Cartilage mediates long bone growth during endochondral ossification and bears the mechanical joint load to maintain mobility throughout life. Increasing evidence links defects in mitochondrial respiration to skeletal abnormalities and degenerative cartilage diseases, but the underlying pathomechanism is not yet defined.

Here, a transgenic mouse model was used to understand the molecular consequences of mitochondrial dysfunction for cartilage development and aging. These mice express a mutant form of the mitochondrial helicase Twinkle in a cartilage-specific manner to progressively reduce the mitochondrial DNA copy number and thus impair respiratory chain function in cartilage.

Seahorse measurements indicated a decreased respiratory chain activity in chondrocytes of one-month-old mice, phenotypically leading to a premature cartilage vascularization and cartilage to bone transition. Premature blood vessel infiltration was associated with changes in HIF-1α levels during development, while VEGF expression, protein amounts and secretion remained unchanged. The production of reactive oxygen species was decreased and cartilaginous tissue did not show signs of inflammation. Further, the composition of the immune cell population in primary and secondary lymphoid organs was not altered. Fluorometric and mass spectrometry analysis showed that loss of respiratory chain activity led to increased NADH levels and an accumulation of citric acid cycle intermediates and distinct amino acids. Concomitantly, changes in extracellular matrix protein levels could be detected, whereas matrix protein distribution did not appear to be affected. Among the altered metabolites succinate levels were the highest, associated with an increased expression of its receptor, which may trigger the activation of the MAPK/ERK signaling cascade and thereby contribute to premature cartilage ossification. In addition to these molecular changes, the importance of the respiratory chain for bone regeneration after injury was demonstrated by a delay in chondrocyte-mediated fracture healing.

In summary, mitochondrial respiration is essential for the cartilage to bone transition. Respiratory defects in cartilage lead to an accumulation of citric acid cycle intermediates and amino acids, which could result in an altered signal transduction and premature ossification.