MUSCLE AND BONE, A COMMON WORLD?

Biological pathways in muscle and bone adaptation to mechanical loading

General summary
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The natural life span within our population is increasing, as well as the number of people suffering from metabolic syndrome or other chronic disorders, e.g. due to overweight or sedentary lifestyle. Disuse conditions such as impaired mobility and limited physical activity often result in a loss of muscle mass and strength as well as impaired bone mass and structure. Exercise can counteract these negative effects by improving and maintaining musculoskeletal health. However, exercise is difficult for people with impaired mobility, as the load-taking capacity in these people is low. Therefore alternative treatments are needed to improve and to maintain bone and muscle mass. This requires insight in the mechanisms by which bone and muscle respond to mechanical loading, resulting in adaptation of muscle and bone mass.

During exercise, muscle and bone tissue are subjected to increased mechanical loads, leading to biological adaptation of both bone and muscle tissue mass and structure. Adaptation to mechanical loading results in a higher force generating capacity of muscle, and improved resistance of bone against loading demands. In bone, remodeling is responsible for the reshaping and/or replacement of the tissue, and it allows bone to adapt to its mechanical environment. The mechanosensing osteocytes tightly orchestrate the activity of the bone resorbing osteoclasts and bone forming osteoblasts in the process of bone remodeling. In muscle, the muscle fibers and their satellite cells transduce mechanical stimuli into biochemical signaling cascades, resulting in changes in protein turnover within the muscle fiber and in an increase in muscle fiber size.

The process by which a mechanical stimulus is transduced by the cell into a biochemical signal is known as mechanotransduction. Although the known mechanisms of mechanotransduction within muscle and bone seem quite similar, there are also marked differences. It has been hypothesized that bone and muscle form an operational unit, and that not only a mechanical interaction, but also a biological interaction between muscle and bone exists. As yet, it is unknown whether mechanical loading of the mechanosensor cells in bone express signaling molecules affecting muscle hypertrophy, and whether signaling molecules produced by mechanically stimulated muscle cells affect the effector cells in bone, the osteoblasts and osteoclasts. Therefore, the aim of this thesis was two-fold: 1) to investigate whether biochemical communication between muscle and bone in response to mechanical loading is possible, by identifying signaling molecules produced by bone cells that could affect muscle and vice versa, and 2) to identify novel signaling pathways potentially involved in the adaptation of bone mass and muscle fiber size in response to mechanical loading.

The prime anabolic and metabolic factors regulating muscle fiber size are insulin-like-growth factors-I (IGF-I Ea), mechano-growth factor (MGF), myostatin, vascular endothelial growth factor (VEGF), and hepatocyte growth factor (HGF). In chapter 2 we first observed that MLO-Y4 osteocytes expressed mRNA of VEGF, HGF, IGF-I Ea, and MGF, but not myostatin. Furthermore we demonstrate that mechanical loading by pulsating fluid flow (PFF) enhances mRNA expression and/or protein release of IGF-I Ea, MGF, VEGF, and HGF by MLO-Y4 osteocytes. Our results suggest that IGF-I, MGF, VEGF, and HGF are possible candidates for biochemical communication between osteocytes and muscle cells. Although expression levels of
IGF-I Ea and MGF or myostatin were very low or absent in MLO-Y4 osteocytes, it is known that IGF-I Ea, MGF and myostatin affect the activity of osteoblasts and/or osteoclasts. The abundant expression levels of these factors in muscle cells, in combination with low expression levels in MLO-Y4 osteocytes provides a possibility that growth factors expressed in muscle could affect signaling in bone cells.

It is known that some factors, which are expressed in muscle and bone alike i.e. cyclooxygenase-2 (COX-2), bone morphogenetic proteins (BMPs), and Wnt signaling proteins, play an important role in adaptation of bone mass to mechanical loading. However, it is unknown whether some crucial bone mass regulatory factors, such as receptor activator of nuclear factor kappa-B ligand (RANKL), are expressed in muscle. In chapter 3 we demonstrate that cyclic uni-axial strain affects gene expression of COX-2 and Wnt signaling proteins, as well as RANKL by C2C12 myotubes. As these typical osteo-anabolic and osteo-catabolic factors are produced by muscle cells in response to mechanical loading, they may play a role in the regulation of muscle fiber size, as well as affect bone remodeling via paracrine and endocrine signaling.

In response to mechanical loading, skeletal muscle produces numerous growth factors and cytokines that enter the circulation and may reach bone cells. In chapter 4 we show that unloaded myotubes produce soluble factors that inhibit osteoclast formation. We also show that mechanically-loaded myotubes secrete soluble factors, amongst others IL-6, which affect osteoclast formation. These results suggest that muscle could potentially affect bone homeostasis in vivo via production of growth factors and/or cytokines.

Mechanotransduction is crucial in the adaptation of bone and muscle mass to mechanical loading. This thesis shows how physiologically relevant and currently unknown information is gained from extrapolating knowledge from bone mechanotransduction to muscle mechanotransduction and vice versa. In chapter 5 we demonstrate that shear stress, a mechanical stimulus extensively studied in bone, may also be relevant in skeletal muscle. Shear stress resulting from PFF treatment of myotubes activates signaling pathways, i.e. NO-production, and changes the expression of muscle anabolic and metabolic factors. The shear stress-induced NO production by myotubes requires an intact glycocalyx and functional stretch activated ion channels (SACs). Therefore shear stress may activate signaling pathways in skeletal muscle via membrane-bound mechanoreceptors. In bone, it is currently believed that shear stress is the main mechanical stimulus in the response of osteocytes to mechanical loading resulting in bone adaptation. As a result of deformation of the load-bearing bone-matrix, osteocytes experience tensile strain as well. In contrast to osteocytes, osteoblasts on the surface of the bone matrix are likely not exposed to high fluid shear stresses, but may experience tensile strain. In chapter 6 we observed that MC3T3-E1 osteoblasts are more sensitive to tensile strain than MLO-Y4 osteocytes with respect to gene expression of IGF-I Ea, which is an important anabolic growth factors in bone as well as in muscle. This suggests that the increase in IGF-I Ea expression in osteocytes is likely due to fluid flow in the canaliculi rather than tensile strain. Multiple mechanical stimuli (e.g. deformation, shear stress) affect bone cells and muscle cells during physical activity. Studies on the effects of these stimuli on muscle cells and bone cells are highly important, since this will lead to a broader insight into the biological responses and
mechanical properties that are involved in mechanotransduction in muscle and bone.

Since our population gets older and continues a sedentary lifestyle, the prevalence of musculoskeletal disorders will increase. This leads to a decreased quality of life and high costs. In order to maintain musculoskeletal health, a physically active lifestyle is of utmost importance. The existence of biochemically mediated muscle-bone interactions in the adaptive response of muscle and bone to mechanical loading asks for new therapeutic approaches. These approaches preferably need to be focused on skeletal muscle and bone as one unit, as the adaptive response of skeletal muscle and bone are similar to a large extent. The experimental information provided in this thesis fits with the hypothesis that biochemical communication exists between muscle and bone during physical activity. This, together with new insights in the molecular pathways involved in muscle and bone adaptation, improves our understanding of how muscle and bone cells sense and respond to mechanical loading, and how a biochemical interaction may exist between muscle and bone. These insights could contribute to the development of therapies and exercise programs to maintain existing bone mass and aid the gain of new bone mass in people that are likely to develop osteoporosis and sarcopenia.