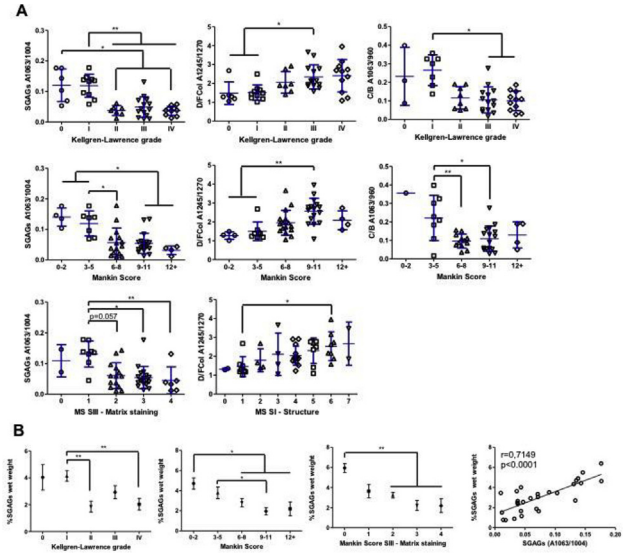

Figure 1. A: Scatter dot plots (mean + SD) for SGAGs, D/FCol and C/B Raman ratios for different categories of K-L grade, Total MS, MS-SI and MS-SIII. B: Biochemical results for SGAGs (wet weight percentage) for different categories of KL grade, total MS, and MS-SIII, and correlation between SGAGs ww% and SGAGs 1063/1004 Raman ratio.*p < 0.05 and **p<0.01.

PG, 1245/1270 for defective/functional collagen-D/FCol and 1063/960 for cartilage-to-bone ratio-C/B) were calculated, after intensity normalization to 1004 cm^{-1} band. SGAGs wet weight percentage (ww%) was quantified using the Blyscan kit according to manufacturer' instructions (Biocolor). Kruskal-Wallis tests and Spearman's correlation coefficients (rho) between spectral data and K-L, MS and SGAGs ww% were calculated using R statistical open software (version R 3.5.1). $P<0.05$ were considered statistically significant.

Results: In this work, we analyzed the potential of specific RS signals related to cartilage main components, namely SGAGs and collagen as well as the cartilage functional matrix to phosphate mineralization (C/B) ratio as optical biomarkers using an *ex vivo* model. We found that the relative content of SGAGs (Figure 1A) decreases with the increase in disease severity (K-L), more significantly for KL I vs ≥II. Similar results were found when analyzed against histopathological MS (total and SIII) and consistent with SGAGs biochemical analysis (Figure 1B). Correlations for this parameter were moderate to strong (rho= -0,632 vs KL, -0.642 vs total MS and 0.7149 vs SGAGs ww%, all with p<0.001). Other signals related with PG (1375/1004) presented fair to moderate correlations (rho= -0.532 vs KL, -0.484 vs total MS and 0.549 vs SGAGs ww%, all with p<0.01). D/FCol parameter revealed an increase in collagen structure disorganization (defective col) with the increase in either KL or MS (total and MS-SI) with moderate correlations found (rho= 0.529 vs KL and 0.593 vs total MS, both p<0.001). We also confirmed a C/B ratio decrease with increasing KL and TMS, with a fair correlation regarding KL (rho= -0.426 and p=0.005) whilst no significant correlation was found between C/B and total MS.

Conclusions: With this study we have demonstrated significant evidence of molecular alterations involving SGAGs, collagen and C/B ratio during OA progression, validated against both a well-established clinical scoring system and cartilage histological and biochemical quantification. According to these results we suggest the use of the described parameters as an optical biomarker profile for osteoarthritis diagnosis.

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SERUM AGGRECAN FRAGMENT ARGS NEOEPITOPE IS ASSOCIATED WITH AGE AND STRUCTURAL SEVERITY IN OSTEOARTHRITIS

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Purpose: ARGS neoepitope (ARGS) originates from aggrecan, a proteoglycan abundant in cartilage of high importance for structure and function. ARGS is a product of A Disintegrin and Metalloproteinase with Thrombospondin Motifs 4 and 5 (ADAMTS-4, -5) enzymatic degradation of aggrecan. ARGS has mainly been investigated in synovial fluid (SF) of knee trauma subjects, where it has been observed to increase following injury. Furthermore, studies have indicated that ARGS in SF may also be increased in knees affected by OA. However, studies on ARGS as a biochemical marker in serum are sparse. We aimed to investigate the correlations between sARGS and OA patient characteristics.

Methods: The data is a cross-sectional analysis of the baseline data of a randomized cross-over trial (EFEX-OA) where OA patients were included in different sessions of physical activity. ARGS neoepitope was measured in the serum of patients with x-ray verified primary OA of the knee. Samples were acquired before noon. Participants were required to be in fasting state. sARGS levels were measured by sandwich ELISA. Correlation analyses were made using multiple regression analysis adjusting for age, sex, body mass index (BMI), Kellgren Lawrence (KL) grade and sARGS where applicable. Univariate graphs were plotted for the purpose of visualization.

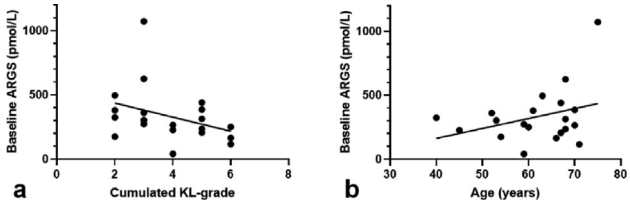

Table 1. Subject characteristics

	OA subjects (N = 20)
Mean age, years (R)	61.8 (9.1)
Male sex, n (%)	5 (25)
Mean BMI (kg/m^2) (SD)	26.3 (3.4)
Highest KL-grade at baseline, n (%)	1: 3 (15) 2: 8 (40) 3: 9 (45)
Mean ARGS (pmol/L) (SD)	332 (214)
Mean KOOS Pain (100-0) (SD)	73.0 (12.6)

Results: The subject characteristics are shown in Table 1. Mean age was 61.8 years, 25% were male, mean BMI was 26.3 kg/m^2, KL grade ranged 1-3, mean sARGS level was 332 pmol/L and the mean KOOS pain score was 73.0. In a multivariate analysis age ($r = 0.67$, $p = 0.003$) and cumulated KL grade ($r = -0.69$, $p = 0.002$) correlated with sARGS, indicating that higher age is associated with higher ARGS, and higher total KL grade is associated with lower sARGS. Correlations are shown in figure 1a-b.

Conclusions: In this study, we found a positive correlation between age and sARGS and a negative correlation between cumulated KL-grade and sARGS. The results suggest that sARGS may be a useful biomarker in OA research. The biomarker may play a role in identification of progressors and in monitoring target engagement with DMOAD candidates such as aggrecanase inhibitors.

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CARTILAGE BIOMARKERS S-COLL2-1 AND S-COLL2-1NO2 ARE HELPFUL IN IDENTIFYING KNEE OSTEOARTHRITIS PATIENTS AT RISK OF DISEASE WORSENING

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Purpose: To identify if biochemical markers s-Coll2-1 and s-Coll2-1NO2 are associated to knee osteoarthritis (OA) phenotypes, focusing on pain, function as well as structural features assessed by MRI in various knee compartments and to assess their ability at predicting knee OA worsening.

Methods: 116 subjects with knee OA were followed during one year with pain, function and MRI evaluation (PRODIGE study, NCT02070224). Type II collagen-specific biomarker Coll2-1 and its nitrated form Coll2-1NO2 were directly measured in serum using immunoassays at baseline and after three, six and twelve months follow-up.

Results: sColl2-1 and sColl2-1NO2 were associated to several baseline knee features quantified with Whole-Organ Magnetic Resonance Imaging Score (WORMS). S-Coll2-1 was significantly correlated with bursitis (r=0.29, P<0.01), bone attrition (r=0.25, P=0.01), cysts (r=0.24, P=0.02) and cartilage (r=0.23, P=0.03) WORMS sub-scores for the whole joint as well as with the medial femorotibial joint sum score (r=0.26, P=0.01) and medial femorotibial joint cartilage (r=0.23, P=0.02). s-Coll2-1NO2 was correlated with WORMS total score (r=0.23, P=0.02), WORMS scores in the patellofemoral (r=0.23, P=0.02) and medial femorotibial compartments (r=0.21, P=0.03) and with osteophytes scores (r=0.27, P<0.01). Baseline s-Coll2-1NO2 was higher in subjects with a pain worsening (426.4 pg/mL, 278.04-566.95) as compared to non-progressors (306.84 pg/ml, 200.37-427.84) over one year (AUC=0.655, P=0.015).
Conclusions: Cartilage biomarkers s-Coll2-1 and s-Coll2-1NO2 are associated to several knee OA features quantified with WORMS scoring system on MRI. Serum values of Coll2-1NO2 are also associated to a worsening of target knee pain over one year. Coll2-1 and Coll2-1NO2, in association with other structural features, pain and function, could help at identifying OA phenotypes and patients at risk of OA worsening.

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ASSOCIATION OF INFLAMMATION, INSULIN RESISTANCE, DYSGLYCEMIA, ADIPOKINES WITH MULTIPLE JOINT OSTEOARTHRITIS : OSTEOARTHRITIS INITIATIVE

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Purpose: Knee and hip OA are largely mechanical driven, but it has been hypothesized that hand OA and multiple joint OA (MJOA) may be associated with abnormal metabolic processes such as inflammation, insulin resistance, dysglycemia and adipokines. We tested this hypothesis in a sample of the Osteoarthritis Initative (OAI).
Methods: In a sub-cohort of OAI participants oversampled for incident hand OA (n=1770) bilateral knee and hips xrays and hand xrays in the dominant hand were performed and read at baseline. Hand OA was defined as at least one joint with Kellgren-Lawrence (KL)≥2 on at least two rays excluding the carpalmetacarpal joint ; Hip OA was defined as a modified Croft Grade ≥ 2 in either hip, Knee OA as KL≥2 in either knee. Biomarkers of inflammation (hs-CRP, cytokines interleukin-1 β, IL-5,7,8,18), insulin resistance and dysglycemia (glucose, insulin, glycated serum protein(GSP), HOMA-IR), adipokines (resistin, adiponectin, leptin) were measured using standard methods at the *HNRCA Clinical and Analytical Core Laboratory* which was blinded to OA status . Biomarkers were log transformed for analysis and untransformed in the table below for ease of interpretation. We used analysis of covariance adjusting for age, race and sex to test for associations between levels of biomarkers and type of OA.

Variable	Total (n=1770)	No OA (n=449)	Hand Only (n=273)	Knee or Hip (n=511)	Hand AND (Knee or Hip) (n=472)	Hand, Knee & Hip (n=65)	p-value	trend
Glucose (mg/dL) [MDL 10 mg/dL]	99.5 (1.30)	98.7 (1.30)	98.4 (1.30)	100.0 (1.30)	101.3 (1.30)	99.2 (1.30)	0.11	0.49
hs-CRP (mg/L) [MDL 0.2 mg/L]	1.69 (8.84)	1.39 (9.08)	1.66 (8.75)	1.96 (9.01)	1.81 (8.73)	1.69 (8.71)	**0.001**	0.15
Insulin (uIU/mL) [MDL 2.1 uIU/mL]	9.02 (3.38)	7.29 (3.43)	8.72 (3.36)	9.28 (3.41)	9.88 (3.35)	10.25 (3.39)	**<0.001**	**<0.001**
IL-18 (pg/mL) [MDL 2.5 pg/mL]	589.5 (2.45)	580.7 (2.48)	609.7 (2.44)	547.8 (2.47)	610.3 (2.44)	601.7 (2.44)	**0.02**	0.63
IL-7 (pg/mL) [MDL 0.6 pg/mL]	13.11 (1.88)	13.26 (1.89)	13.31 (1.87)	12.89 (1.89)	13.30 (1.87)	12.81 (1.87)	0.68	0.55
IL-8 (pg/mL) [MDL 0.1 pg/mL]	14.00 (1.94)	14.33 (1.95)	13.77 (1.93)	13.13 (1.95)	14.29 (1.93)	14.51 (1.93)	**0.002**	0.55
IL-1β (pg/mL) [MDL 0.2 pg/mL]	0.28 (1.89)	0.29 (1.90)	0.29 (1.88)	0.28 (1.90)	0.29 (1.88)	0.28 (1.88)	0.45	0.49
IL-5 (pg/mL) [MDL 0.3 pg/mL]	0.75 (2.85)	0.80 (2.89)	0.78 (2.83)	0.74 (2.87)	0.77 (2.83)	0.68 (2.83)	0.18	0.07
GSP (umol/L) [MDL 7.2 umol/L]	247 (1.41)	252 (1.42)	246 (1.41)	245 (1.42)	245 (1.41)	247 (1.41)	0.30	0.52
Resistin (ng/mL) [MDL 0.2 ng/mL]	8.82 (1.94)	8.87 (1.96)	8.86 (1.94)	9.36 (1.95)	8.73 (1.93)	8.30 (1.94)	**0.05**	0.21
Adiponectin (ng/mL) [MDL 3.9 ng/mL]	7487 (2.87)	8241 (2.91)	7585 (2.85)	7521 (2.89)	7215 (2.84)	6937 (2.85)	**0.03**	**0.02**
Leptin (pg/mL) [MDL 7.8 pg/mL]	15144 (4.16)	12549 (4.23)	15220 (4.15)	16194 (4.21)	15476 (4.12)	16637 (4.11)	**<0.001**	**0.004**
GSP to Insulin ratio	27.40 (3.67)	34.46 (3.71)	28.23 (3.64)	26.44 (3.70)	24.74 (3.63)	24.28 (3.67)	**<0.001**	**<0.001**
Glucose to Insulin ratio	11.08 (3.20)	13.56 (3.24)	11.29 (3.18)	10.79 (3.23)	10.23 (3.18)	9.87 (3.21)	**<0.001**	**<0.001**
HOMA-IR	2.22 (3.77)	1.77 (3.82)	2.12 (3.74)	2.29 (3.80)	2.47 (3.73)	2.56 (3.77)	**<0.001**	**<0.001**
Leptin to Adiponectin ratio	2.02 (7.12)	1.52 (7.29)	2.01 (7.07)	2.15 (7.25)	2.14 (7.03)	2.38 (7.02)	**<0.001**	**<0.001**

Results: Insulin resistance (insulin, GSP/insulin ratio, glucose/insulin ratio, HOMA-IR) and adipokines (adiponectin, leptin, leptin/adiponectin ratio) but not inflammation had monotonically increasing associations for multiple joint OA (See table) None of these association persisted after adjusting for BMI.
Conclusions: MJOA may in part be related to metabolic factors associated with insulin resistance and leptin resistance. Further prospective studies should evaluate these associations and determine if insulin resistance and leptin resistance may be important mechanisms explaining the association of adiposity with multiple joint osteoarthritis.

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NETWORK PHARMACOLOGY BASED INVESTIGATION INTO THE EFFECT AND MECHANISM OF GUIZHI DECOCTION AGAINST OSTEOARTHRITIS

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Purpose: To investigation action and molecular targets for Guizhi decoction (GZD) in the treatment of osteoarthritis(OA).
Methods: The active compounds of GZD were collected and their targets were identified((TCMSP, http://lsp.nwu.edu.cn/tcmsp.php ; DrugBank: https://www.drugbank.ca/). OA-related targets were obtained by analyzing the differential expressed genes between OA patients and healthy individuals(GEO database: https://www.ncbi.nlm.nih.gov/geo/ , Series: GSE1919, Samples: GSM34379, GSM34383, GSM34385, GSM34388, GSM34391, GSM34393, GSM34394, GSM34395, GSM34396, GSM34397). Protein-protein interaction (PPI) data were then obtained and PPI networks of GZD putative targets and OA-related targets were visualized and merged to identify the candidate targets for GZD against OA. Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analysis were carried out (DAVID, https://david.ncifcrf.gov, v6.8). The gene-pathway network was constructed to screen the key target genes.
Results: In total, 113 active compounds and 256 targets of GZD were identified. 2279 differential expressed genes with an were identified between OA patient and normal donor. 79 target genes associated with OA were finally identified. The functional annotations of target genes were found to be related to stress response, cell membrane, protease activity, and so on.TNF signaling pathway, Toll-like receptor signaling pathway, and apoptosis were significantly enriched. CALM1 was the core gene in the treatment of OA.

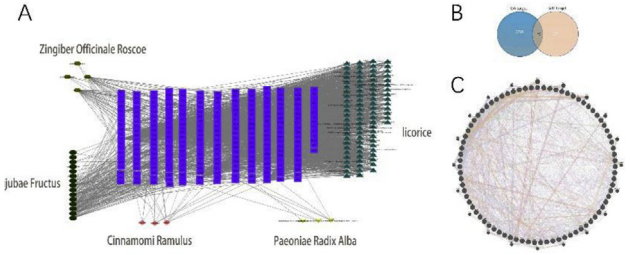

Figure 1 | A:Compound- target network of GZD. B:Distribution of GZD potential targets and OA targets. C: The interactive PPI network of GZD putative targets and OA-related targets

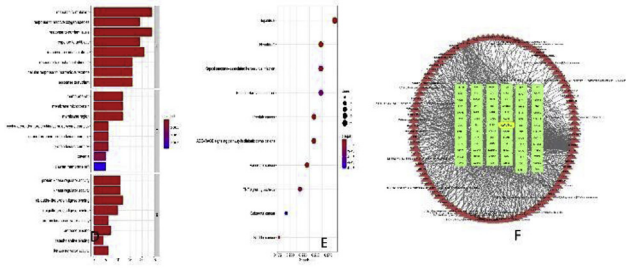

Figure 2 | D:Gene Ontology (GO) analysis.E:Reactome pathway analysis of genistein targets. F: Target-compound Network of GZD against OA.