The Effect of Phosphate Availability on Chondrocyte Metabolic Function

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Introduction: Phosphate is essential for normal fracture healing and bone growth. Previous studies have established that mice given a phosphate deficient diet demonstrate delayed cartilage maturation and callus mineralization, and changes in gene expression consistent with oxidative phosphorylation dysfunction during fracture healing. It has also been established that ATDC5 chondroprogenitor cells grown in differentiating media containing β-glycerolphosphate express genes associated with chondrocyte differentiation and develop hydroxapatite mineral deposits. The main goal of this study was to examine the role of inorganic and organic phosphate availability in chondrocyte differentiation, mineralization, and metabolic function.

Methods: Cell model. ATDC5 murine chondrocytes were expanded in growth medium (DMEM/F12 1/1 with 5% FBS and 1% penicillin/streptomycin) and were seeded on XF24 plates (Seahorse Bioscience) at 1500 cells/well. Once cells had achieved confluence (day 0) they were switched to differentiating media: (Full Pi) medium (αMEM supplemented with 5% fetal bovine serum, 1% penicillin/streptomycin, 1X insulin-transferrin-selenium and 0.2mM ascorbate) or half inorganic phosphate (Half Pi) medium (αMEM without sodium bicarbonate or sodium monophosphate supplemented with 5% FBS, 1X insulin-transferrin-selenium, 0.2mM ascorbate, 2.5 mM sodium bicarbonate and 0.5 mM sodium monophosphate). Half of the wells received 4mM β-glycerolphosphate (+βGP), while the remaining wells did not have any β-glycerolphosphate (-βGP). Oxygen consumption rate, DNA content, and protein content were assessed at days 7, 14, 21, and 28. DNA and Protein Quantification. DNA and protein were extracted using 4M guanidine HCl with 1% Triton in 1X TE buffer. DNA was quantified using the PicoGreen dsDNA fluorescence assay. Protein was quantified using MicroBCA protein assay kit. Measured protein concentration was normalized to DNA content.

Results: Both differentiation and the availability of inorganic and organic sources of phosphate affect the oxidative function of chondroprogenitor cells. All measures of oxidative function increased as cells differentiated between d’and d21 (p<0.0001). From d21 to d28, BR, RC, and ATP (p=0.0265) decreased significantly. Inorganic phosphate availability significantly affected maximum RC. The cells cultured in Full Pi medium had higher RC than those in Half Pi medium, regardless of the availability of βGP (p=0.0064). RC was not different between Full Pi +/βGP conditions or between Half Pi +/βGP conditions (p>0.99). ATP was significantly higher in Full Pi+βGP than in Half Pi+βGP and Half Pi –βGP (p=0.0213). NMR was significantly higher in Full Pi +βGP than in Half Pi –βGP and was higher in Half Pi +βGP than Half Pi –βGP (p<0.0019). H+ leak was not significantly affected by either Pi or βGP availability (p>0.05). The availability of βGP affected total protein content. Total protein content was higher under the Full Pi –βGP media condition than under Full Pi +βGP (p=0.0007). Similarly, protein content was higher under Half Pi –βGP than under Half Pi +βGP (p=0.0321). The availability of βGP affected mineral accumulation. MA was higher under the Full Pi+βGP condition than under Full Pi+βGP (p=0.0001); MA was higher under the Half Pi+βGP condition than under Half Pi+βGP (p=0.0001). MA was not different between Full Pi+βGP and Half Pi+βGP (p=0.97) or between Full Pi+βGP and Half Pi+βGP (p=0.98).

Discussion: The results indicate that chondrocyte differentiation is associated with an overall increase in mitochondrial respiration. While chondrocyte oxidative function is affected by the availability of both inorganic and organic sources of phosphate, the most significant differences appeared to be a result of inorganic phosphate deficiency. Of all oxygen consumption measures, respiratory capacity was the most affected by the inorganic phosphate deficiency. Groups with half the inorganic phosphate demonstrated a significant reduction in maximum respiration. There was no effect from organic phosphate on respiratory capacity, suggesting that these changes in respiratory capacity were independent of mineralization since mineralization only occurred in the media containing organic phosphate. Inorganic phosphate deficiency also appeared to cause a reduction in the rate of ATP turnover, while organic phosphate availability did not. Cells grown in media without the organic phosphate accumulated significantly more protein than those with organic phosphate, suggesting that mineralization is associated with reduced protein accumulation. These results suggest that the interaction between oxidative metabolism and systemic phosphate metabolism may be the common mechanistic link that is affected by the major co-morbidities – such as obesity and diabetes – that are associated with delayed fracture healing.

Significance: This study assessed the interaction between differentiation and extracellular organic and inorganic phosphate on chondrocyte mineralization, and metabolic function. This study showed that chondrocyte differentiation is associated with increasing oxidative metabolism and that inorganic phosphate deficiency leads to a significant decrease in oxidative metabolic function, while the absence of organic phosphate leads to a deficiency in matrix mineralization. In our studies of fracture healing we show that dietary phosphate deficiency leads to decreased expression of oxidative metabolic gene expression and delayed fracture healing.

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Key Words: phosphate, metabolism, chondrocyte, mineralization

Figure 1. The Effect of Phosphate on Proteins and Mineral Accumulation.

Figure 2. The Effect of Phosphate on Proteins and Mineral Accumulation.

Figure 3. The Effect of Phosphate on Proteins and Mineral Accumulation.