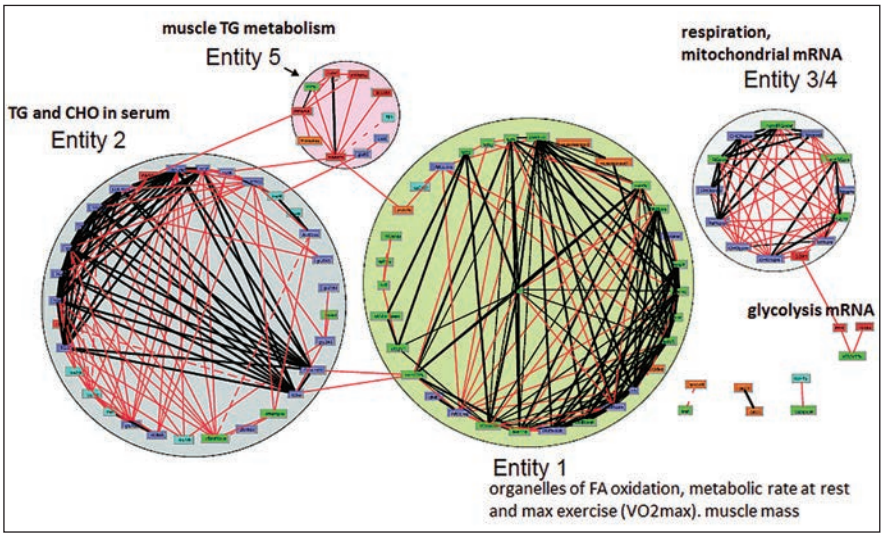


Fig. 7 Network of linear relationships defining metabolic health. Abbreviations: CHO, carbohydrate; mRNA, messenger ribonuclei acid; TG, triglyceride. Modified from Osterhof *et al.* (2010)

Fig. 7 represents topology analysis of the main, correlated features of metabolic processes after growth hormone-replacement therapy of patients. Relationships that are lost with adiposity are indicated by red lines. Three entities (1, 2 and 5) are identified which enhance metabolic fitness by establishing a stoichiometry between muscle and whole body lipid metabolism.

Through this approach, it is aimed to expose the larger picture of the connected network of processes that govern mechanical and metabolic muscle functioning and to identify potential single factors (i.e. biomarkers) that represent bottlenecks in the cure of the detached rotator cuff muscle. We hope this will lead to the identification of new targets for future pharmacological or physiological interventions of diseased muscle in the orthopaedic clinics. An important step in this direction is the experimental collaboration with the team of von Rechenberg of the CABMM.

Outlook

With defining the time course of molecular adaptations we expect to expose possible venues of intervention. Specifically, we expect that in order to halt fatty degeneration of skeletal muscle with tendon rupture, one must halt the transition of a muscle from a mechanically stiff tissue to a softer, fat-muscle composite. Therefore, first intervention will likely be targeted to prevent the degradation of adhesive structures that regulate and maintain mechanical properties of skeletal muscle.

HORIZON 2020

Professor Dr Martin Flück
Balgrist University Hospital
Professor Dr med vet Brigitte von Rechenberg
Center for Applied Biotechnology and Molecular Medicine

BROWSE www.balgrist.ch
www.cabmm.uzh.ch