non-naturally distributed biomarker outcomes (MMP3) and days between injury and ACLR.

**Results:** Days between ACL injury and surgery ranged from 9 to 67, with a mean 31.0 ± 14.4 days. Greater days between ACL injury and ACLR was associated with greater C2C:CPII ratios at the six-month follow-up exam after ACLR (0.15 ± 0.02, r = 0.46, P = 0.03). All other associations between biochemical markers and days between injury and ACLR were weak (r ranged between -0.006 and -0.075, all p > 0.05).

**Conclusions:** Individuals who waited a greater number of days between ACL injury and ACLR and increased C2C:CPII at 6 months after ACLR. It will be examined after ACLR (0.15 ± 0.02, r = 0.46, P = 0.03). All other associations between biochemical markers and days between injury and ACLR were weak (r ranged between -0.006 and -0.075, all p > 0.05).

**Purpose:** The discovery and validation of arthritis-related biomarkers and establishment of methodology for proteomic studies in osteoarthritis (OA) are needed. Proteomics strategies have identified many proteins that may relate to pathological mechanisms of OA, however targeted approaches are required to validate the roles of these proteins. This study aimed to use mass spectrometry and western blotting to identify peptides from several proteins in the secretome of chondrocytes, cartilage explants and osteochondral biopsies treated with inflammatory cytokines over a 2-week period, to evaluate their potential as biomarkers of OA progression.

**Methods:** Healthy cartilage was obtained from fetlock joints of skel- etally mature horses, euthanized for unrelated veterinary reasons. Cartilage explants were isolated using a 6 mm biopsy, with discs placed into wells (3 discs per 1 ml DMEM + 1% Pen/Strep) before incubation for 24 hours (37 °C, 5% CO₂). After this equilibration period, the media was removed and replaced with either fresh DMEM + 1% Pen/Strep or DMEM supplemented with 1% Pen/Strep containing TNF-α and IL-1β at both 10 ng/ml. Explants were culture for 7–14 days with the cyto- kines replaced every 4th day. For cell based assays chondrocytes were isolated and cultured using 70U protease for 1hr at 37 °C and overnight digestion at 37 °C using a 0.2% collagenase II solution. The cell suspension was filtered and washed before being seeded into culture flasks and cultured until confluence was reached (37 °C, 5% CO₂). Once cultures were established cells were split into two groups: healthy control (DMEM supplemented with 1% Pen/Strep and 10% foetal calf serum) or stimulated cells (DMEM as above plus TNFs and IL-1β both at 10 ng/ml). Chondrocytes were cytokine-stimulated for up to one week. Cells were used in experiments up to the 2nd passage.

**Results:** Mass spectrometry data showed that peptides representative of chondrocyte were found to decrease following 7 days of inflammatory stimulation. Western blotting of secreted proteins in media of cartilage explants or chondrocyte showed that chondrocyte expression was reduced following 7 days of cytokine treatment. Cytosolic matrix metal- loproteinase enzymes MMP1, MMP3 and MMP13, as well as the matrix component cartilage oligomeric protein (COMP) were all found to have an increased abundance in the media of the cytokine treated samples. This data was supported by qPCR for collagen gene expression which showed initially mRNA levels increased 3 days after inflammatory stimulation but expression was lost after 7 days. Western blotting of media from the osteochondral biopsies showed an increase in collagen expression after 7 days of inflammatory stimulation however chondrocyte protein expression could not be detected after 14 days of treatment, indicating a delayed response compared to cartilage tissue alone.

**Conclusions:** The equine chondrocytes, cartilage explant and osteo-ochondral biopsy models exhibited highest chondrocyte secretion in untreated cultures. IL-1β and TNF-α treatment caused a reduction in collagen secretion. Chondrocytes act as a chaperone to aid protein refolding in situations of stress and is constitutively secreted by mammalian cells. IL-1β and TNF-α appear to interrupt collagen secretion and therefore the protection it may offer healthy functioning cells. Previous studies have not reported variable data, with some studies indicating a decrease in collagen in OA, while others indicate an increase in collagen expression. Our results suggest the collagen increases immediately after inflammatory stimulation but is lost after prolonged exposure. Therefore, levels of secreted collagen may be a candidate biomarker for OA progression.