High Systemic LDL Cholesterol Levels during Experimental Osteoarthritis Lead to Increased Synovial Activation and Ectopic Bone Formation at End-Stage Osteoarthritis, While Excessive Levels Accelerate Development of Joint Pathology Already at Early-Stage Osteoarthritis

Wouter de Munter, Msc¹, Martijn H. van den Bosch, MSc¹, Annet W. Sloetjes, BSc¹, Peter M. van der Kraan, PhD¹, Thomas Vogl, PhD², Johannes Roth, PhD², Wim B. van den Berg, PhD¹, Peter L. van Lent, PhD¹.
¹Radboud University Medical Center, Nijmegen, Netherlands, ²Institute of Immunology, Muenster, Germany.


Introduction: A relation between osteoarthritis (OA) and the metabolic syndrome has long been established. One of the characteristics of the metabolic syndrome is increased cholesterol levels. In a recent study, we showed that LDL accumulation by LDL receptor deficient mice resulted in increased ectopic bone formation during experimental osteoarthritis.

In the present study we investigate OA pathology in ApoE deficient (ApoE⁻/⁻) mice with and without a cholesterol-rich diet, which is a model for extremely high systemic LDL cholesterol levels.

Methods: Wild type (WT) and ApoE⁻/⁻ mice received a normal or cholesterol-rich diet for 54 days. At day 18, experimental OA was induced by intra-articular injection of collagenase and animals were sacrificed at day 28 and 54. Joint pathology was investigated by histology. LDL levels were measured in serum and synovial wash-outs.

Results: ApoE⁻/⁻ mice on a normal diet showed markedly higher LDL levels than WT mice (8.90 mmol/L and 0.40 mmol/L, respectively; p<0.001). While no differences between the two groups were found at the early time point (day 28), end point OA (day 54) in ApoE⁻/⁻ mice showed a strong increase of ectopic bone formation, mainly at the medial collateral ligament (fold increase 5.4; p<0.001) compared to WT mice. No significant differences in cartilage damage were found between the two groups; a slight increase in synovial thickening, however, was found in ApoE⁻/⁻ mice (arbitrary score 1.9 versus 1.1 in WT mice; p<0.05). Furthermore, synovial gene expression of both S100A8 and S100A9 (fold increase 1.8 and 1.4, respectively; p<0.05) and S100A8/S100A9 protein levels of synovial wash-outs were increased in ApoE⁻/⁻ mice (fold increase 5.8; p<0.05), suggesting an activated status of synovial lining cells.

In addition, we investigated whether a cholesterol-rich diet could increase joint pathology after induction of OA. The diet increased LDL levels even more in ApoE⁻/⁻ mice (fold increase 2.1, compared to ApoE⁻/⁻ mice on a normal diet; p<0.001). In both ApoE⁻/⁻ and WT mice on a cholesterol-rich diet, excessive bone formation was found in the medial collateral ligament at day 54, however, no significant difference was found between the two groups. Interestingly, at the early time point (day 28; 10 days after OA induction), histological differences between the two groups were observed. Synovial thickening was four times increased (p<0.001) in ApoE⁻/⁻ mice on a cholesterol-rich diet and also ectopic cartilage formation in the medial collateral ligament was strongly increased (fold increase 2.7; p<0.01) compared to WT mice on a cholesterol-rich diet.
**Discussion:** LDL cholesterol accumulation by ApoE deficiency or a cholesterol-rich diet results in increased synovial activation and ectopic bone formation in experimental OA. Excessive LDL levels induced by a combination of ApoE deficiency and a cholesterol-rich diet did not affect joint pathology at end-stage OA, but rather accelerate synovial activation and ectopic bone formation, resulting in early pathology.

**Significance:** It is known that LDL cholesterol, and modifications thereof, can affect immunological processes. Since the metabolic syndrome is associated with OA, it is important to understand the effects of serum cholesterol on OA pathology. Unraveling the etiopathology of OA could lead to new insights into disease development and new therapeutic possibilities.

*ORS 2015 Annual Meeting*
*Poster No: 0423*