HYPERPHOSPHATEMIA IMPAIRS MUSCULAR REGENERATION BY INHIBITING MYOGENIC DIFFERENTIATION

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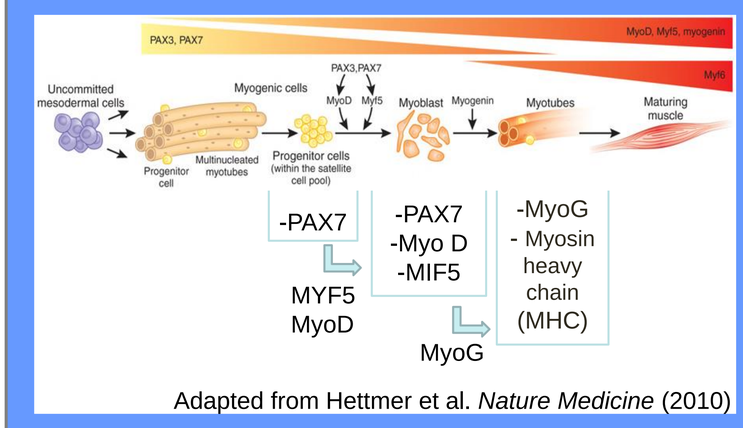
Topic: K2) CKD. Pathophysiology, progression and risk factors.

Introduction:

Hyperphosphatemia has been related to chronic kidney disease (CKD) and aging. A related condition of these pathologies is the loss of mass and muscular force called sarcopenia. Sarcopenic muscle is characterized by loss of regeneration capacity.

Muscle regeneration depends on resident myogenic stem cells known as satellite cells and which function is to repair damaged muscle. The myogenic differentiation of satellite cells into myotubes is regulated by a cascade of transcription factors such as Myogenin. However, this process may be disrupted by micro-environmental conditions like high extracellular phosphate.



Adapted from Hettmer et al. *Nature Medicine* (2010)

Objetive:

To evaluate the effect of high extracellular phosphate concentration on the myogenic differentiation process, analysing the myotube formation and the expression of myogenic factors in cultured myoblast C_2C_{12}.

Methods:

Cultured murine myoblast C_2C_{12} cells were used for all experiments. Cells were grown during seven days with 2% horse serum, to promote myogenic differentiation, in the presence or the absence of 10mM beta-glycerophosphate (BGP, used as a phosphate donor).

Myotube formation was evaluated at several times after desmin and myosin heavy chain (MHC) immunofluorescence staining by confocal microscopy. To analyse the expression of myogenic factors, western blot and immunofluorescence staining were performed, using specific antibodies for PAX-7, MHC and Myogenin.

Summary:

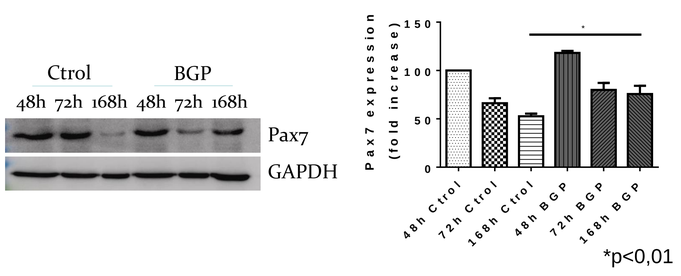
The expression of PAX-7, a marker of satellite cells, was higher in BGP treated cells in all times, whereas PAX-7 expression was reduced in control cells after 72h in culture.

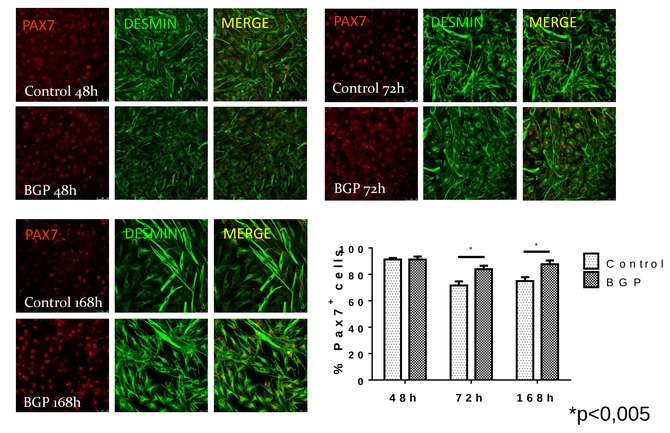
The expression of Myogenin, a transcriptional factor involving in myogenic differentiation, rose in control cells after 72h of culture coinciding with the beginning of myotube formation, whereas Myogenin expression was lower in BGP treated cells at any time, suggesting that BGP was inhibiting the expression of this factor.

C2C12 treated with BGP shown a significant decrease in the number of myotubes (MHC+) formed at all times evaluated with respect to cells treated with vehicle. The expression of MHC was also lower in BGP treated cells after 72h in culture.

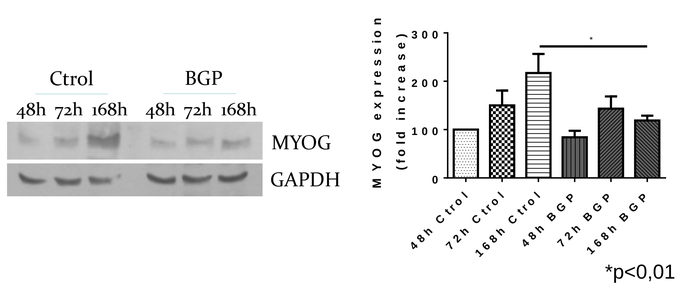
Results:

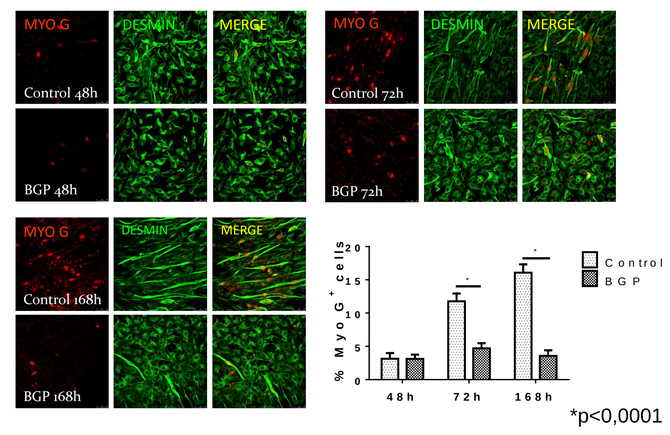
BGP treatment keeps a higher expression of PAX-7 during myogenic differentiation.



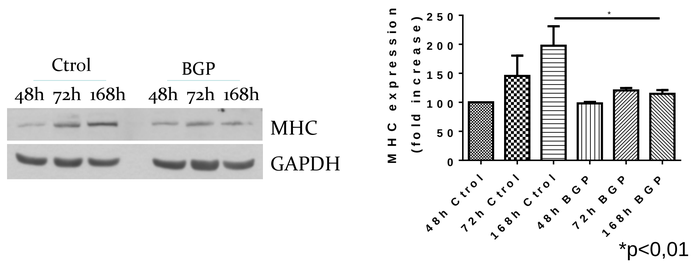


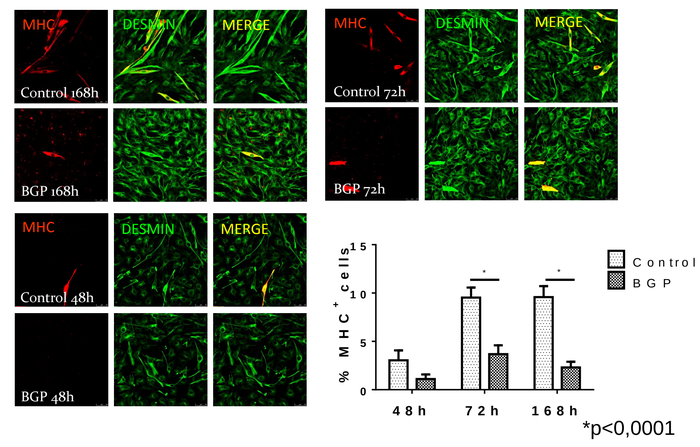
The expression of Myogenin decreases in BGP treated cells during myogenic differentiation.





BGP treatment reduces the myotube formation in culture myoblast





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3. Carrero, J. J., Johansen, K. L., Lindholm, B., Stenvinkel, P., Cuppari, L., & Avesani, C. M. (2016). Screening for muscle wasting and dysfunction in patients with chronic kidney disease. Kidney Int, 90(1), 53-66.

Conclusions:

High extracellular phosphate concentration reduces the myotube formation in culture myoblast, disrupting the myogenic differentiation process. This results point to a role of hyperphosphatemia impairing the muscle regeneration and this could be involved in aging and CKD related sarcopenia.

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