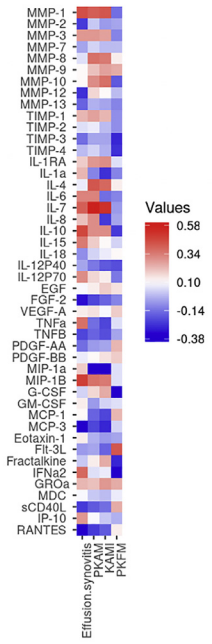

Fig 1. Correlations (Pearson r) between 1-year changes after HTO in synovial fluid biomarkers, effusion-synovitis and external knee moments during walking.

PRESENTATION NUMBER: 435

LIRAGLUTIDE HAS POTENT ANTI-INFLAMMATORY AND ANTI-CATABOLIC ACTIVITY IN TWO CELL-TYPES IMPLICATED IN OSTEOARTHRITIS

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Purpose: Osteoarthritis (OA) is an age-related joint disease affecting 300 million of individuals worldwide which provokes chronic pain and limits mobility leading to increasing cardiovascular-related mortality. The disease progression is associated with inflammatory responses and cartilage degradation. Both chondrocytes, the only cell type present in the cartilage, and macrophages from the synovium, play a major role in OA pathophysiology. Liraglutide is a Glucagon-Like-Peptide 1 Receptor (GLP-1R) agonist widely prescribed for the treatment of type 2 diabetes. Interestingly, immunomodulatory and anti-inflammatory properties of the GLP-1 pathway have been recently reported in various diseases. In this study, we evaluated the anti-inflammatory and anti-catabolic effects of Liraglutide in two *in vitro* models relevant to OA by evaluating surrogate markers of inflammation, cartilage matrix proteolysis and cartilage matrix anabolism.

Methods: Lipopolysaccharide (LPS)-stimulated murine Raw 264.7 macrophages were treated with 10 concentrations (6.6nM-3.4μM) of Liraglutide for 24h. Anti-inflammatory activity was evaluated by measuring the production of nitric oxide (NO) and prostaglandin E_2 (PGE_2) using Griess reaction and ELISA, respectively. Interleukin 1β (IL-1β)-stimulated mouse articular chondrocytes were treated with 10 concentrations (6.6nM-3.4μM) of Liraglutide for 24h. Production of IL-6, matrix metalloproteinase 3 (MMP-3) and glycosaminoglycans (GAG) was measured by ELISA and GAG assay, respectively. RT-qPCR analyses were performed with three selected doses of Liraglutide (13.3nM, 53.1nM and 1.7μM) on both cell types to assess the expression of a panel of genes related to inflammation (IL-6, TNF, iNOS), M1/M2 macrophage phenotype (MCP-1, CD38, ERG-2), catabolism (MMP-13, ADAMTS-5) and anabolism (Sox9, Col2a1, Acan).

Results: Liraglutide induced a dose-dependent inhibition of the LPS-induced production of NO (IC_{50}=45nM) and PGE_2 (IC_{50}=54nM) in Raw 264.7 murine macrophages. Moreover, IL-6 and TNF gene expressions were significantly and dose-dependently decreased in Raw 264.7 cells treated with Liraglutide compared to LPS alone (Table 1). Interestingly, there was a significant dose-dependent reduction of MCP-1 and CD38 (M1 macrophage marker) gene expression in cells treated with the 3 doses of Liraglutide compared to LPS alone while we observed a dose-dependent increase of ERG-2 (M2 macrophage marker) gene expression induced by Liraglutide (Table 1). These results suggest that Liraglutide can polarize M1 macrophages to the M2 phenotype. Liraglutide significantly reduced, in a dose-dependent manner, the IL-1β-induced release of IL-6 (IC_{50}=38nM), cartilage matrix catabolic enzyme MMP-3 (IC_{50}=56nM) and GAG (IC_{50}=47nM) in murine articular chondrocytes. Additionally, Liraglutide treatment dose-dependently decreased the IL-1β-induced gene expression of iNOS, MMP-13 and ADAMTS-5 (Table 2). Finally, IL-1β decreased gene expression of Sox9, Col2a1 and Acan anabolic markers, which could be rescued in a dose-dependent manner by Liraglutide (Table 2).

Conclusions: A shift in M1/M2 macrophage phenotype and the inhibition of chondrocyte expression of several mediators involved in inflammation and cartilage degradation explain, at least in part, our previous results from rodent osteoarthritis models that showed an analgesic, anti-inflammatory and anti-degradative effect of Liraglutide. The fact that Liraglutide is already safely prescribed in another indication allows us to foresee a first trial in humans in the short term.

Marker (fold change)	Vehicle No LPS	LPS 100ng/ml	LPS+ Liraglutide 13.3nM	LPS+ Liraglutide 53.1nM	LPS+ Liraglutide 1.7μM
IL-6	1.0±0.2*	63.6±7.1	58.8±6.5	36.3±8.4*	30.8±2.6*
TNFα	1.0±0.2*	26.8±4.9	17.5±2.6*	9.5±1.8*	4.1±2.1*
MCP-1	1.1±0.6*	91.7±11.2	67.8±4.3*	47.3±6.1*	25.1±5.5*
CD38	1.1±0.6*	103.5±22.3	73.2±12.3*	44.6±8.9*	17.4±3.3*
ERG2	1.0±0.3*	0.3±0.1	1.1±0.5*	2.1±0.6*	3.1±0.2*

Gene expression (fold change) of genes related to inflammation and M1/M2 macrophage phenotype

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Marker (fold change)	Vehicle No IL-1β	IL-1β 2ng/ml	IL-1β +Liraglutide 13.3nM	IL-1β +Liraglutide 53.1nM	IL-1β +Liraglutide 1.7μM
iNOS	1.0±0.2*	47.8±17.6	34.2±15.5	18.9±8.2*	11.8±2.9*
MMP-13	1.0±0.2*	9.8±2.0	7.6±1.0	4.6±0.7*	2.5±0.4*
ADAMTS5	1.2±0.8	2.7±0.6	2.0±0.6	1.6±0.3	1.1±0.2
Sox9	1.0±0.1*	0.4±0.2	0.4±0.2	0.6±0.0	0.7±0.2
Col2a1	1.0±0.2*	0.3±0.1	0.3±0.1	0.5±0.3	0.8±0.3*
Acan	1.2±0.8*	0.2±0.1	0.1±0.0	0.5±0.1*	0.7±0.2*

Gene expression (fold change) of genes related to inflammation, cata- and anabolism in chondrocytes

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