INTRODUCTION:
Traditionally, obesity has been viewed primarily as a mechanical risk factor for osteoarthritis (OA) through increased joint loading and altered kinematics. The last decade, however, has produced a rising understanding of the systemic role of adiposity in numerous inflammatory disorders, including rheumatoid arthritis, and of the roles of biologically active adipokines in the development of OA [1]. The infrapatellar fat pad (IFP) and synovium have been identified as major sources of proinflammatory factors including IL-6, leptin, and visfatin [2]. In addition, elevated levels of adipokines in both the serum and synovial fluid are associated with increased progression or severity of OA [3]. Co-culture of cartilage and synovium profoundly reduced cartilage sGAG synthesis, which was not affected by IL-1 or TNF-alpha blockade [4], hinting that unidentified factors secreted by the synovium can alter cartilage homeostasis. To the authors’ knowledge, there has not been previous investigation of the direct interaction between the IFP and articular joint tissues. The aim of this study was to determine how factors secreted by the IFP may alter cartilage and meniscus matrix production through the co-culture of different tissue types.

METHODS:

Tissue Culture: Articular cartilage (AC) and meniscal fibrocartilage (MFC) explants (4mm diameter) were obtained from the medial femoral condyle and meniscus of two immature bovine stifles. Fat tissue was excised from the infrapatellar fat pad. Following a 24hr culture period in high glucose DMEM supplemented with HEPE, NEAA, ITS, ascorbate-2-phosphate, and antibiotic/antimycotic, AC and MFC explants were trimmed to 2mm thickness leaving the surface layer intact. To obtain a constant volume of fat for each sample, the fat tissue was cut to fill custom wells sized 6mm in diameter and 3mm in depth. Tissue explants were allocated to five groups (Fat, MFC, MFC+Fat, AC, AC+Fat; Fig. 1) with eight samples per group (four from each animal). The explants were cultured for 14 days in medium composed of high glucose DMEM supplemented with HEPE, NEAA, ITS, ascorbate-2-phosphate, and gentamicin with media changes every 48hrs. Biological assays: At the end of the experiment, wet and dry masses of explants were measured. Conditioned media were assayed for sulfated glycosaminoglycan (sGAG) content using the DMMB assay with chondroitin sulfate standards. Sulfated glycosaminoglycan release rate for each well was determined via linear regression to the cumulative release data vs. time. Data are presented as mean +/- SEM. Data were analyzed using general linear models after Box-Cox transformation, with Bonferroni’s test for pairwise comparisons and significance at p<0.05.

RESULTS:
There was progressive release of sGAG from all groups throughout the 14-day culture with clear differences among the sample groups (Fig. 2). Release rates for Fat (0.78±0.09ug/day) and MFC (1.56±0.40ug/day) explants cultured alone did not significantly differ, while the release rate for AC explants cultured alone (11.8±1.2ug/day) was significantly higher than for other tissues. The sGAG release rate for the MFC+Fat co-culture (4.13±0.33) was significantly higher than either tissue cultured alone, and was nearly double the combined rates of release from isolated MFC and Fat. Likewise, the sGAG release rate for the AC+Fat co-culture (24.2±1.7 ug/day) was significantly greater than either tissue cultured alone, and was also nearly double the combined rates of release from isolated AC and Fat. All cultured Fat explants lost approximately 60% of their initial wet mass over the two-week culture period, with no significant differences in wet or dry mass among culture groups (Fig. 3). Cultured MFC explants did not significantly differ in wet or dry mass from baseline (Day 0) or between isolated and co-cultured groups. Cultured AC explants swelled significantly over the two weeks to nearly double their initial wet mass, and the cultured groups’ dry masses were notably greater than baseline. Neither wet nor dry mass varied notably between isolated and co-cultured AC explants.

SIGNIFICANCE:
Obesity is a growing epidemic currently affecting over a third of U.S. adults and 50% of adults over 65 years have diagnosed arthritis. In light of new understanding regarding the systemic impact of adipose tissue, this study provides insight into the connection between these two diseases and the pathology of OA.

REFERENCES:

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