Production of proinflammatory cytokines by infrapatellar fat pad of patients with knee osteoarthritis is further increased by interleukin 1β and inhibited by activation of peroxisome proliferator activated receptor α activation

Methods
Explants of infrapatellar fat pads from four OA patients obtained with approval of the Local Ethical Committee (number MEC 2004-322), were preincubated during 24h and then cultured again for 48 hours with(out) 10 ng/ml IL1β as a pro-inflammatory stimulus with(out) 10 and 100 μM Wy-14643, a potent and selective PPARα agonist. Culture media were analyzed with LumineX for monocyte chemoattractant protein (MCP)1, tumor necrosis factor(TNF)α, IL10 and IL6. Analysis of mRNA expression was performed for these cytokines on all explants. A mixed linear model was used to analyze the mRNA expression data. To investigate whether PPARα activation depends on the IL1β response of explants, IL1β response was added as covariable.

Results
Infrapatellar fat expresses TNFα, MCP1, IL6 and IL10 (Figure 1). These cytokines can also be detected in the culture media by LumineX. The addition of 100 μM Wy-14643 showed a trend to a decrease of TNFα mRNA expression (p=0.06), but the mRNA expression of IL6, IL10 and MCP1 were not significantly influenced by 10 or 100 μM Wy-14643 (Figure 1).

The incubation during 3 days decreased mRNA expression at day 2 of TNFα to 26% (p=0.001), MCP1 to 68% (p=0.01), IL10 to 22% (p=0.001) compared to the mRNA expression in explants after harvesting (day -1). Only IL6 was increased to 811% (p=0.02) of the initial expression (day -1). By adding IL1β to explants of infrapatellar fat pad the mRNA expression of TNFα increased to 930% (p<0.001), MCP1 to 670% (p<0.001), IL6 to 930% (p<0.001) and IL10 to 176% (p=0.06) compared to the explants cultured during 3 days without IL1β.

Conclusion
The IPFP is an active osteoarthritic joint tissue that produces inflammatory cytokines that have been demonstrated to alter cartilage metabolism. Evenmore, it can be activated by inflammatory cytokines such as IL1β that can be present in synovial fluid of arthritic joints. PPARα activation seems to counteract IL1β induced responses, but only in explants of patients with a strong response to IL1β.

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Figure 1. Effect of W-14643 on mRNA expression in infrapatellar fat pad explants incubated during 48h with interleukin 1β and with(out) PPARα agonist Wy-14643. N=4. * indicates a p value of p<0.05 and *** p<0.001.

Figure 2. Effect of Wy-14643 on mRNA expression in infrapatellar fat pad explants incubated during 48h with interleukin 1β and with(out) PPARα agonist Wy-14643. N=4. ** indicates a p value of p<0.05 and *** p<0.001.

In the presence of IL1β, the effect of 100 μM Wy-14643 on infrapatellar fat pad explants was highly dependent on the response of the explants to IL1β for TNFα (p=0.01), MCP1 (p=0.001) and IL10 (p=0.01) (Figure 2). For these cytokines, IL1β stimulation was counteracted by 100 μM Wy-14543 only in the adipose tissue explants of donors that had a strong response to IL1β by producing more cytokines. No effect of 100 μM Wy-14643 was seen on IL6 gene expression (p=0.93). Similar results were observed when 10 μM Wy-14643 was added for TNFα (p=0.001), MCP1 (p<0.001), IL10 (p=0.001) and IL6 (p=0.01).

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