Differential leptin expression in osteoarthritis of major joints; effect on cartilage metabolism

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Materials and Methods: Articular cartilage samples were obtained from femoral heads, femoral condyles and tibial plateau of patients with primary OA undergoing hip or knee replacement surgery. A total of 17 patients were included in the study. From each patient two distinct areas, one with mild and one with advanced OA, were taken. Macroscopic findings were validated by histological studies. Normal cartilage was obtained from 5 individuals, undergoing fracture repair surgery.

RNA was isolated using Trizol reagent. mRNA expression levels of leptin and Ob-Rb were measured by real-time quantitative RT-PCR (Light Cycler Instrument, Roche Molecular Systems, Alameda, CA). Protein levels of leptin and Ob-Rb were measured by western blot analysis.

Primary chondrocytes cultures, normal and osteoarthritic, were stimulated with leptin (0-100ng/mL) and cell proliferation as well as leptin-induced IL-1β production were measured, by the use of an MTT assay and a commercially available ELISA kit respectively. MMP-9 and 13 mRNA and protein levels were also evaluated. Serum and SF leptin concentrations were also measured with ELISA technique.

Results: An intrajoint differential expression of leptin and Ob-Rb mRNA was observed between advanced and mild OA cartilage; a statistically significant increase in leptin mRNA expression was observed from the mild (0.064 ± 0.068 leptin/PBGD copies) to the severely (0.20 ± 0.093 leptin/PBGD copies) affected OA cartilage (t(15)=6.798, p<0.001). Regarding the Ob-Rb mRNA expression levels, a statistically significant increase (t(15)=4.154, p<0.001) was observed from the mild (0.018 ± 0.030 Ob-Rb/PBGD copies) to severely damaged OA cartilage (0.212 ± 0.183 Ob-Rb/PBGD copies). Similar findings were obtained when we studied the protein levels of leptin and Ob-Rb in OA and normal chondrocytes.

Discussion: Leptin has been directly associated with obesity, a potent risk factor for knee OA development. The link between obesity and OA may involve complex interactions of biomechanical and metabolic factors. For the first time to our knowledge, using quantitative real-time PCR, we observed leptin and leptin's receptor isoform (Ob-Rb) mRNA and protein expression in chondrocytes of osteoarthritic cartilage. Furthermore, we observed intrajoint differences in leptin and Ob-Rb mRNA and protein expression between advanced and minimally affected knee and hip osteoarthritic cartilage, which along with the higher observed leptin concentrations in SF than in paired serum samples, indicate a local role of leptin in joint tissues. We also observed increased leptin's mRNA expression in obese compared to normal weight patients suggesting that mechanical overload may alter chondrocytes' phenotype. The observed decrease in chondrocyte's proliferation after induction with leptin points toward a long term detrimental effect on cartilage, while the observed IL-1β production and MMP-9 and 13 mRNA and protein upregulation suggests an inflammatory and catabolic role of leptin, which may account for cartilage degradation. Furthermore, it can be suggested that in patients with osteoarthritis there is a unique microenvironment in the cartilage characterized by enhanced locally produced leptin levels, which induce IL-1β and MMP-9 and MMP-13 production by chondrocytes, reinforcing the scenario that leptin acts as a pro-inflammatory cytokine with a catabolic role on cartilage metabolism. However, further studies are required to elucidate on leptin's catabolic role on cartilage metabolism.