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enzymes potential targets for studying cartilage oxidative homeostasis. Our ongoing studies are assessing the changes.

Table 1. Antioxidant Proteins

Protein Name	Abbreviation	Detected
Aldose reductase	Akrib1	X
Aldehyde dehydogenase	Aldh2	X
Catalase	Cat	X
Glutathione peroxidase 1	Gpx1	
Phospholipid hydroperoxide glutathione peroxidase	Gpx4	X
Glutathione reductase	Gsr	X
Glutathione S-transferase alpha-3	Gsta3	X
Glutathione S-transferase Mu 1	Gstm1	X
Glutathione S-transferase P	Gstp1	X
Peptide methionine sulfoxide reductase	Msra	
NAD(P) transhydrogenase	Nnt	
Peroxiredoxin 1	Prdx1	X
Peroxiredoxin 2	Prdx2	X
Peroxiredoxin 3	Prdx3	X
Peroxiredoxin 4	Prdx4	X
Peroxiredoxin 5	Prdx5	X
Peroxiredoxin 6	Prdx6	X
Superoxide dismutase-1	Sod1	X
Superoxide dismutase-2	Sod2	X
Thioredoxin	Txn1	X
Thioredoxin reductase 1	Txnrd1	

453
NOVEL BIOINFORMATIC APPROACHES FOR IDENTIFYING PUTATIVE OA BIOMARKERS: LABEL-FREE QUANTIFICATION OF PROTEINS IN THE SECRETOME OF ARTICULAR CARTILAGE

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Purpose: The aim of this study was to develop bioinformatic methods for label-free quantification of proteins identified in the secretome of canine articular cartilage using high throughput tandem mass spectrometry.
Methods: Cartilage was obtained from animals euthanized for purposes other than research. Canine cartilage explants were pre-incubated in serum-free DMEM supplemented with 2% penicillin and streptomycin in a CO_2 incubator for 24 hours at 37°C. The explants were then incubated alone (control media), or with recombinant canine IL-1β (10 ng/ml), the non-steroidal anti-inflammatory drug carprofen (Rimadyl, Pfizer Animal Health, 1 mg/ml) or carprofen and IL-1β combined (1 mg/ml and 10 ng/ml respectively). After 5 days in culture, cell-free supernatants were removed and representative samples were selected for proteomic analysis. Mascot was used to analyze the data from each sample, with the Uniprot database, and the results were imported into the Trans-Proteomic Pipeline (TPP). TPP includes both PeptideProphet and ProteinProphet for evaluation of the Mascot assignments. Two label-free spectral counting based quantification software, PepC and APEX, were then used to analyze the output of ProteinProphet. From the APEX results classification models were built, using WEKA, to differentiate between control and IL-1β samples. A number of different classifiers were tested including Naive Bayes, support vector machines, C4.5, IBk and Random Forest and were evaluated using 10-fold cross validation.
Results: The label-free quantification methods identified a number of proteins as significantly different between the treatments. In particular matrix metalloproteinase-3 (MMP-3) and thrombospondin-1 (TSP-1) were increased in the IL-1β treated samples when compared to the controls. This supports previously primary experimental data from equine and canine explant models of articular cartilage. Other proteins increased in IL-1β samples included cartilage oligomeric matrix protein (COMP) and triosephosphate isomerase (TPIS). The classification models built using WEKA were able to accurately label the control and IL-1β samples. The Naive Bayes method performed best and correctly assigned all samples to their respective treatments. Classification was also performed on all four treatments, however the similarity between the control and carprofen only treated samples decreased the classification accuracy.
Conclusions: The label-free quantification methods for analysis of mass spectrometry data discussed here have been found to be suitable for determining potential biomarkers of OA and for differentiating between control and IL-1β treated samples using machine learning. The methods may now be implemented on larger datasets to support these results and determine any further potential biomarker. The classification method developed may also be used to identify novel biomarkers of OA, as the proteins used in the machine learning models were found in significantly different quantities across the different treatments.

Rehabilitation

454
NEUROMUSCULAR ADAPTATION IN PEOPLE WITH KNEE OA

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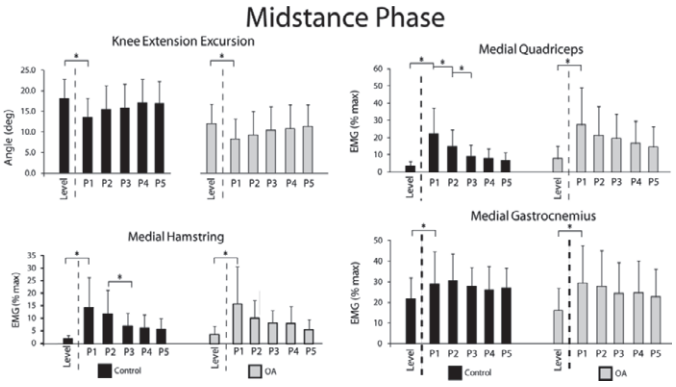
Purpose: Treatment interventions aimed at restoring dynamic knee stability are gaining popularity for people with knee OA. These "neuromuscular training" programs involve activities that challenge knee stability in a safe and controlled manner. The underlying premise is that patients use trial and error practice to learn joint control strategies that can transfer to daily activities. When a perturbation is applied to the limb during locomotion two types of responses occur. Reactive responses rely on sensory feedback during or shortly after the disturbance in order to restore balance. Proactive responses are those that occur prior to the onset of the next disturbance and are thought to minimize the destabilization brought on by the disturbance. Proactive responses represent the ability of the nervous system to predict the effect of the disturbance and adapt motor output accordingly. Sensory input is important for both responses, so people with OA who have been shown to have impaired sensory feedback from the knee joint may be less able to adjust their motor patterns in response to destabilizing events making neuromuscular training less effective. The purpose of this study was to investigate to better understand the ability of the nervous system to adapt motor responses in spite of impaired sensory feedback from the knee.
Methods: Knee joint motion and muscle activation patterns were collected from 14 OA and 17 control subjects as they walked over a platform that translated laterally 5.8 cm at a speed of 40 cm/s at heel strike. Ten trials in which the platform remained locked and 30 trials in which the platform translated were collected. Linear envelopes were created from EMG from the quadriceps, hamstrings and gastrocnemius muscles and averaged over 100 msec prior to HS (preactivation) and midstance (MSt). A Group by Condition Mixed ANOVA was used to make the comparisons.
Results: Both OA and C subjects reacted to the first experience of the translation by landing in more flexion with higher EMG responses ($p \leq 0.002$); no differences were observed between OA and Controls ($p > 0.05$). During MSt a main effect for trial number was observed for all variables ($p = 0.000$) (Figure) but no group differences or interaction effects were observed ($p > 0.05$). When comparing the adaptation that occurred in knee extension excursions and levels of muscle activation no differences were observed by group ($p > 0.05$).



Conclusion: The results demonstrate similar reactive and proactive responses to perturbations that challenge the knee in both OA and Control subjects which suggests that sufficient afferent feedback was present to allow all of the subjects to detect the error associated with