by RT-PCR. The levels of c-fos and NFATc1 protein were assessed by western blot. Also the mitogen-activated protein (MAPK)s and important signal pathways were measured using Western blot analysis. Osteoclast function was evaluated with resorption pit assay and osteoblastic effects of Cldn11 was evaluated with new bone formation of mouse calvaria. With LPS and co-treated Recombinant protein of Cldn11 on mouse calvaria, we evaluated the effects of Cldn11 on LPS induced bone loss by using histologic analysis.

Results: We found that Cldn11 played a negative role in receptor activator of nuclear factor kappa B ligand dependent osteoclast (OC) differentiation by down-regulating the phosphorylated form of extracellular signal-regulated kinase (ERK), Bruton's tyrosine kinase, and phospholipase C gamma 2, in turn impeding c-Fos and nuclear factor of activated T cells c1 expression. Osteoblast (OB) differentiation by positive feedback of Cldn11 was achieved through the phosphorylation of Smad1/5/8, ERK, and c-Jun amino-terminal kinase. Importantly, this Cldn11-dependent dual event arose from targeting EphrinB2 ligand reverse signalling into OC and EphB4 receptor forward signalling into OB. In agreement with these *in vitro* effects, subcutaneous injection of Cldn11 recombinant protein exerted similar effects on local calvarial regions in mice.

Conclusions: These findings suggest that Cldn11 is a novel regulator in bone homeostasis.

Disclosure of Interest: None declared

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OP0264 GLUCOSEPANE : A NEW BIOMARKER OF THE SEVERITY OF OSTEOARTHRITIS

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Background: Glycation, oxidation and nitration of proteins are reactions involved in accelerated ageing of tissues. The products of these reactions are used as biomarkers of chronic pathologies such as diabetes or chronic inflammatory states.

Objectives: In this work, we studied by mass spectrometry the levels of amino acids and glycated, oxidised or nitrated proteins in culture media of chondrocytes cultivated in multi-layers and in the blood of guinea pigs or osteoarthritis patients.

Methods: Sixty male, 3-week-old Dunkin-Hartley guinea pigs were used in this work. At 4-weeks-old and 8 week intervals until week 36, twelve animals were sacrificed and histological severity of knee osteoarthritis evaluated and cartilage rheological properties. Human patients with early and advanced osteoarthritis and healthy subjects were recruited. Human chondrocytes cultured in multilayers were treated for 10 days with interleukin (IL) -1 β . Amino acids and glycated, oxidised and nitrated proteins were analysed in the serum of guinea pigs, osteoarthritis patients and in the culture medium conditioned by chondrocytes by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry using the Acquity UPLC system.

Results: Severity of osteoarthritis increased progressively in guinea pigs with age. Glycated, oxidised and nitrated amino acids were increased markedly at week 36. Glucosepane and dityrosine increased progressively from weeks 20

and 28, respectively. Glucosepane was positively correlated with the OA histological severity ($r=0.58$, $p<0.0001$) and the Young's modulus ($r=0.52-0.56$, $p<0.0001$). Oxidation free adducts were positively correlated with OA severity ($p<0.0009-0.0029$) and hydroxyproline with cartilage thickness ($p<0.0003-0.003$). In the clinical study, plasma glucosepane was increased 38% in patients with early osteoarthritis ($p<0.05$) and 6-fold in patients with advanced osteoarthritis ($p<0.001$) compared to healthy subjects. IL-1 β increased the release of glycated, oxidised and nitrated products from chondrocytes *in vitro*.

Conclusions: The glycation, oxidation and nitration of proteins are reactions related to the severity of osteoarthritis. The products of these reactions are measurable in blood by mass spectrometry and could be biomarkers of osteoarthritis. More specifically, glucosepane is an advanced glycation product very strongly increased in the severe form of the disease. In conclusion, serum glucosepane is a potential biomarker for diagnosis and progression of osteoarthritis.

Disclosure of Interest: None declared

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OP0265 LOCAL REACTIVATION OF GLUCOCORTICOIDS BY 11 β -HYDROXYSTEROID DEHYDROGENASE TYPE 1 MEDIATES THE DEVELOPMENT OF GLUCOCORTICOID-INDUCED BONE LOSS

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Background: Due to the potent immunomodulatory and anti-inflammatory nature of glucocorticoids (GCs) they are routinely used in the treatment of inflammatory diseases such as rheumatoid arthritis. However their therapeutic potential is limited due to the prevalence of adverse side effects associated with long term GC exposure such as osteoporosis, insulin resistance and obesity. 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) is a bi-directional enzyme that primarily converts inactive GCs to their active counterparts. Previously, local reactivation of GCs by 11 β -HSD1 has been shown to play a major role in the metabolic side effects associated with GC excess.

Objectives: We aim to assess whether local reactivation of GCs by 11 β -HSD1 mediates the adverse effects of therapeutic GCs on bone.

Methods: Wild-type (WT) mice and transgenic mice with a global 11 β -HSD1 knockout (11 β KO) were treated with the active murine GC corticosterone (CORT) (100 mg/ml) for 4 weeks. Tibia and humerus bones were excised post-mortem for micro-CT analysis, gene expression analysis and three point flexure strength (TFS) tests. Serum was collected from mice for ELISA analysis of TRAcP-5b and P1NP.

Results: Micro-CT analysis of bone volume to tissue volume (BV/TV), trabecular thickness (TT) and trabecular number (TN) found no significant differences between untreated WT and 11 β KO mice (BV/TV: WT 8.5% $\pm$ 0.66 vs 11 β KO 7.5% $\pm$ 0.76, NS; TT: WT 96.5 μ m $\pm$ 3.8 vs 11 β KO 95.8 μ m $\pm$ 6.4, NS; TN: WT 0.0009 1/ μ m $\pm$ 0.00004 vs 11 β KO 0.0008 1/ μ m $\pm$ 0.00004, NS). Humerus TFS tests of WT and 11 β KO animals also showed no significant differences (WT 51.2 MPa $\pm$ 15.1 vs 11 β KO 49.2 MPa $\pm$ 4.9, NS). All bone parameters were decreased in CORT fed WT mice indicating the development of osteoporosis, whilst 11 β KO mice were protected against many of the detrimental effects of CORT (BV/TV: WT 4.2% $\pm$ 0.38 vs 11 β KO 7.2% $\pm$ 0.71, $p\leq 0.05$; TN: WT 0.0006 1/ μ m $\pm$ 0.00004 vs 11 β KO 0.0009 1/ μ m $\pm$ 0.00008, $p\leq 0.001$; HBS: WT 27.1 MPa $\pm$ 5.6 vs 11 β KO $\pm$ 50 MPa $\pm$ 5.1, $p\leq 0.05$). ELISA analysis of mouse serum showed no significant differences in the bone resorbing osteoclast marker TRAcP-5b amongst the groups, whereas analysis of the bone forming osteoblast marker P1NP revealed a significant increase in CORT fed 11 β KO mice compared with CORT fed WT mice (11 β KO 158.6 ng/ml $\pm$ 53.1 vs WT 31.4 ng/ml $\pm$ 7.4, $p\leq 0.05$). Gene expression of the mature osteoblast markers ALP (alkaline phosphatase) and BGLAP (osteocalcin) showed significant increases in CORT fed 11 β KO animals compared to CORT fed WT animals (ALP: 11 β KO 0.0074 AU $\pm$ 0.0012 vs WT 0.0022 AU $\pm$ 0.0007, $p\leq 0.01$; BGLAP: 11 β KO 0.27 AU $\pm$ 0.04 vs WT 0.02 AU $\pm$ 0.01, $p\leq 0.001$). No significant differences were observed between untreated WT and 11 β KO animals.

Conclusions: These data suggest that local reactivation of GCs by 11 β -HSD1 mediates the development of glucocorticoid-induced osteoporosis by inhibiting osteoblastic bone formation.

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