Purpose: There is currently no cure for osteoarthritis (OA) and no therapy that will slow or arrest progression of this disease. Our previous studies identified a transcriptional regulator, Cbp/p300 Interacting Transactivator 2 (CITED2) that mediates chondroprotection, at least in part by suppressing expression of proteases including the matrix metalloproteinase MMP-13 and the aggrecanase ADAMTS5. Using a targeted screening approach, we found four nutraceuticals (carvacrol [C], curcumin [C], green tea polyphenol Epigallocatechin gallate [E], and oligomeric proanthocyanidins [O]), that individually increased the expression of CITED2 and reduced the expression of MMPs and ADAMTS in human chondrocytes in vitro. The objective of the present study was to determine the efficacy of the combination of these nutraceuticals (C-CEO) in chondroprotection in vitro and in vivo using an OA disease model that includes symptom modification.

Methods: Human chondrocyte culture: Human chondrocytic cells (C28/I2) were treated with Carvacrol (C, 1μM), Curcumin (C, 1μM), EGCG (E, 10μg/mL), OPC (O, 50μg/mL), or C-CEO (combination of all 4 compounds) for 24 hours in the presence of IL-1β (10ng/ml). Relative mRNA levels of MMPs 1, 3, 13, TnF-α, ADAMTS5, TNF-α, and CITED2 were analyzed by real-time PCR. n=6, * p<0.05 using one-way ANOVA and Tukey post-hoc test. Mice (C57BL/6, male, 6 months-old, n=8/group) were subjected to destabilization of the medial meniscus (DMM) and treated daily (7 days/week) with Carvacrol (C, 50mg/kg), Curcumin (C, 50mg/kg), Epigallocatechin gallate (E, 5mg/kg), oligomeric proanthocyanidins (O: 50mg/kg), C-CEO (combination of all 4 compounds), or vehicle for 8 weeks via oral gavage with naïve mice as an additional control. OA assessments: Arthritic pain was assessed at 8 weeks following DMM surgery using assays of von Frey (mechanical allodynia) and open field. OA severity was evaluated by OARSI scoring of Safranin O-stained tissue sections from decalciﬁed, formalin-ﬁxed hind limbs. Immunohistochemistry was used to detect cleaved aggrecan and type II collagen in the cartilage extracellular matrix and MMP-13, ADAMTS5, and CITED2 positive chondrocytes.

Results: In vitro, the combination treatment, C-CEO, was more potent than any individual compound in reducing mRNA levels of MMP-3, MMP-13, ADAMTS-5 and TNF-α and increasing CITED2 mRNA (Fig 1), indicating a synergistic chondroprotective effect of the 4 compounds as a potential OA therapeutic. In vivo, while each individual compound reduced OA disease progression, C-CEO-treated mice exhibited the lowest OA score and best preservation of the articular cartilage from thinning and surface damage compared to that observed in vehicle-treated DMM mice (Fig 2A). C-CEO also protected against type II collagen and aggrecan cleavage, and reduced MMP-13 and ADAMTS5 immunostaining (Fig 2B). Furthermore, C-CEO treatment restored the percentage of CITED2-positive chondrocytes from 28±3% (vehicle) to 55±6% (C-CEO; C-CEO vs. vehicle p<0.05), similar to that in naïve mice (51±3%, Fig 2C). With regards to pain, C-CEO-treated DMM mice, like naïve controls, required a higher force to elicit paw withdrawal (p<0.05, Fig 3A), traveled a longer distance (p<0.05, Fig 3B), and increased rearing (p<0.05, Fig 3C), indicating that C-CEO exerted a signiﬁcant relief of pain symptoms related to OA.

Conclusions: A novel nutraceutical formulation, C-CEO, increases levels of CITED2 and is effective in slowing OA progression and in relieving OA-related pain in an OA animal model. These ﬁndings provide a rational basis for advancing this product as an intervention for OA prevention and treatment.
Results: Participants reported taking (−) (n = 300) or not taking (−) (n = 300) OA treatment (analgesics/NSAIDs) over 24 months, with or without Glu/CS. The participants with meniscal extrusion had more knee OA structural damage at baseline and more knee OA progression as assessed by both X-rays and qMRI. Patients in both − and + analgesics/NSAIDs groups with meniscal extrusion had significantly greater cartilage volume loss in the medial compartment compared to those without meniscal extrusion.

No significant effect on JSW loss was found at T24 between − and + analgesics/NSAIDs groups, regardless of the presence or absence of meniscal extrusion and of the consumption or not of Glu/CS, whereas significant differences emerged for cartilage volume loss when examined by qMRI.

In the +analgesics/NSAIDs group (mild disease) with meniscal extrusion (more progressive disease) participants taking Glu/CS had less cartilage volume loss in the plateau (p = 0.01). Multivariate analyses further confirmed in the +analgesics/NSAIDs group, that those with meniscal extrusion who took Glu/CS had significantly less cartilage volume loss in the medial plateau (p = 0.009).

In the +analgesics/NSAIDs group, at T24, participants without meniscal extrusion (moderate disease) taking Glu/CS had less cartilage volume loss in the global (p < 0.002, univariate and multivariate analyses) and medial and lateral plateaus (p = 0.034, p = 0.013, respectively, multivariate analysis). However, in participants with meniscal extrusion (severe disease), although those taking Glu/CS had significantly less cartilage volume loss in the medial condyle at T24 in univariate analysis, this finding was not confirmed in multivariate analysis.

Conclusions: The presence of meniscal extrusion is an important factor that can influence the drug effect on cartilage volume. X-rays were found to be much less sensitive than MRI at documenting the protective effect of treatment on structural changes. Indeed, with qMRI, the presence of meniscal extrusion (moderate disease) taking Glu/CS had less cartilage volume loss in the medial condyle at T24 in univariate analysis, this finding was not confirmed in multivariate analysis.

Methods: Human synovial fibroblasts and chondrocytes were isolated from Dr. Martin K Lotz (The Scripps Research Institute, USA). Normal human knee synovial fibroblasts and chondrocytes were isolated from autopsy donors as leftover de-identified material and with no interactions with subjects, and therefore with no informed consent required. The human chondrocyte and synovial fibroblast was plated at 5 x 10^4 per well in 24 well plates. These cells were treated with 1, 5, 10, 25, 50 μM CA for 24 h. The expression of HO-1 and autophagy marker LC3 was analysed using immunoblotting. We examined whether the expression of CA-related genes COL2a1, AGGRECAN, IL-1β, IL-6, MMP-3, -13 and ADAMTS-5 can be regulated by CA, when synovial fibroblasts and chondrocytes were stimulated with IL-1β (1 ng/ml) for 24 h. To examine whether CA effects on prevention of cartilage degradation, we performed Glycosaminoglycan (GAG) release assay using mouse cartilage explants. Femoral heads were harvested from 4-week-old C57BL/6 mice and incubated at 37 °C for 72 h in 48-well plates. The cartilage samples were cultured for 72h in 500 μl of serum-free DMEM containing CA 10 or 50 μM with or without IL-1β. For studies on CA drinking, we evaluated the levels of HO-1 in response to CA drinking in the knee joints by immunohistochemistry. Furthermore, the senescence-accelerated mouse (SAMP8) was used as a primary OA, and 3-months old male SAMP8 mice were bred with free access to drinking water including CA (50 μM) for 10-months. The knee joints were stained with Safranin O and grade by the OA scoring systems.

Results: The expression of HO-1 was upregulated in human synovial fibroblasts and chondrocytes treated with CA in a dose-dependent manner. LC3 was not induced by CA. CA had no effect on the expression of Col2a1 and AGGRECAN in chondrocytes. Although the expression of IL-1β, IL-6, MMP-3, -13 and ADAMTS-5 was upregulated by IL-1β in the human synovial fibroblasts and chondrocytes, the expression of IL-1β, MMP-3, -13 was significantly downregulated by CA. In GAG release assay using cartilage explants, CA decreased proteoglycan release. Finally, to determine whether CA would be effective in OA model mice as a supplement, we performed the histologic evaluation of knee joints in SAMP8. Although the severity of cartilage destruction was trend towards differences in the CA drinking group compared with control group, none of the differences were statistically significant. HO-1 was not markedly induced by orally administered CA in joint tissues including cartilage, however, it suppressed age-related hepatic steatosis.

Conclusions: This study showed that CA induced HO-1 in synovial fibroblasts and chondrocyte and suppressed proteoglycan release from cartilage via downregulation of IL-1β, MMP-13. However, orally administered CA could not prevent spontaneous age-related OA. These findings suggest that CA is an effective HO-1 inducer and have a potential to be supplement for OA prevention.