To investigate the long-term effects (12 days) of avocado/soybean unsaponifiables (ASU) on the metabolism of human osteoarthritic (OA) chondrocytes cultured in alginate beads.

**Methods:** Enzymatically isolated OA chondrocytes were cultured in alginate beads in a well-defined culture medium for 12 days, in the presence or not of $10^{-10}$ M IL-$\beta$-$\beta$. The DNA content was measured according to a fluorimetric method. Aggrecan (AGG), stromelysin-1 (MMP-3), tissue inhibitor of metalloproteinases-1 (TIMP-1), macrophage inflammatory protein-1 beta (MIP-1$\beta$), interleukin (IL)-6, -8 and TGF-$\beta$1 production were assayed by specific enzyme amplified sensitivity immunoassays (EASIA). Prostaglandin (PG) E$_2$ was measured by a specific radioimmunoassay and nitrite by a spectrophotometric method based upon the Griess reaction. Avocado and soybean unsaponifiable residues were mixed in the ratio 1:2 (A$_1$S$_2$) or tested separately in a range of concentrations between 0.625 and 40 $\mu$g/ml.

**Results:** After twelve days of incubation, A$_1$S$_2$ increased in a dose- and time-dependent manner AGG synthesis and accumulation in alginate beads. Furthermore, A$_1$S$_2$ promoted the recovery of aggrecan synthesis after a three days period of IL-1$\beta$ treatment (figure 1). ASU were potent inhibitors of both basal and IL-1$\beta$-stimulated MMP-3 productions, and stimulators of TGF-$\beta$1 synthesis. They also partially reversed the inhibitory effect of IL-1$\beta$ on TIMP-1 production (figure 2). Furthermore, ASU inhibited MIP-1$\beta$, IL-6, IL-8, NO and PGE$_2$ basal production by OA chondrocytes and partially counteracted the IL-1-stimulating effect on PGE$_2$. In A$_1$S$_2$ mixture, A and S had an additive effect on AGG synthesis and acted in synergy to inhibit IL-8 and NO production (data not shown).

**Conclusions:** From this work, we can conclude that ASU stimulated aggrecan production and recovered aggrecan production after IL-1$\beta$ treatment, probably through the production of TGF-$\beta$1. In parallel, ASU decreased MMP-3 production and stimulated TIMP-1 production. Taken together, these findings give an explanation to the recently demonstrated structure-modifying effects of ASU in hip osteoarthritis (1).

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