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Understanding the endocrine crosstalk between bone and muscle: molecular investigation of the impact of myokines on osteogenesis using C2C12 myoblast and 2T3 osteoblast cell lines

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Bones and skeletal muscles interact mechanically to allow motor activity in vertebrates and even invertebrates. Until the last decade of research, bone-muscle interactions had been gathered under the umbrella of the "mechanical coupling" theory, where muscles are the load suppliers and bones provide the attachment sites [1]. However, bones and skeletal muscles have recently been identified as endocrine organs, that secrete cytokines and chemokines, through which they interact to promote the motor activity. This molecular and biochemical interplay has been named "bone-muscle crosstalk". The bi-directional flow of signals between bone and muscle has been investigated experimentally by differentiating bone or skeletal muscle progenitor cells in a medium conditioned by myotubes or osteocytes respectively [2][3]. These studies have demonstrated that osteocyte and myotube secreted factors (osteokines and myokines, respectively) have a reciprocal inhibitory influence on myogenesis and osteogenesis, since they reduce the majority of the mRNA levels of genes associated with differentiation. We propose to study the effects of myokines on osteogenesis by differentiating 2T3 osteoblastic cells in a medium conditioned by either early (3-5 days) or late (7-10 days) myotubes. The study includes: *i)* analysis of mRNA and protein levels of marker genes of differentiation, to establish the effect of early and late patterns of myokines; *ii)* characterization of the differentiation process from the functional viewpoint by studying alkaline phosphatase activity and the deposition of mineralized matrix. As expected results, early and late myotube-conditioned media should affect differently the osteoblast lineage in the course of differentiation. The study includes also the successive identification of the metabolomic profile of the conditioned medium, to identify the cytokines most abundantly expressed. This first set of results will pave the way for further experiments of myoblast/osteoblast co-cultures aimed at a real-time tracking of the bi-directional signaling between bone and muscle tissues and its impact on all stages of differentiation. The results of this study will deepen the understanding of how the muscle secretome protects osteocytes and preserves their function and *vice versa* how bone factors maintain muscle function. Such knowledge will help to identify potential new target therapies for bone and muscle diseases, especially when they co-exist, as is the case of the twin syndrome of osteoporosis and sarcopenia.

References

[1] Brotto, M.; Bonewald, L., Bone and muscle: Interactions beyond mechanical. Bone 2015, 80, 109-14.
[2] Johnson, R. W.; White, J. D.; Walker, E. C.; Martin, T. J.; Sims, N. A., Myokines (muscle-derived cytokines and chemokines) including ciliary neurotrophic factor (CNTF) inhibit osteoblast differentiation. Bone 2014, 64, 47-56.
[3] Wood, C. L.; Pajevic, P. D.; Gooi, J. H., Osteocyte secreted factors inhibit skeletal muscle differentiation. Bone Rep 2017, 6, 74-80.

Keywords
Bone-muscle crosstalk, osteokines/myokines, osteogenesis, myotube, conditioned medium