

Oxygen, Nitric Oxide, and Osteoarthritis

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INTRODUCTION: Increased nitric oxide (NO) production is associated clinically with osteoarthritis (OA)¹, and the severity of disease is significantly decreased *in vivo* using nitric oxide synthase (NOS) inhibitors². Mechanical stress has strong influences on cartilage metabolism and plays an important role in the maintenance of articular cartilage in both health and disease³. Pro-inflammatory cytokines and mechanical stress can increase the production of inflammatory mediators such as NO by articular cartilage⁴. Articular cartilage, however, is avascular and exists at a reduced oxygen tension⁵, the superficial zone at approx 6% O₂ and the deep zone at 1% O₂. As the production of NO is an oxygen dependent process, our studies have investigated the effects of oxygen tension on mechanically and cytokine induced NO production.

METHODS: Full thickness explants of articular cartilage were harvested from the femoral condyles of 2-3 yr old female pigs and cultured in DMEM with 10% FBS, non-essential amino acids, HEPES, penicillin and streptomycin. Treated and control explants were paired from adjacent sites in the joint. After 72 hrs in culture at 37°C, 5% CO₂, 95 % air, explants were incubated in culture media supplemented with 37.5 µg/ml ascorbate-2-phosphate at either 5% CO₂, 95% air, or 5% CO₂, 5% O₂, 90 N₂ or 5% CO₂, 1% O₂ and 94% N₂.

The effects of recombinant porcine IL-1 α (rpIL-1 α , R&D Systems) and oxygen were tested on site-matched paired explants cultured in control or experimental culture media and incubated at different oxygen tensions for 72 hrs.

The effects of mechanical compression were tested by applying intermittent compressive loads, at 0.5 Hz (1sec on, 1 sec off) for 24 hrs, using a modified version of the Biopress system™ (Flexcell International), at 20%, 5% or 1% O₂.

The role of NOS2 was determined using the selective NOS2 inhibitor 1400W (2 mM, Alexis). NO production was measured via the Griess reaction and cell viability determined

using a fluorescent live/dead assay (Molecular Probes).

RESULTS:

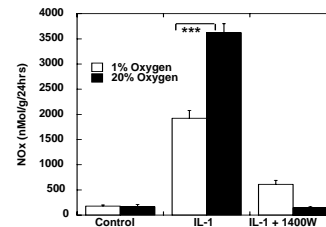
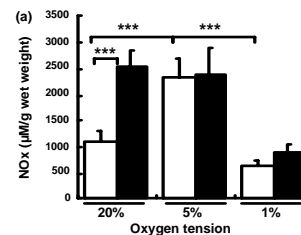


Figure 1: Nitric oxide production from articular cartilage explants in 1% v 20% O₂ in the presence



or absence of rpIL-1 α and 1400W. Mean \pm SEM, n=12 ***p<0.001.

Figure 2: Nitric oxide production from articular cartilage explants compressed at 0.05 MPa, 0.5 Hz for 24 hrs at either 20% O₂, 5% O₂ or 1% O₂, Mean \pm SEM, n = 18, ***p<0.001.

DISCUSSION & CONCLUSIONS: Oxygen tension significantly alters endogenous NO production in articular cartilage, as well as the stimulation of NO in response to both mechanical loading and pro-inflammatory cytokines. In other studies, we have shown that oxygen tension also influences the production of peroxynitrite, the production of ATP, and the mechanical properties of articular cartilage. These findings suggest that the relatively low levels of oxygen within the joint may have significant influences on the metabolic activity and inflammatory response of cartilage as compared to ambient levels.

REFERENCES: ¹Amin *et al* (1999); *Current Opinion in Rheumatology* 11: 202; ²Pelletier *et al* (1998); *Arthritis & Rheumatism* 41: 1275; ³Guilak *et al* (1997) *Basic Orthopaedic Biomechanics*: 179; ⁴Fermor *et al* (2002) *Osteoarthritis and Cartilage* 10: 792; ⁵Silver (1975) *Phil.Trans. Royal Soc.* 271: 261.

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