without an interaction between condition and experiment. Because this effect was observed for all conditions, the observed marker kinetics support the theory that load affects the immediate mechanoresponse of sCOMP in the walking stress test.Differences in median relative sCOMP increase after the walking stress between the 120%BW and 100%BW condition in both experiments agree with our expectations. Smaller median differences in load-induced changes in sCOMP in experiment 2 than in experiment 1 were surprising. Walking speed was self-selected before each experiment and kept constant for all walking stress tests within each experiment. Although not statistically significant, the mean self-selected walking speed was 0.08 m/s faster in experiment 2 than in experiment 1 resulting in a small but significant reduction in stance time. We assume that such a subtle difference in stance time will not alter the characteristics of the applied load during the walking stress. Because the used load modification framework enables us to systematically alter applied load without changing other load characteristics and reproduces similar spatiotemporal gait parameter (cadence, step time), this framework is suitable to study biomarker kinetics at repeated time points. The consistently greater mechanoresponse for the 120% than the for the 100%BW condition further strengthens the value of employing these conditions in future studies on in vivo mechanosensitivity of articular cartilage. The results shown here suggest that applying additional load results in greater and more consistent effects on the mechanoresponse of sCOMP than unloading conditions. In daily life, healthy persons rarely apply partial weight bearing possibly explaining inconsistent differences in the mechanoresponse of sCOMP to this condition compared to normal body weight.Overall, we cannot attribute the observed variability between experiments to any specific factor. It remains unknown whether the observed differences in the mechanoresponse of sCOMP to the walking stress between experiments are caused by true changes in the participants' physiology or metabolism. Future studies should address short-term repeatability of this experimental framework and elucidate the effect of physical activity, injury, age or other potential confounders on sCOMP kinetics after a walking stress. Although we presented first evidence for long-term repeatability of load-induced changes in sCOMP concentration for different loading conditions, this study should be considered as pilot study because it was limited to only eight subjects. Nonetheless, the data presented here is critical for interpreting data on the mechanoresponse of sCOMP to different loading regimes in a research and clinical context.

Acknowledgements: The authors thank Karin Wild, David Koch and Michael Hächler for their help in the experiments.

Funding: This project was funded in part by the Department of Orthopaedics and Traumatology (University Hospital Basel, Switzerland) and the Swiss National Science Foundation, Switzerland (#320030_184912). The funding sources were not involved in any aspect of the study.

PRESENTATION NUMBER: 183
CHARACTERIZING THE EFFECT OF APPA ON TISSUE TURNOVER IN CARTILAGE AND BONE TISSUE CULTURES

C.S. Thudium [1], A.-C. Bay-Jensen [1], T. Gantzel [2], M.H. Dziegiel [3], N. Larkins [4], A. Reynolds [4]. [1] *Nordic BioSci. A/S, Herlev, Denmark;* [2] *Gentofte Univ. Hosp., Gentofte, Denmark;* [3] *RigsHosp.et, Copenhagen Univ. Hosp., Copenhagen, Denmark;* [4] *AKL Res. and Dev. Limited, Stevenage, United Kingdom*

Purpose: Osteoarthritis is a debilitating disease characterized by gradual deterioration of the structural components of the joint, including cartilage and bone. Currently no disease modifying osteoarthritis drugs are available to halt or change the course of the disease. APPA is being developed for the treatment of osteoarthritis (OA) and other pain-related and inflammatory conditions. It consists of a combination of apocynin and its isomer paeonol. The objective of the study was to investigate the effects of APPA on structural tissue turnover assessed by quantifying biochemical markers of extra-cellular matrix remodeling in an ex vivo model of human OA cartilage and an in vitro model of human osteoclast differentiation and resorption.

Methods: Full depth human cartilage explants (HEX) were treated were stimulated with 10 ng/ml oncostatin M and 20 ng/ml TNF-α (O+T) with or without APPA in a 3-fold dose range from 6 mM to 0.22 mM three times a week for three weeks. Metabolic activity assessed by alamar blue was assessed weekly and at termination. The biomarker AGNx1 (aggrecanse mediated aggrecan degradation) was assessed by ELISA at day 0, 7, 14, and 21. The MMP inhibitor GM6001 was used as inhibitor control. Osteoclasts were generated from human CD14+ monocytes isolated from buffy coats. Monocytes were stimulated 3 days with 25ng/ml M-CSF followed by differentiation into mature osteoclasts by treatment with additional 25 ng/ml RANKL with or without APPA. APPA was added in a 3-fold dose range from 6mM to 25μM three times a week for 11 days. Differentiation was assessed by measuring TRAP activity in the media and TRAP stained cells were qualitatively assessed for number of mature osteoclasts. Bone resorption was assessed in mature osteoclasts by reseeding mature osteoclasts on bovine cortical bone after differentiation with M-CSF and RANKL. Osteoclasts were then treated for up to 5 days in a setup similar to that of the differentiation setup. Osteoclast numbers were assessed by TRAP activity and staining, and bone resorption was assessed by CTX-I on day 5 of bone resorption.

Results: In human explants O+T increased metabolic activity at day 7 and 14. The highest dose of APPA reduced metabolic activity compared to both O+T and unstimulated explants, while a dose dependent but non-significant reduction was observed for the remaining doses, with levels maintained above those of unstimulated explants. APPA significantly and dose dependently reduced AGNx1 by approximately 50-80% at day 7 in all doses except 0.22mM. A similar trend was observed at day 14 although this only reached statistical significance for the 6mM dose. In differentiating osteoclasts APPA significantly reduced metabolic activity at 6 and 2 mM after 11 days by 100% and 68% respectively, while no effect was observed for the remaining doses. TRAP activity was significantly and dose dependently decreased by approximately 20-50% with APPA in all but the 25μM dose, indicating impaired osteoclastogenesis even at the lower doses. In mature osteoclasts seeded on cortical bone, APPA reduced metabolic activity at 6 and 2mM by 89% and 57%, respectively. APPA reduced TRAP activity significantly and dose dependently in all tested doses. The reduction in TRAP activity was associated with a dose dependent reduction in TRAP stained mature osteoclasts. Similarly, a dose dependent reduction in CTX-I was observed for the 6mM (80%) %, 2mM (73%) and 0.67mM (46%) doses, while a dose dependent trend was seen for the remaining doses.

Conclusions: APPA reduces inflammation derived tissue turnover in human cartilage explants and inhibit RANKL mediated osteoclastogenesis and bone resorption by human osteoclasts. These findings indicate that APPA modulates chondrocyte and osteoclast cellular function and may inhibit pathological mechanism associated with joint degradation in arthritic disease.

PRESENTATION NUMBER: 184
THE NC1 FRAGMENT OF TYPE X COLLAGEN MEASURED IN SERUM AS A POTENTIAL BIOMARKER OF OSTEOARTHRITIS

Y. He, M. Karsdal, A. Bay-Jensen. *Nordic BioSci., Herlev, Denmark*

Purpose: The chondrocytes taking a terminal differentiation route of hypertrophy, invasion of blood vessels from the subchondral bone, apoptosis, and calcification of cartilage has been observed in experimental models of OA and human osteoarthritis (OA). Hypertrophy-like changes in chondrocytes are recently believed to play a role in the initiation and progression of cartilage degeneration. Type X collagen, a well-known marker of hypertrophy chondrocytes, plays a role in facilitating and regulating endochondral ossification of cartilage. We aimed to develop an immunoassay that detects the released NC1 fragment of type X collagen in the serum of healthy donors and OA patients.

Methods: A sandwich chemiluminescence immunoassay, designated COL10NC, detecting the cleaved NC1 domain of type X collagen was developed. A biotinylated antibody against a newly discovered cathepsin K-generated neoepitope ^{479}GIATKG (one amino acid apart from the well-known cleavage site ^{480}IATKG generated by collagenase) was employed as a capture. A horseradish peroxidase (HRP) labeled antibody to target the C-terminus of the NC1 domain was used as a detector. The COL10NC assay was validated to assure the reliability of the measurement. To characterize the biological relevance of the assay, 69 serum samples from normally growing infants and children from birth