# Lutein and β-Cryptoxanthin Inhibit Inflammatory Mediators in Human Chondrosarcoma Cells Induced with IL-1β

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**Abstract:** *Objective:* Studies have shown that lutein (Lu) and β-cryptoxanthin (βCr) may down-regulate factors involved in inflammation associated with osteoarthritis and rheumatoid arthritis. We studied the possible protective effects of Lu and βCr *in vitro* against human chondrocyte dysfunction using a human chondrosarcoma cell line.

*Methods:* SW-1353 human chondrosarcoma cells were cultured for 24 hr in supplemented medium containing 0, 0.01, 0.1 or 1.0 μmol/L of Lu or βCr and subsequently stressed for 24 hr in the presence of 10 μg/L IL-1β. The resulting conditioned medium was analyzed for matrix-metalloproteinase-13 (MMP-13), cytokines (IL-1α, IL-2, IL-4, IL-10, IFN-γ , IL-6, IL-8, and TNF-α), and PGE2. Nuclear extract from the harvested cells was analyzed for NFκB.

*Results:* Lu (1.0 μmol/L; $P<0.05$) but not βCr decreased MMP-13. Both Lu (1.0 μmol/L; $P<0.05$) and βCr (0.1 and 0.01 μmol/L; $P<0.01$) inhibited $PGE_2$ production. All concentrations of βCr suppressed ($P<0.05$) IL-1α, IL-2 and IFN-γ production while Lu increased concentrations of these cytokines. Lu increased ($P<0.05$) while βCr decreased IL-4 and IL-10 concentrations. NFκB p50 production was suppressed ($P<0.01$) by both Lu and βCr, with Lu being more inhibitory.

*Conclusion:* Therefore, Lu and βCr protected against IL-1β-induced chondrocyte dysfunction by down-regulating NFκB activation and inhibiting inflammatory response, albeit through somewhat different pathways.

**Keywords:** Lutein, β-Cryptoxanthin, Arthritis, Human chondrosarcoma, Inflammatory mediators.

## INTRODUCTION

Osteoarthritis (OA) is a musculoskeletal disorder that involves the degeneration of articular cartilage, intra-articular inflammation with synovitis, and alterations in peri-articular and subchondral bone [1]. Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the localization of inflammatory cells in the synovial lining, leading to joint erosion and progressive bone and articular cartilage destruction [2]. In 2005, OA and RA affected 27 and 1.3 million adults, respectively [3,4]. OA originates in the cartilage and may spread to synovial tissue encompassing the joint, while RA begins in the synovial tissue adjacent to the joint before spreading to cartilage [5]. Despite differing pathogenesis, the activation of matrix-degrading proteinases and the production of inflammatory cytokines and mediators, specifically those produced by chondrocytes in articular cartilage, are very similar.

Research has shown that IL-1β is the key force in promoting an imbalance between cartilage degradation and repair [6,7]. Several studies have implicated reactive oxygen species (ROS) in IL-1-mediated cellular responses [8,9]. Induction of apoptosis in chondrocytes by IL-1β leads to increased cellular generation of reactive oxygen species (ROS), and the ensuing upregulation of NFκB and production of matrix-degrading proteinases, inflammatory cytokines, and mediators.

Lutein (Lu) and β-cryptoxanthin (βCr), naturally occurring, food-derived carotenoids [10], must be derived from the diet. Lu has been shown to prevent systemic inflammation in response to lipopolysaccaride (LPS), and to modulate immune and inflammatory responses by regulating redox potentials [11,12]. In OA and RA, upregulation of NFκB results in increased production of pro-inflammatory cytokines and mediators, along with collagen degrading proteinases. Lu is effective in reducing intracellular accumulation of the highly potent ROS $H_2O_2$ in murine macrophages, resulting in downregulation of NFκB [2]. βCr has been found to be associated with a reduced risk of developing inflammatory polyarthritis [13]. Even though the protective effects of Lu and βCr on the cellular mechanism in arthritic chondrocytes is not known, it is likely that βCr is involved in downregulating NFκB activation through its potent antioxidant activity.

The SW-1353 cell line has been shown to be a suitable model for examining pathological factors in OA and RA. It has strong similarities to primary human chondrocytes with respect to catabolic effects after treatment with IL-1β, including activation of the transcriptional regulator NFkB and subsequent production of MMP-1, MMP-3, and MMP-13 [14]. IL-1β can activate the three MAPK pathways p38, ERK, and JNK in SW-1353 cells, and leads to downstream activation of the transcriptional factor AP-1 [15,16]. It was recently shown that p38 regulates IL-1β stimulation of both MMP-1 and MMP-13 [17], which is consistent with previous reports of a dose-dependent decrease in the production of MMP-13 and MMP-1 when SW-1353 cells were treated with p38 kinase inhibitor [18]. This indicates that the SW-1353

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1874-2882/12 

cell line is an appropriate model for investigating IL-1β-induced joint degeneration.

The objective of this study is to assess the protective effect of the carotenoids, Lu and βCr, on NFκB activation, inflammatory response, and proteinase production in SW-1353 human chondrosarcoma cells induced with IL-1β. We hypothesize that Lu and βCr will reduce inflammatory response and proteinase production by downregulating NFκB.

## MATERIALS AND METHODOLOGY

### Materials

Human SW-1353 chondrosarcoma cells were purchased from the American Type Culture Collection (Manassas, VA). DMEM, penicillin G, streptomycin sulfate, amphotericin B, trypsin-EDTA, tetrahydrofuran, and the carotenoids lutein and β-Cryptoxanthin were purchased from Sigma Aldrich (St. Louis, MO), newborn calf serum from HyClone (Logan, UT), and the Micro BCA Protein Assay Kit was from Pierce (Rockford, IL). The ELISA for MMP-13 was purchased from AnaSpec (San Jose, CA), $PGE_2$ from R&D Systems (Minneapolis, MN), cytokines from Quansys (Logan, UT), and NFκB (human p50) from Cayman Chemical (Ann Arbor, MI).

### Cell Culture

Human SW-1353 chondrosarcoma cells (ATCC; Manassas, VA) were cultured in DMEM supplemented with 100 units/mL penicillin G, 100 μg/mL streptomycin sulfate, 0.25 μg/mL amphotericin B, and 10% newborn calf serum, at 37°C in a humidified 5% $CO_2$ atmosphere. All experiments were conducted using cells within 2 passage numbers**.** Cells were plated at $2 \times 10^5$ cells/well in 6 well plates and incubated for 48 h. Cell medium was removed and replaced with supplemented medium containing 0, 0.01, 0.1, or 1.0 μmol/L Lu (Expt. 1) or βCr (Expt. 2). The carotenoids were prepared immediately before use by solubilizing in tetrahydrofuran (THF), gradually adding to NCS with continual stirring, followed by addition of DMEM to give a final concentration of 5% serum. (0.1% THF final concentration). Control cultures contained 0.1% THF but no carotenoid; previous research has shown tetrahydrofuran is a suitable solvent for carotenoids in cell culture [19]. The cells were incubated with carotenoid for 24 h, medium removed, 10 ng IL-1β/mL DMEM added, and incubated for an additional 18 h. Cells that received 0 μmol/L carotenoid and no IL-1β served as negative controls, and cells that received 0 μmol/L carotenoid and 10 ng IL-1β/mL served as positive controls. Conditioned medium (CM) following incubation with IL-1β was collected and stored at -80°C until assay. The adherent cells were dissociated from the wells with trypsin-EDTA and counted using a Coulter counter (Beckman Coulter, Brea, CA). Cell pellets were snap-frozen and stored at -80°C.

### Assays

Total latent and active matrix metalloproteinase-13 (MMP-13) in the CM were analyzed using a commercially available ELISA kit (SensoLyte MMP-13 ELISA Kit, AnaSpec, San Jose, CA, USA). Since MMP-13 is a key enzyme facilitating breakdown of cartilage in arthritis, it is useful in examining potential inhibitors of this process. The lower limit of detection for MMP-13 was ≤ 6 pg/ml.

$PGE_2$ was analyzed by ELISA (Parameter $PGE_2$, R & D Systems, Minneapolis, MN, USA) and the lower limit of detection for $PGE_2$ was 8.5 pg/ml.

Pro-inflammatory cytokines IL-1α, IL-2, IFN-γ, IL-6, IL-8, TNF-α and anti-inflammatory cytokines IL-4, IL-10 were analyzed in CM by chemiluminescent array format ELISA (Quansys Q-Plex Cytokine Array, Logan, UT). This assay simultaneously measures multiple cytokines. Digital images (Canon EOS 40D, Canon, Irvine, CA, USA) were acquired using imaging capture software (Digital Imaging Professional 3.3, Canon, Irvine, CA, USA). Data obtained was analyzed using Quansys Q-View 2.5.2 software (Quansys Q-Plex Cytokine Array, Logan, UT). The lower limit of detection was 4.10, 1.10, ≤ 1.0, ≤ 1.0, 1.36, 2.11, 1.36, and ≤ 1.0 pg/mL for IL-1α, IL-2, IFN-γ, IL-6, IL-8, TNF-α, IL-4, and IL-10, respectively.

Nuclear extract NFκB p50 was analyzed in the cell pellets using a commercially available ELISA kit (NFκB (human 50) Transcription Factor Assay, Cayman Chemical, Ann Arbor, MI, USA). This assay requires purification of cellular nuclear extracts. Due to inadequate cell numbers, cell pellets from 3 samples in each treatment were pooled for analysis of NFκB. All buffer reagents were purchased from Sigma Aldrich (St. Louis, MO, USA). Percent change was calculated by dividing the optical density of each treatment sample by the corresponding positive control (IL-1β only).

Total protein in the nuclear extract were analyzed using a colorimetric assay (Micro BCA Protein Assay Kit, Pierce, Rockford, IL, USA). The lower limit of detection for protein was 0.5 μg/ml.

### Statistical Analysis

Data was analyzed by one-way analysis of variance (ANOVA) using the General Linear Models procedure of SAS, and treatment means were compared with protected LSD. A probability value of $P<0.05$ was considered statistically significant.

## RESULTS

### Matrix Metalloproteinase

MMP-13 concentrations in the cell supernatants was expressed as ng total MMP-13/$10^6$ to standardize amounts between cultures. Basal concentrations of total MMP-13 in SW-1353 cell cultures without IL-1β stimulation (negative control) averaged 0.027 ± 0.003 and 0.031 ± 0.008 ng/$10^6$ cells for the Lu and βCr experiments, respectively (Fig. **1**). In the presence of IL-1β (positive control), MMP-13 production increased ($P<0.01$), with concentrations reaching 4.97 ± 0.50 ng/$10^6$ cells for Lu and 4.21 ± 0.30 ng/$10^6$ cells for βCr. Pre-incubating cultures with Lu decreased MMP-13 concentration in a dose-dependent manner following IL-1β-induced stress. Concentrations of MMP-13 in cultures pre-incubated with 1.0 μmol/L Lu were approximately 35% ($P<0.02$) those without Lu. Conversely, βCr did not influence MMP-13 production, although MMP-13 concentrations in the presence of 0.01 μmol/L βCr tended to be lower than cultures without βCr.

### $PGE_2$ Production

Addition of IL-1β slightly stimulated $PGE_2$ production (Fig. **2**), when compared with negative control. Production

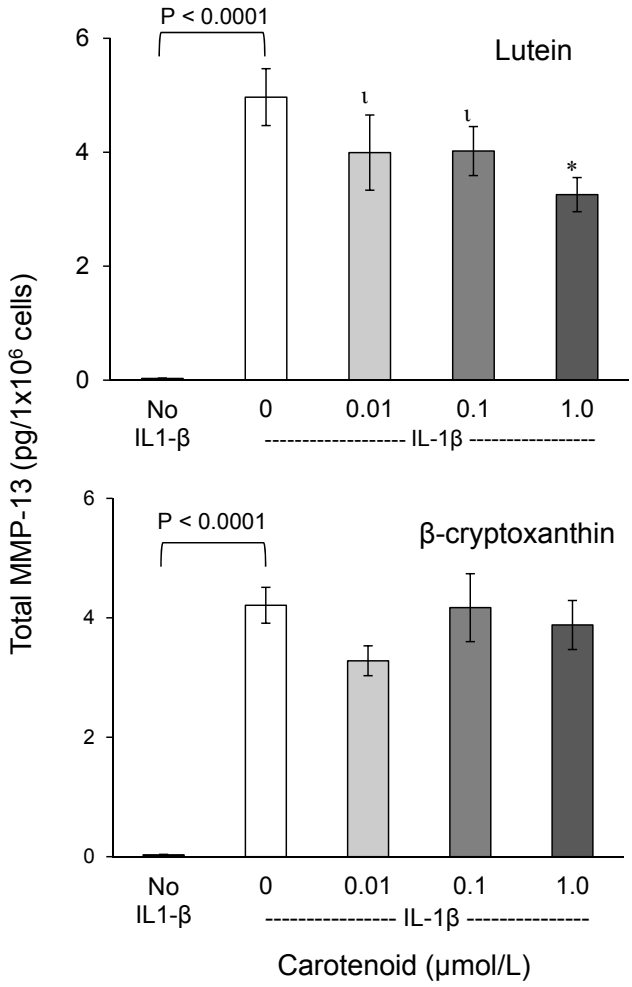


**Fig. (1).** Production of MMP-13 (mean ± SEM) by SW-1353 cells incubated in the presence of 0, 0.01, 0.1, or 1.0 μmol/L lutein or β-cryptoxanthin and stimulated with 10 ng/mL IL-1β. Negative control wells (no IL-1β) were incubated with neither carotenoid nor IL-1β. Data were analyzed by ANOVA. $^{*}$ $^{\tau}$ Statistically different from 0 μmol/L carotenoid control ($^{*}P$<0.05, $^{\tau}P$<0.06).

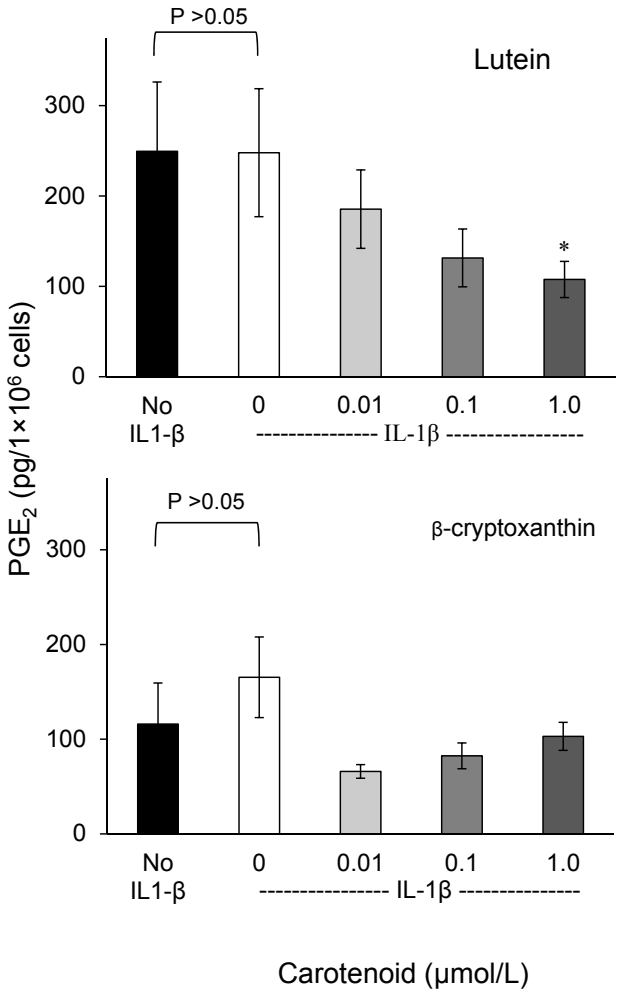


**Fig. (2).** Production of $PGE_2$ (mean ± SEM) by SW-1353 cells incubated in the presence of 0, 0.01, 0.1, or 1.0 μmol/L lutein or β-cryptoxanthin and stimulated with 10 ng/mL IL-1β. Negative control wells (no IL-1β) were incubated with neither carotenoid nor IL-1β. Data were analyzed by ANOVA. $^{*}$ Statistically different from 0 μmol/L carotenoid control ($P$<0.05).

of $PGE_2$ decreased in a dose-dependent manner when cells were cultured in the presence of Lu, with a significant suppression of $PGE_2$ following incubation with 1.0 μmol/L Lu ($P$<0.05). There was also a significant (P<0.05) decrease in $PGE_2$ production with 0.01 and 0.1 μmol/L βCr but not with the highest concentration.

**Cytokine Production**

The effects of Lu on the production of the pro-inflammatory cytokines IL-1α, IL-1β, IL-6, IL-8 and TNF-α are presented in Table **1**. Addition of IL-1β into cultures without the carotenoids increased ($P$<0.01) IL-1α production one to two fold. While Lu did not influence IL-1α production, the highest concentration of βCr inhibited (P<0.05) IL-1α concentrations (Table **2**).

The presence of IL-1β alone increased ($P$<0.05) IL-2 concentrations 85 (Lu) to 250% (βCr). At 0.1 μmol/L, Lu increased ($P$<0.01) IL-2 concentrations by 85%. Conversely, addition of βCr, especially at 0.1 and 1.0 μmol/L produced a dose-dependent decrease in IL-2 concentrations.

IL-1β stimulated ($P$<0.05) IFN-γ production by SW-1353 cells in both studies. At the highest concentration, Lu increased ($P$<0.06) IFN-γ concentration. In contrast, 0.1 and 1.0 μmol/L βCr inhibited IFN-γ production.

In the presence of IL-1β alone, production of IL-6, IL-8, and TNF-α increased significantly ($P$<0.01). Neither Lu nor βCr significantly influenced IL-6, IL-8, or TNF-α production.

The effects of Lu and βCr on the production of the anti-inflammatory cytokines IL-4 and IL-10 are presented in Tables **1** and **2**. The presence of IL-1β alone stimulated (P<0.01) IL-4 production 166% and 283% in Lu and βCr, respectively. While Lu increased (P<0.01) IL-4 production in a dose-dependent manner, βCr had no significant effect.

IL-1β alone stimulated ($P$<0.01) IL-10 production, and βCr was inhibitory, with a significant (P<0.05) inhibition observed with 1.0 μmol/L. In contrast, Lu had no significant effect on IL-10 production.

**Table 1. Cytokine Response (pg/mL) by SW-1353 Cells Incubated in the Presence of 0, 0.01, 0.1, or 1.0 µmol/L Lutein and Stimulated with 10 ng/mL IL-1β**

| | | Lutein (µmol/L) | | | |
|---|---|---|---|---|---|
| | | 0 | 0.01 | 0.1 | 1.0 |
| Cytokine | No IL-1β | ---------- IL-1β ---------- | | | |
| IL-1α | 4.7 ± 0.8 | 9.2 ± 1.0 | 9.1 ± 0.7 | 9.9 ± 0.7 | 13.1 ± 2.6 |
| IL-2 | 10.2 ± 0.9 | 18.9 ± 1.4 | 25.1 ± 3.0 | 35.0 ± 4.2* | 21.9 ± 2.0 |
| IL-4 | 3.8 ± 0.3 | 10.1 ± 1.8 | 17.7 ± 1.1 | 22.3 ± 2.1* | 23.0 ± 6.7* |
| IL-10 | 4.5 ± 0.6 | 13.9 ± 2.7 | 19.2 ± 2.9 | 16.5 ± 2.5 | 11.9 ± 1.4 |
| IFN-γ | 3.2 ± 0.2 | 4.9 ± 0.5 | 6.6 ± 0.7 | 7.0 ± 0.5 | 8.7 ± 2.5 |
| TNF-α | 8.1± 0.7 | 37.5 ± 3.6 | 41.7 ± 5.0 | 42.3 ± 2.5 | 42.2 ± 4.5 |

Values (n=6) are mean ± SEM.
*Statistically different from control ($P$<0.05).

**Table 2. Cytokine Response (pg/mL) by SW-1353 Cells Incubated in the Presence of 0, 0.01, 0.1, or 1.0 µmol/L β-Cryptoxanthin and Stimulated with 10 ng/mL IL-1β**

| | | β-Cryptoxanthin (µmol/L) | | | |
|---|---|---|---|---|---|
| | | 0 | 0.01 | 0.1 | 1.0 |
| Cytokine | No IL-1β | ---------- IL-1β ---------- | | | |
| IL-1α | 3.0 ± 0.5 | 8.9 ± 1.3 | 6.4 ± 0.7 | 7.9 ± 1.1 | 6.1 ± 0.6* |
| IL-2 | 7.6 ± 0.4 | 26.7 ± 7.6 | 17.6 ± 1.5 | 14.8 ± 1.0* | 14.7 ± 1.3* |
| IL-4 | 3.0 ± 0.1 | 11.5 ± 2.8 | 10.8 ± 2.5 | 10.1 ± 1.8 | 7.0 ± 0.9 |
| IL-6 | 7.0 ± 5.3 | 294.1 ± 32.8 | 377.6 ± 75.9 | 293.7 ± 39.7 | 347.4 ± 63.4 |
| IL-8 | 28.5 ± 12.2 | 226.2 ± 11.6 | 228.1 ± 12.8 | 209.9 ± 9.3 | 215.7 ± 7.9 |
| IL-10 | 3.0 ± 0.4 | 10.8 ± 2.2 | 8.7 ± 1.3 | 7.6 ± 0.7 | 6.1 ± 0.9* |
| IFN-γ | 2.2 ± 0.0 | 11.5 ± 1.3 | 7.2 ± 1.0 | 4.4 ± 0.5 | 4.5 ± 0.5 |
| TNF-α | 5.7 ± 0.6 | 32.5 ± 4.5 | 35.8 ± 2.6 | 34.2 ± 5.2 | 26.1 ± 1.2 |

Values (n=6) are mean ± SEM.
*Statistically different from control ($P$<0.05).

## NFKB (P50)

The addition of IL-1β to the chondrocyte culture stimulated NFκB expression 79% (Lu) to 270% (βCr), when compared to negative control. However, 0.01 and 0.1 µmol/L Lu significantly down-regulated (P<0.01) p50 expression by approximately 32 to 39%, respectively, compared to control cultures (Fig. **3**). Only the highest concentration (1 µmol/L) of βCr reduced (P<0.01) p50 expression to approximately the same magnitude.

## DISCUSSION

We studied the possible protective effects of Lu and βCr against IL-1β-induced inflammation, proteinase production and NFκB (p50) expression *in vitro* using human chondrosarcoma cells. Overall, preincubation of cells with Lu and βCr resulted in a protection against IL-1β-induced inflammatory response.

NFκB, present in chondrocyte cytosol in the inactive form, IκB, can be activated by inflammatory stimuli such as IL-1β, TNF-α, and lipopolysaccaride (LPS), and this upregulation of NFκB leads to increased production of pro-inflammatory cytokines, pro-inflammatory mediators ($PGE_2$ and NO), and type II collagen degrading proteinases (such as MMP-13) [2,20]. Similarly, Li and Engelhardt showed that ROS, such as NO and $H_2O_2$, can activate the NFκB pathway. Intracellular signals activate the IκB kinase (IKK) complex inducing phosphorylation and degradation of IκB [21], nuclear appearance of p50 and p65, and the subsequent transcriptional induction of inflammation-associated genes that encode the pro-inflammatory cytokines, iNOS, COX-2, and MMP-13 [2].

Preincubation of IL-1β-stimulated SW-1353 cells with the carotenoids Lu and βCr significantly down-regulated nuclear p50 expression, with Lu being 100 fold more suppressive than βCr. Similarly, Kim *et al*., reported that 10 µmol/L Lu inhibited NFκB activation in LPS-induced RAW 264.7 cells [2]. Yamaguchi and Uchiyama showed that 0.1 µmol/L βCr was not able to downregulate NFκB activation in osteoclastic cells [22]. This indicates that βCr is required

in higher concentrations (1.0 μmol/L in this study) to prevent p50 translocation into the nucleus.

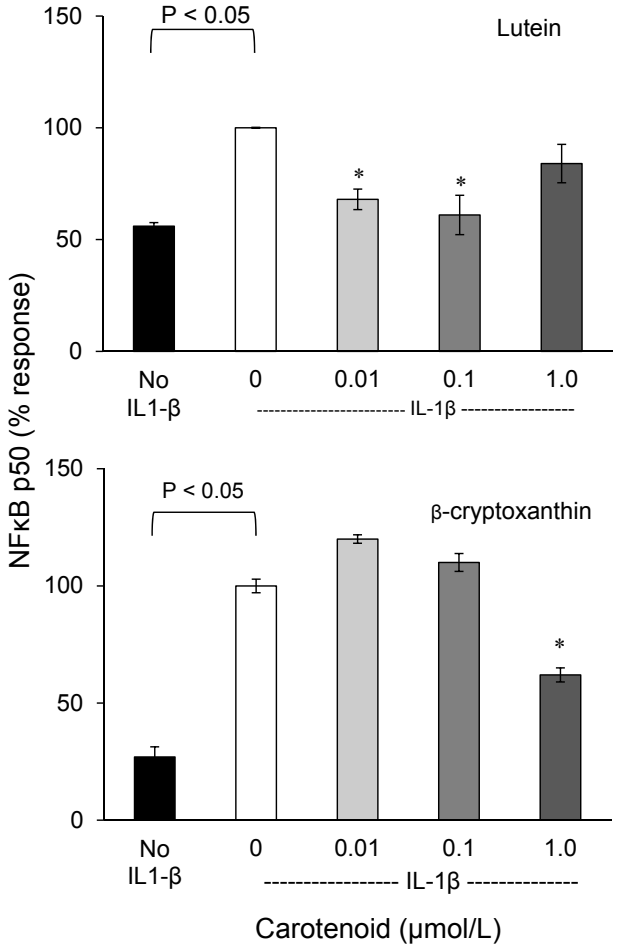


**Fig. (3).** Production of NFκB p50 (mean ± SEM) in SW-1353 cells incubated in the presence of 0.01, 0.1, or 1 μmol/L lutein or β-cryptoxanthin and stimulated with 10 ng/mL IL-1β. Negative control wells (no IL-1β) were incubated with neither carotenoid nor IL-1β. Data were analyzed by ANOVA. * Statistically different from 0 μmol/L carotenoid control ($P$<0.05).

The ability of antioxidants to down-regulate NF-κB activation is likely due to their potent scavenging of ROS [23], as was seen in human chondrocytes pretreated with carotenoids, which resulted in clearance of ROS and inhibition of ligands required for downstream NFκB activation. In our studies, ROS generated in response to IL-1β were indeed reduced in cultures that were pre-incubated with both Lu and βCr (unpublished data).

In OA and RA cartilage, NFκB activation induces MMP-13 production, resulting in the loss of type II collagen, tensile properties, and structural integrity of the affected joint. Numerous studies have demonstrated the ability of IL-1β to induce MMP-13 production in chondrocytes [24,25]. Gebauer *et al*., confirmed that SW-1353 cells and primary human adult articular chondrocytes were also similar in producing increased amounts of MMP-13 in response to IL-1β stimulation [14]. The present study shows that physiological concentrations (0.01-1.0 μmol/L) of Lu can decrease IL-1β-induced production of MMP-13 in SW-1353 cells and thereby protect against type II collagen degradation. In contrast, βCr did not exert a similar effect.

$PGE_2$ is a major pro-inflammatory mediator in arthritic diseases, and has been reported to not only potentiate inflammation but also inhibit proteoglycan synthesis [26]. Nitric oxide (NO) contributes to the development of OA and RA by mediating catabolic responses mediated by pro-inflammatory cytokines such as IL-1β. The IL-1β-induced upregulation NFκB in chondrocytes results in production of $PGE_2$ and NO through COX-2 and iNOS activation, respectively [27]. In this study, both Lu (1.0 μmol/L) and βCr (0.01 and 0.1 μmol/L) decreased $PGE_2$ concentrations, suggesting that Lu, and especially βCr, can alleviate $PGE_2$-modulated inflammation and joint degeneration common in OA and RA. Retinoic acid has also been shown to inhibit $PGE_2$ production in IL-1-induced human primary chondrocytes, and suppress nitric oxide, and iNOS and COX-2 expression, suggesting that the action is mediated through the suppression of JNK-AP-1 signaling pathway [27]. While βCr has provitamin A activity, lutein does not; therefore the actions of these carotenoids are unlikely due to prior conversion to vitamin A.

IL-1β can also induce chondrocytes to produce several pro-inflammatory cytokines including IL-1α, IL-2, IFN-γ, IL-6, IL-8 and TNF-α [28]. Lu increased IL-2 secretion by SW-1353 cells but had no significant effect on other pro-inflammatory cytokines studied. On the other hand, βCr inhibited IL-1 IL-2 and IFN (0.01 and 0.1 μmol/L). At 0.1 μmol/L, Lu tended to increase IL-1α, IL-2 and IFN-γ secretion. IL-1α, IL-2, and IFN-γ are generally classified as Th1-modulating cytokines due to their ability to induce immune responses that lead to autoimmune diseases such as RA. We showed that βCr, but not Lu, has anti-inflammatory properties. These results paralleled the down-regulation of NFκB in the nuclear fractions of cells cultured with 1.0 μmol/L βCr, suggesting that the down-regulation of NFκB led to decreased expression of the pro-inflammatory cytokines IL-1α, IL-2, and IFN-γ. The lack of a similar response with Lu cannot be explained. IL-6, IL-8 and TNF-α are pivotal in driving acute rather than chronic inflammation [29]. The lack of effect of Lu and βCr on IL-6, IL-8 and TNF-α perhaps suggests that these two carotenoids are better suited to handle chronic inflammatory responses, as seen with OA and RA.

IL-4 and IL-10 are generally regarded as Th-2-modulatory cytokines and inhibit many of the catabolic processes induced by pro-inflammatory cytokines. IL-4 is undetectable in arthritic tissue, suggesting that an imbalance in Th1/Th2-modulated cytokines drives disease progression [30]. It has also been shown that IL-4 can inhibit MMP production in cartilage explants [31]. In this study, Lu but not βCr stimulated IL-4 production. Joosten *et al*. demonstrated that IL-4 treatment reduced collagen and bone destruction in DBA/-1J/Bom mice [30]. Even though IL-4 has tissue-protective properties, it is not able to act as a potent anti-inflammatory cytokine. This is consistent in our study; while Lu increased IL-4 production, it decreased production of collagen degrading factors such as MMP-13 and $PGE_2$. Previous research indicates that IL-10 is chondroprotective by downregulating MMP and pro-inflammatory cytokines such as IL-6 [31]. In this study, βCr but not Lu increased IL-10 production. Human chondrosarcoma cells lack functional IL-

10 receptors due to a defect in IL-10R1 surface expression, leaving cells unresponsive to IL-10 induction, therefore provides a possible explanation for the upregulation of IL-10 production by βCr [25].

In conclusion, the carotenoids Lu and βCr protect against degenerative factors upregulated by IL-1β-induced in SW-1353 cells, likely by scavenging ROS required for NFκB activation. In general, downregulation of NFκB resulted in decreased production of cytokines, mediators, and proteinases which are upregulated in OA and RA. The action of Lu and βCr seem to be mediated through somewhat different pathways: Lu tended to downregulate factors involved with cartilage destruction (MMP-13 and $PGE_2$) and upregulate factors involved in tissue-protection (IL-4) at the expense of downregulating pro-inflammatory cytokines; βCr tends to downregulate factors associated with pro-inflammatory response (IL-1α, IL-2, and IFN-γ) and cartilage destruction ($PGE_2$), while failing to downregulate matrix-degrading proteinases. This is expected because the two carotenoids differ structurally, influencing location and orientation and interaction at the membrane. βCr is more polar than Lu and therefore may exert different biological actions within the cell. These findings offer new perspectives from therapeutic approaches for treating both the symptomatic and degenerative processes characteristic of OA and RA.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

M.M.D., B.D.M., J.S.P, and B.P.C designed the experiments, conducted experiments and analyzed data. M.M.D. wrote the first draft and B.D.M., J.S.P, and B.P.C. revised the paper. All authors read and approved the final manuscript.

## ACKNOWLEDGEMENTS

This work was supported by a grant from the Agriculture Research Center, Washington State University, Pullman.

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Received: April 22, 2011 Revised: January 31, 2012 Accepted: February 02, 2012