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Long term consumption of flaxseed enriched diet decreased ovarian cancer incidence and prostaglandin E_2 in hens ☆☆

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HIGHLIGHTS

- Flaxseed enriched diet decreased ovarian cancer incidence and severity in hens
- Long-term consumption of flaxseed reduced the expression of COX-2 in hen ovaries
- The concentration of PGE2 was reduced in ovaries of hens fed flaxseed

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ABSTRACT

Objective. Ovarian cancer is the most lethal gynecological malignancy. Prevention may be the best approach to reduce ovarian cancer. Flaxseed is the richest vegetable source of omega-3 fatty acids which may be effective in the prevention of ovarian cancer. Prostaglandin E_2 (PGE_2) is the most pro-inflammatory ecoisanoid and one of the downstream products of two isoforms of cyclooxygenase (COX) enzymes: COX-1 and COX-2. Our objective was to determine if long-term consumption of a flaxseed enriched diet decreased ovarian cancer severity and incidence in the laying hen and to investigate its potential correlation with the expression of COX enzymes and PGE_2 concentration.

Methods. White Leghorn hens were fed 10% flaxseed-enriched or standard diet for 4 years. The severity and incidence of ovarian cancer were determined by gross pathology and histology. COX-1 and COX-2 protein and mRNA expression and PGE_2 concentrations in ovaries were measured by Western blot, quantitative real-time PCR and ELISA, respectively.

Results. The results demonstrated that there was a reduction in ovarian cancer severity and incidence in hens fed flaxseed diet. In correlation with decreased ovarian cancer severity and incidence, concentration of PGE_2 and expression of COX-2 were diminished in ovaries of hens fed flaxseed.

Conclusions. Our findings suggest that the lower levels of COX-2 and PGE_2 are the main contributing factors in the chemo-suppressive role of long-term flaxseed consumption in ovarian cancer in laying hens. These findings may provide the basis for clinical trials of dietary intervention targeting prostaglandin biosynthesis for the prevention and treatment of ovarian cancer.

Introduction

Prevention of ovarian cancer is the best approach for reducing the impact of this deadly disease. Progress in the treatment and prevention of ovarian cancer has been hampered due to the lack of a valid and appropriate animal model, and absence of effective chemoprevention strategies. Promising findings come from studies with the laying hen, a robust model of spontaneous ovarian cancer that recapitulates the human disease. Dietary intervention with flaxseed, the richest vegetable source of omega-3 fatty acids (OM-3FAs) and phytoestrogen lignans, demonstrate the potential for effective prevention and amelioration of ovarian cancer by targeting inflammatory prostaglandin pathways.

Cyclooxygenase (COX) is the rate limiting enzyme in catalyzing the conversion of arachidonic acid (AA) to prostaglandin H_2. Specific prostaglandin synthases act on PGH_2 to produce prostaglandins and thromboxanes. Two identified isoforms of cyclooxygenase are: COX-1 and COX-2 which have similar structure and function, but encoded

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with different genes (PTGS1 and PTGS2) and show distinct expression patterns. Both COX enzymes may be involved in tumor establishment [1] and maintenance of existing tumors [2]. Up-regulation of COX-2 has been reported in many malignancies, however, we and others have shown that COX-1 is over-expressed in ovarian cancer [2–4]. Prostaglandins (PGs) are downstream products of cyclooxygenase enzymes and PGE_2 is commonly elevated in different human cancers including colon, lung, breast, and head and neck cancers [5].

Dietary intervention with flaxseed to prevent cancer was first proposed by Budwig in the 1950s [6]. OM-3FA consumption has been associated with the prevention of many cancers. Inhibitory effects of flaxseed on prostate [7], intestine and colon cancer [8] have been reported. The most well understood mechanism for the chemopreventive action of OM-3FAs is their suppressive effect on the production of arachidonic acid-derived prostanoids, particularly PGE_2 [9]. Flaxseed is the richest plant sources of the OM-3FA alpha-linolenic acid (ALA, C18:3 ω-3). ALA is elongated to form eicosapentaenoic acid (EPA, C20:5 ω-3) and docosahexaenoic (DHA, C20:6 ω-3) acid in the intestine. Along with the OM-3FAs, the flaxseed lignan secoisolariciresinol diglucoside (SDG) is metabolized in the digestive tract to phytoestrogens enterodiol (END) and enterolactone (ENL), which are potent antiestrogens. In addition to the potential anti-cancer actions of flaxseed via estrogen antagonism, the lignan metabolites are also potent antioxidants with the ability to inhibit lipid peroxidation and DNA scission, and scavenge hydroxyl radicals [10].

Ovarian cancer is the fifth leading cause of cancer death among women and remains as the most lethal gynecological malignancy. Regardless of the origin of ovarian cancer, there is evidence to suggest that women with fewer numbers of ovulations during their reproductive life are less prone to develop ovarian cancer. While the role that ovulation might play is controversial, it is evident that ovulation is important to the initiation of the disease [11]. Multiparity, duration of lactation and use of birth control pills all decrease the risk of epithelial ovarian cancer [12]. The domestic hens ovulate almost daily and spontaneously develop ovarian adenocarcinomas that are similar in histological appearance to human ovarian carcinomas and share similar symptoms of the disease, such as perfuse ascites fluid and peritoneal metastatic dissemination [13,14].

We reported that old laying hens which were fed a flaxseed enriched diet for one year experienced a significant decrease in the severity of ovarian cancer and increased survival. The long-term efficacy of dietary intervention with flaxseed on ovarian cancer incidence, and the mechanisms through which flaxseed works is unclear. The aims of this study were 1) to determine if long term consumption (>4 years) of a flaxseed enriched diet decreases ovarian cancer incidence as well as severity; and 2) to investigate the correlation between ovarian COX enzyme expression, PGE_2 tissue concentrations, flaxseed consumption and cancer incidence in old laying hens. The results of this study demonstrate that long term consumption of flaxseed decreases the incidence and severity of ovarian cancer in hens. Moreover, COX-2 expression, but not COX-1, and PGE_2 levels in the ovaries were significantly reduced by flaxseed consumption. The results suggest that flaxseed targets the prostaglandin pathway to prevent and treat this highly lethal malignancy.

Material and methods

Animals

1200 single comb white Leghorn hens, aged 6 months, were randomly divided into two groups (n = 600). The first group was fed control diet and second group was fed 10% flaxseed enriched diet. The composition of the diets has been shown previously [15]. A percentage of both corn and soy were removed from the experimental diet to keep the diets isocaloric with the addition of the flaxseed. The hens were fed 110 grams of feed a day and were provided water ad libitum. Hens on the flaxseed diet consumed around 11 grams of flaxseed per day, or 6.2 g/kg body weight. Hens were maintained three per cage and exposed to a photoperiod of 17 h light: 7 h dark, with lights on at 05:00 h and lights off at 22:00 h. Animal management and procedures were reviewed and approved by the Institutional Animal Care and Use Committees at the University of Illinois at Urbana-Champaign, University of Illinois at Chicago and Southern Illinois University at Carbondale.

Collection of tissue

At the age of 12, 19, 24, 31, 36 and 45 months, twenty hens from each of control and flaxseed group were randomly selected, euthanized using CO_2 asphyxation and necropsied. Samples from chickens aged 19, 31 and 45 months were designated as 1.5, 2.5 and 3.5 years old, respectively. The ovarian tissues were processed as previously described [16].

Histology

Ovarian tissues fixed in NBF were processed and paraffin embedded as previously described [3,14,17]. Four micrometer sections were cut and mounted on SuperFrost Plus microscope slides. Slides were deparaffinized and rehydrated through xylene and graded ethanol solutions. Hematoxylin and eosin staining were performed as described [18].

RNA extraction and analysis and quantitative real-time PCR

Total RNA was extracted from ovarian tissue using Trizol reagent. The quantification and the quality check was performed as previously described [16]. RNA samples were treated with RQ1 RNase-free DNase prior to reverse transcription reaction. cDNA was synthesized from DNase treated RNA with the high capacity cDNA archive kit. The chicken-specific primers and plasmid standards used for each gene were designed and qRT-PCR was conducted as previously shown [16].

Western blot

The proteins were extracted from snap frozen ovarian tissue samples and the western blot was performed to detect COX-1 and COX-2 as described previously [16].

PGE_2 EIA

The amount of PGE_2 in ovarian tissues from hens in both control and flaxseed fed groups was measured using a specific enzyme immunoassay as shown previously [16].

Statistical analysis

All experiments were performed in duplicate at each time-point (n = 20) and differences in data from groups were analyzed with GraphPad InStat by using One-way ANOVA with Student–Newman–Keuls. Dichotomous data were analyzed using the Chi Square test in GraphPad InStat. Outcome differences with a pr A value of $P < 0.05$ was considered significant whereas a value of $P < 0.01$ was considered highly significant.

Results

Flaxseed decreased the incidence of ovarian cancer

Upon necropsy of 20 hens from each group at each time-point, ovaries were classified as normal, cancerous or suspected abnormalities. Ovaries with suspected abnormalities were analyzed by histology to

confirm the cancerous ovarian tissue (Fig. 1A-F). Tumors from both control diet and flaxseed enriched diet groups were analyzed and characterized according to previously published tumor classification method [15]. Fig. 1G presents the percentage of hens with ovarian cancer. No malignancy was detected in ovaries of hens fed a flaxseed enriched diet and hens fed control diet aged 1, 1.5 and 2 years; however, 6 month later, 10% of 2.5 year old chickens fed control diet (n = 2) had ovarian cancer whereas 5% of hens fed a flaxseed diet had ovarian cancer (n = 1). Sixty five percent of 3 year old hens fed control diet (n = 13) had ovarian cancer but flaxseed enriched diet, reduced the prevalence of ovarian cancer to 45% in the hens of the same age (n = 9), however, the difference was not statistically significant (P > 0.05). Only 20% of 3.5 year old fed a flaxseed enriched diet (n = 4) had ovarian cancer whereas fifty percent of 3.5 year old hens fed control diet (n = 10) had ovarian cancer indicating that flaxseed significantly reduced the incidence of ovarian cancer in 3.5 year old hens (P < 0.05). Hens fed flaxseed had less late stage tumors indicative of the chemosuppressive actions of flaxseed (Fig. 1H). In contrast,

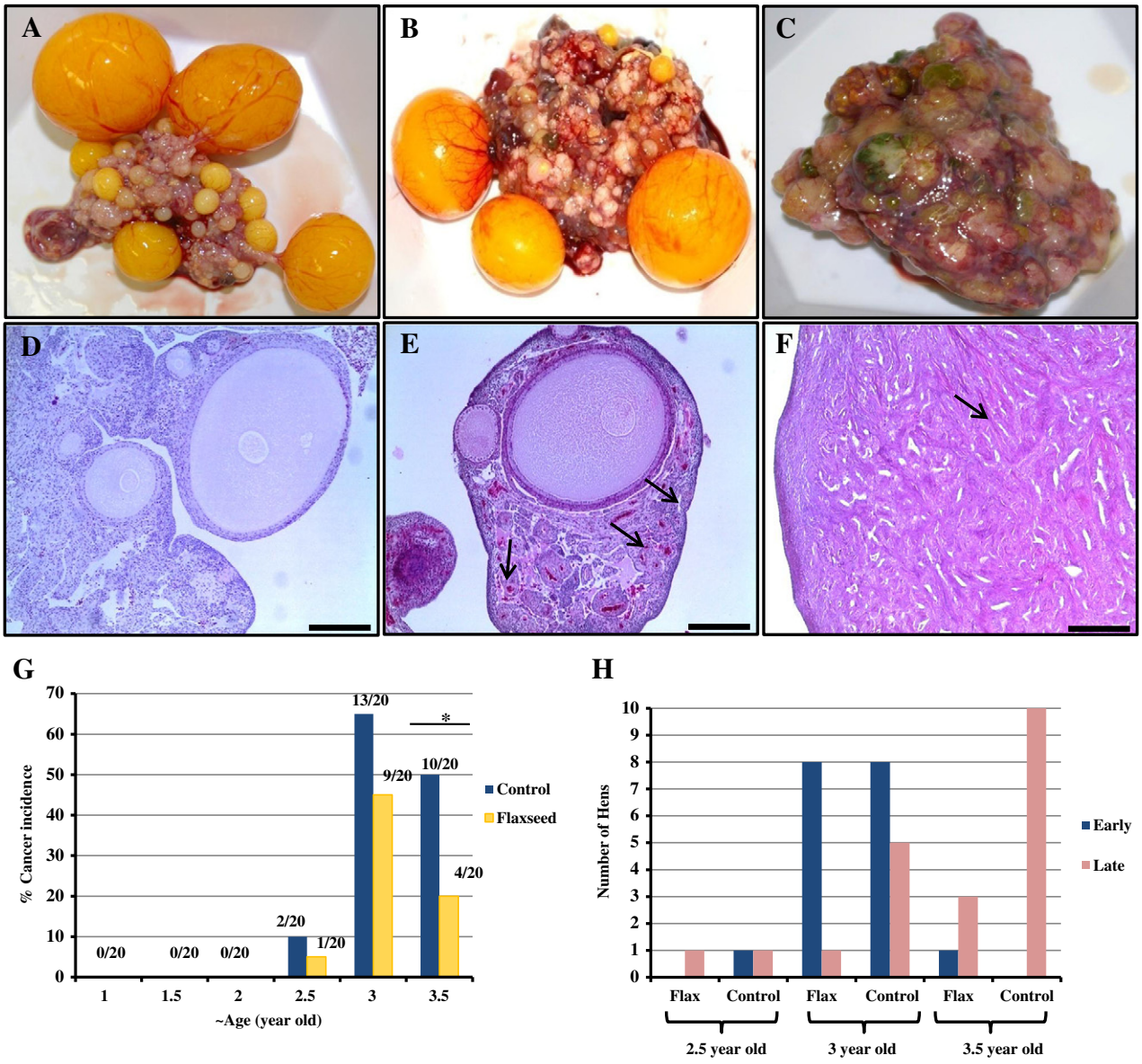

Fig. 1. (A) Gross anatomy of normal ovary with a hierarchy of developing follicles; (B) Ovary classified as a suspected; (C) Ovary taken from a hen with metastatic late stage ovarian cancer; (D) H&E stain of normal ovary with developing small follicles; (E) H&E stain of suspected ovary showed a disassociated stoma with unusually high numbers of blood vessels and formation of focal lesions in the stroma below the ovarian surface was apparent (arrow); (F) H&E stain of metastatic late stage ovarian cancer with solid areas composed of slit-like sheets containing cells with high-grade nuclear atypia and a few tiny glands were also seen without any papillae. Calibration Bar, 20 μm (G) Percentage of ovarian cancer incidence in hens fed flaxseed enriched and control diet. Necropsy of hens was performed at different time-points (n = 20) and ovaries were classified as normal, cancerous or questionable. Questionable ovarian tissues were analyzed by histology to confirm the cancerous ovarian tissue. No ovarian malignancy was detected in 1 (n = 0/20), 1.5 (n = 0/20) and 2 (n = 0/20) year old hens in both flaxseed and control groups; however, 10% of 2.5 year old (n = 2/20) hens fed control diet had ovarian cancer whereas 5% (n = 1/20) of hens fed flaxseed had ovarian cancer. There was a difference in ovarian cancer incidence in 3 years old hens fed control diet (n = 13/20) (65%) compared to hens fed a flaxseed enriched diet aged the same (n = 9/20) (45%), but it was not statistically significant. Flaxseed enriched diet significantly reduced the incidence of ovarian cancer in 3.5 year old hens compared to hens fed control diet (P < 0.05). (H) Comparison of early and late stage ovarian cancer between hens fed a flaxseed enriched diet and hens fed control diet. The cancerous ovary of 2.5 year old hen fed a flaxseed enriched diet was at the late stage but one early and one late stage ovarian cancer were found in hens aged the same on control diet. At the age of 3 year old, 8 early stage and only 1 late stage ovarian cancer were detected in hens fed flaxseed diet; similarly, 8 early stage ovarian cancer were observed in 3 year old hens on control diet but there was more late stage ovarian cancer (n = 5). One early and 3 late stage ovarian cancer were observed in 3.5 year old hens fed a flaxseed enriched diet but interestingly, all of 10 ovarian cancers detected in 3.5 year old hens fed control diet were at the late stage. *, P < 0.05.

hens on the control diet had more late stage tumors (T3/T4) that presented with ascites fluid and metastasis compared to the flaxseed group.

Effect of flaxseed on COX-1expression

No significant differences were detected when the expression of COX-1 protein and mRNA in normal ovaries of hens fed a flaxseed enriched diet at each time-point compared to COX-1 protein and mRNA expression in normal ovaries of hens fed control diet aged the same ($P > 0.05$; Fig. 2). There was no difference in expression of COX-1 protein and mRNA between cancerous ovaries of hens fed a flaxseed enriched diet and cancerous ovaries hens fed control diet at each time-point ($P > 0.05$; Fig. 3).

Effect of flaxseed on COX-2 expression

There were no significant differences in COX-2 protein and mRNA expression in normal ovaries of 1, 1.5 and 2 year old hens fed a flaxseed enriched diet compared to normal ovaries of 1, 1.5 and 2 year old hens fed control diet, respectively ($P > 0.05$; Fig. 4 A and B). Normal ovaries of 2.5 ($n = 19$), 3 ($n = 11$) and 3.5 ($n = 16$) year old hens fed a flaxseed enriched diet had significantly lower expression of COX-2 protein compared to normal ovaries of 2.5 ($n = 18$), 3 ($n = 7$) and 3.5 ($n = 10$) year old hens fed control diet, respectively (Fig. 4A and B). Similar to the protein expression, normal ovaries of 2.5 ($n = 19$), 3 ($n = 11$) and 3.5 ($n = 16$) year old hens fed a flaxseed enriched diet had lower COX-2 mRNA expression compared to normal ovaries of hens fed control diet aged the same ($P < 0.05$; Fig. 4 C).

A significant difference in COX-2 protein and mRNA expression was found between cancerous ovaries of 3 year old hens fed a flaxseed enriched diet ($n = 9$) versus cancerous ovaries of hens fed control diet aged the same ($n = 13$; $P < 0.05$; Fig. 5). The 3.5 year old chickens fed a flaxseed enriched diet ($n = 4$) had lower COX-2 protein and mRNA expression in their cancerous ovaries compared to the cancerous ovaries of 3.5 year old hens fed control diet ($n = 10$; $P < 0.05$; Fig. 5).

Flaxseed decreases PGE_2 concentrations in ovaries

PGE_2 concentrations were measured in ovaries of hens as a possible function of flaxseed enriched diet. Significantly lower amounts of PGE_2 were found in normal ovaries of 1 ($n = 8$), 1.5 ($n = 8$) and 2 ($n = 8$) year old hens fed a flaxseed enriched diet compared to normal ovaries of 1 ($n = 8$), 1.5 ($n = 8$) and 2 ($n = 8$) year old hens fed control diet, respectively ($P < 0.05$; Fig. 6A). The normal ovaries of 2.5 year old hens fed a flaxseed enriched diet ($n = 8$) had significantly lower PGE_2 concentrations compared to normal ovaries of 2.5 year old hens fed control diet ($n = 8$; $P < 0.01$). Furthermore, flaxseed enriched diet decreased the PGE_2 concentrations in normal ovaries of 3 ($n = 8$) and 3.5 ($n = 8$) year old hens compared to normal ovaries of 3 ($n = 7$) and 3.5 ($n = 8$) year old hens fed control diet, respectively ($P < 0.001$).

The concentrations of PGE_2 in cancerous ovaries of 3 ($n = 8$) and 3.5 ($n = 4$) year old hens fed a flaxseed enriched diet were decreased compared to cancerous ovaries of age matched hens fed control diet ($n = 8$) ($P < 0.001$; Fig. 6B).

Discussion

The goals of our study were to investigate the effect of long term consumption of flaxseed enriched diet on the incidence and severity of ovarian cancer and to compare the expression of COX-1, COX-2 and concentration of PGE_2 in normal and cancerous hen ovaries to determine any potential correlation. Here, we report that long-term consumption of 10% flaxseed enriched diet decreases the incidence and severity of ovarian cancer in the laying hen. The expression of both COX-2 protein and mRNA was decreased in normal ovaries of 2.5, 3 and 3.5 year old hens fed a flaxseed enriched diet compared to normal ovaries of age-matched hens on control diet. Flaxseed down-regulated the COX-2 protein and mRNA expression in cancerous ovaries of 3 and 3.5 year old hens compared to cancerous ovaries of 3 and 3.5 hens fed control diet, respectively. At all ages, normal ovaries of hens fed a flaxseed enriched diet had lower PGE_2 concentrations compared to normal ovaries of hens fed control diet indicating

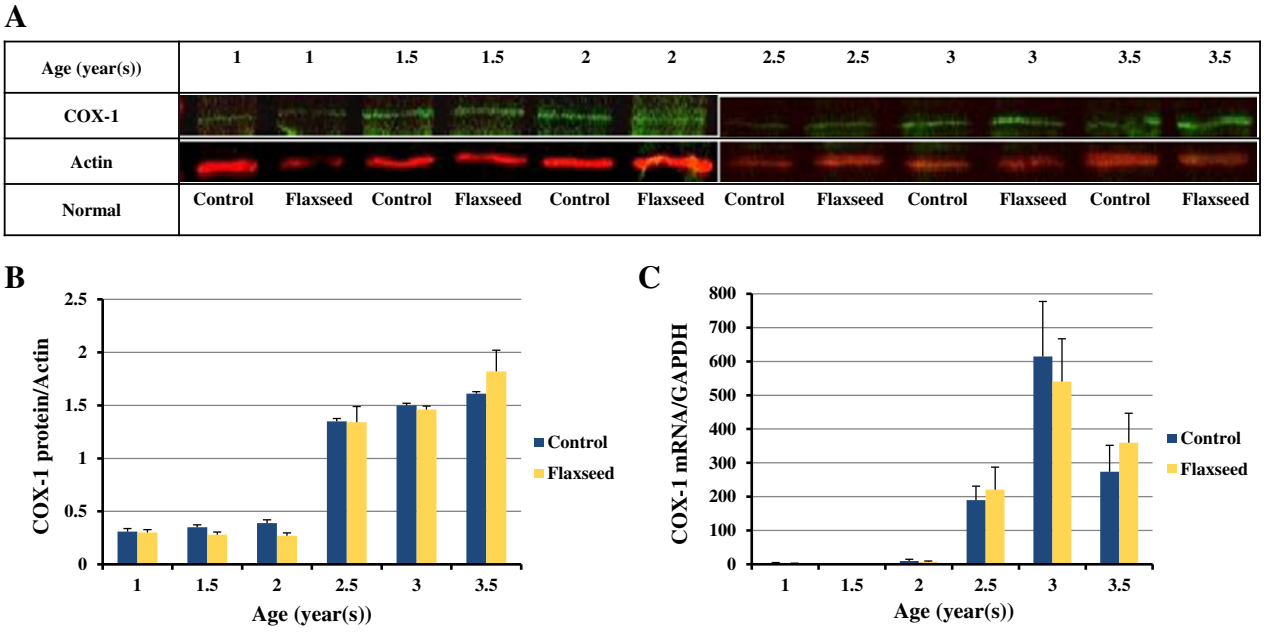

Fig. 2. Expression of COX-1 protein and mRNA was measured in normal ovaries of hens fed a flaxseed enriched and normal ovaries of hens fed control diet at approximately 6 month intervals. (A& B) No significant differences in COX-1 protein expression in normal ovaries of hens fed either flaxseed enriched diet or control diet were observed at all ages ($P > 0.05$). (C) Similar to the protein expression, flaxseed diet did not change the COX-1 mRNA expression in normal ovaries of hens compared to ovaries of hens fed control diet ($P > 0.05$). Bars indicate standard error.

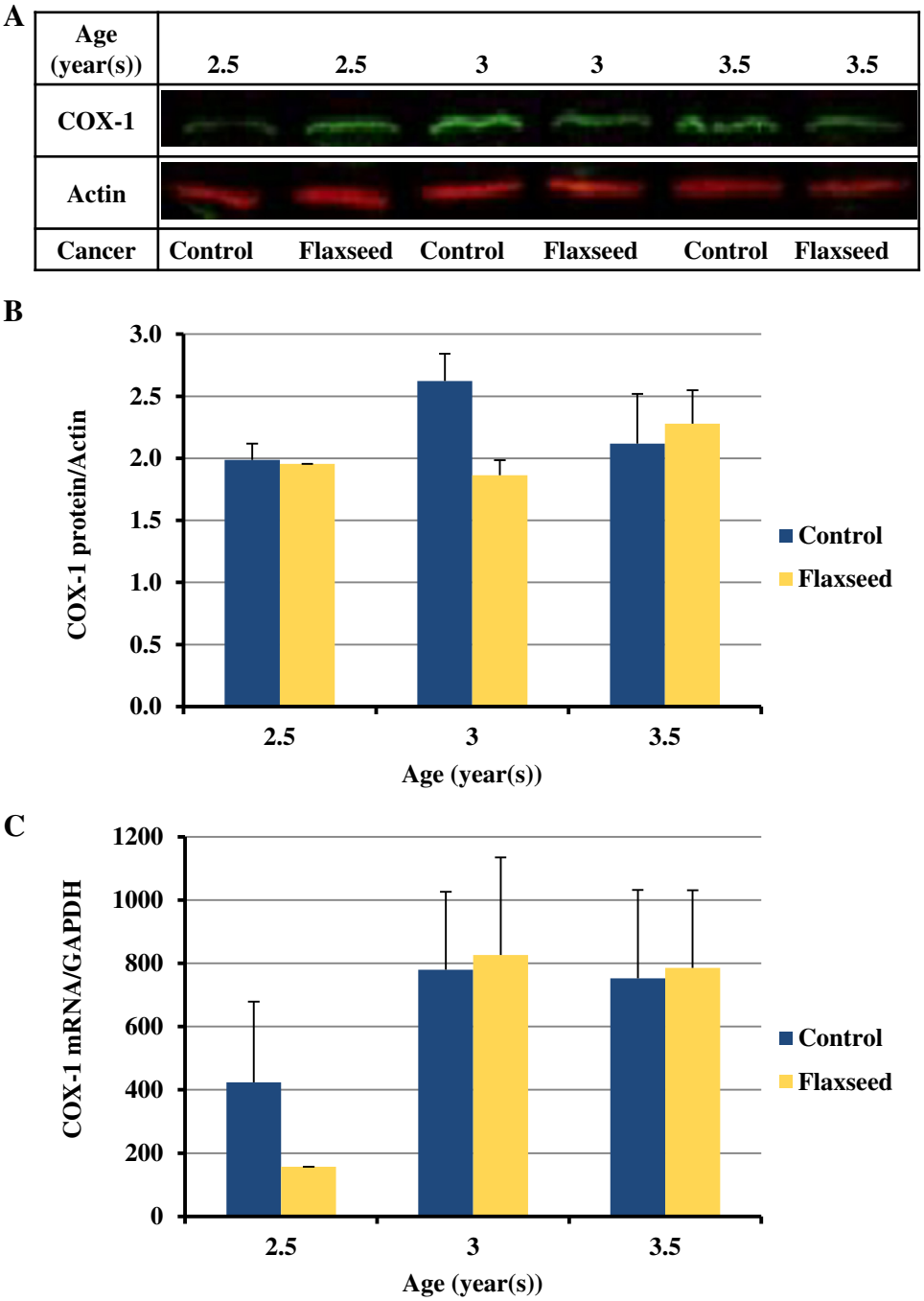

Fig. 3. Expression of COX-1 protein and mRNA was measured in cancerous ovaries of hens fed a flaxseed enriched diet and cancerous ovaries of hens fed control diet at approximately 6 month intervals. There was no significant difference in COX-1 protein (A&B) and mRNA (C) expression in cancerous ovaries of 3 and 3.5 year old hens fed a flaxseed enriched diet versus age matched hens fed control diet ($P > 0.05$). Bars indicate standard error.

a negative correlation between flaxseed consumption and PGE_2 concentrations in the ovaries. In correlation with decreased incidence and severity of ovarian cancer, concentration of PGE_2 was decreased significantly in cancerous ovaries of 3.5 year old hens fed flaxseed compared to cancerous ovaries of hens on control diet at the same age.

Cancer appears in hens starting at 2–2.5 years of age [16]. We have shown that when hens of this age are fed a flaxseed enriched diet for one year, there was a significant amelioration of ovarian cancer, markedly reducing the severity of the disease in affected hens [15]. The overall wellbeing of the flaxseed-fed hens was also improved by several criteria including a significant reduction in mortality. However, the overall incidence of cancer was not reduced in the flaxseed-fed hens compared to age-matched control diet-fed hens. It is likely that the older hens had already experienced significant ovulation induced inflammatory damage to their ovaries and were harboring occult disease. The anti-inflammatory actions of the flaxseed enriched diet reduced the progression of the disease so that at time of necropsy the affected hens presented with much less advanced disease. The present study was conducted so that hens would be provided flaxseed diet from the time of their first lay (around 6 months of age) for 4 years, until they reached the age of the old hens used in the first study. The results of the present study demonstrate that there was a significant reduction in the incidence of ovarian cancer, as well as a reduction in the severity of ovarian cancer in the few hens which did

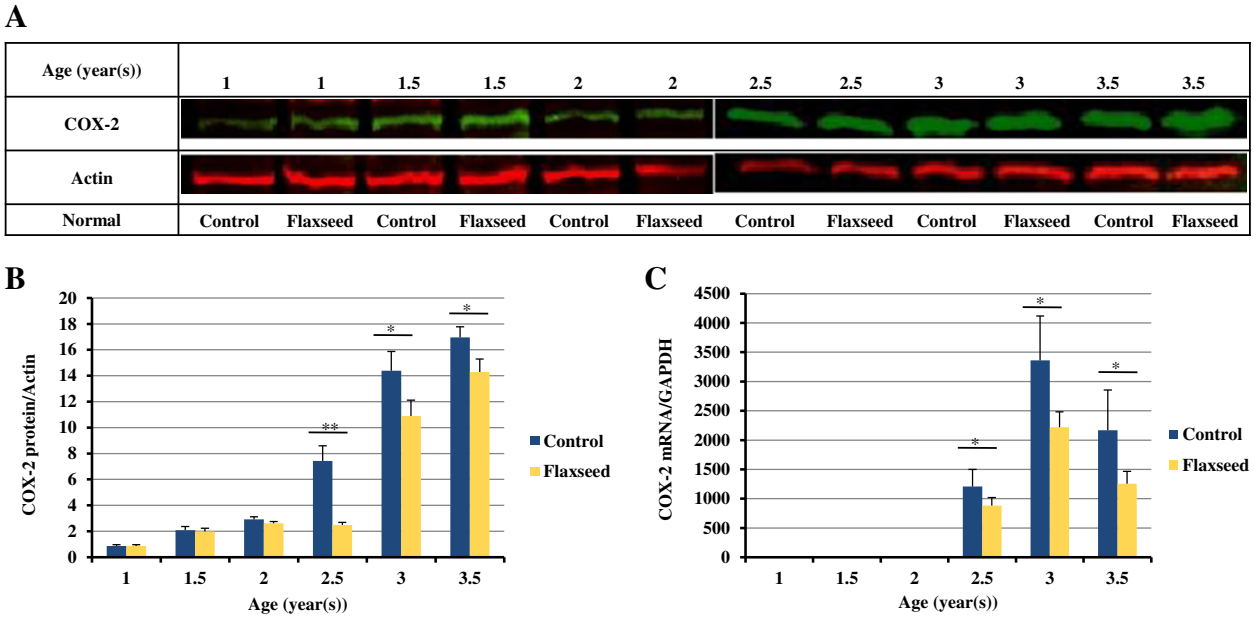

Fig. 4. Expression of COX-2 protein and mRNA was measured in normal ovaries of hens in both flaxseed and control groups at different ages. Normal ovaries of 2.5 (n = 19), 3 (n = 11) and 3.5 (n = 16) year old hens fed a flaxseed enriched diet had significantly lower expression of COX-2 protein (A&C) and mRNA (C) compared to normal ovaries of 2.5 (n = 18), 3 (n = 7) and 3.5 (n = 10) year old hens fed control diet. *, $P < 0.05$; **, $P < 0.01$; Bars indicate standard error.

present with cancer. Therefore long-term intervention with flaxseed was effective in chemo-prevention as well as suppression, whereas one-year was not an adequate length of time on diet to achieve effective prevention. These results were in agreement with a study that showed flaxseed reduced the incidence, number and growth of tumors in a carcinogen-induced breast cancer model in rats [19,20]. Moreover, a randomized, placebo controlled, clinical trial in postmenopausal women with newly diagnosed breast cancer also found that the daily consumption, from the time of diagnosis to surgery, of 25 g flaxseed in a muffin formulation, reduced tumor cell proliferation and increased apoptosis, whereas placebo muffin did not [21]. This is the first report describing the long term therapeutic efficacy of flaxseed enriched diet on the prevention of ovarian cancer in the laying hen. Our finding has a therapeutic potential because women at high risk for developing ovarian cancer could supplement their diet with flaxseed which may prevent ovarian cancer or significantly delay its progression.

We and others have shown that COX-1 is over-expressed in ovarian cancer [2–4]. The regulation of PGE_2 synthesis by COX-1 in ovarian cancer cell lines [22] and hen ovaries with tumor [4] has been previously shown. These findings indicate that targeting COX-1 activity may be a mechanism of flaxseed action to attenuate and even suppress the ovarian cancer. We found no changes in COX-1 protein and mRNA expression in the normal or cancerous ovaries of laying hens on flaxseed diet compared to the hens on control diet indicating that flaxseed does not affect the expression of COX-1 enzyme in hen ovaries. However, it is likely that the omega-3 fatty acids in flaxseed decrease COX-1 enzyme activity by modulating substrate levels, as described below, resulting in a decrease in PGE_2.

Many years of ovulation, longer exposure to environmental factors, ovarian aging and accumulated DNA damage might be associated with increased rate of ovarian cancer with age. We have shown an increase in the ovarian cancer incidence in the laying hen after two years of ovulation, concomitant with increased PGE_2 levels [16]. Moreover, we have shown that while COX-1 increases with cancer, COX-2 increases with age [3]. We propose that the age-related increase in PGE_2 may help to establish a pro-carcinogenic environment which helps to promote ovarian carcinogenesis.

Elevation of PGE_2 in ovarian cancer has been previously reported [23]. Prostaglandins are potent mediators of intercellular communication, and high concentrations of PGE_2 are believed to be immunosuppressive of T cell-mediated immunity [24], increase angiogenesis [25] and stimulate cell proliferation and inhibit apoptosis in ovarian cancer cell lines [26]. Consumption of the flaxseed-enriched diet decreased the concentration of PGE_2 in both normal and cancerous ovaries of the hens, regardless of their age. Flaxseed has been reported to reduce PGE_2 levels in kidney tissue in rats [27], injured skin in mice [28] and skeletal muscle in chicks [29]. Moreover, Urick et al. showed that treatment of the hens with a diet containing 0.1% aspirin for one year reduced PGE_2 concentrations in the liver and decreased the stage but not the incidence of ovarian cancer [30]. The mechanisms by which the OM-3FAs inhibit PGE_2 remain to be fully elucidated. Dietary OM-3FAs may modulate substrate pools available to COXs and lipoxygenases (LOX), thereby controlling the downstream eicosanoid formation and subsequent receptor activation [31]. The OM-6FA arachidonic acid (AA) is the predominant substrate for both COX enzymes. The omega-3 fatty acid EPA can compete with AA and act as an alternative substrate for COX enzymes [32]. COX enzymes convert OM6-FAs to 2-series PG products such as PGE_2 whereas the end products of COX enzymes activity on OM-3FAs are 3-series prostaglandins such as PGE_3. The 3-series PG products are generally less pro-inflammatory than the 2-series products [33–35]. Although a reduction in tissue levels of pro-tumorigenic PGE_2 alone could explain the anti-cancer properties of EPA, it is possible that PGE_3 per se could also contribute to the anti-tumorigenic activity of EPA [36].

COX-2 initiates the inflammatory process [37] and is related to the formation and promotion of tumors and inhibition of apoptosis, angiogenesis and the metastatic process [38]. Consumption of OM3-FAs enriched diets had inhibitory effects on COX-2 and prostaglandin production in both plasma and experimentally induced tumors [39] but the effect of flaxseed, as a plant source of OM-3FAs on COX-2 expression in ovarian cancer is unknown. Our data indicated that flaxseed enriched diet decreases the COX-2 expression in normal ovaries of hens aged 2.5, 3 and 3.5 years compared to age matched hens fed control diet. Furthermore, flaxseed diet decreased the COX-2 expression in cancerous ovaries of hens aged 3 and 3.5 years compared to cancerous ovaries of age-matched hens fed control diet. Our results are in agreement with previous reports that have shown the dietary flaxseed reduces COX-2 expression in colon cancer in rats [40] and the dietary ALA reduces COX-2

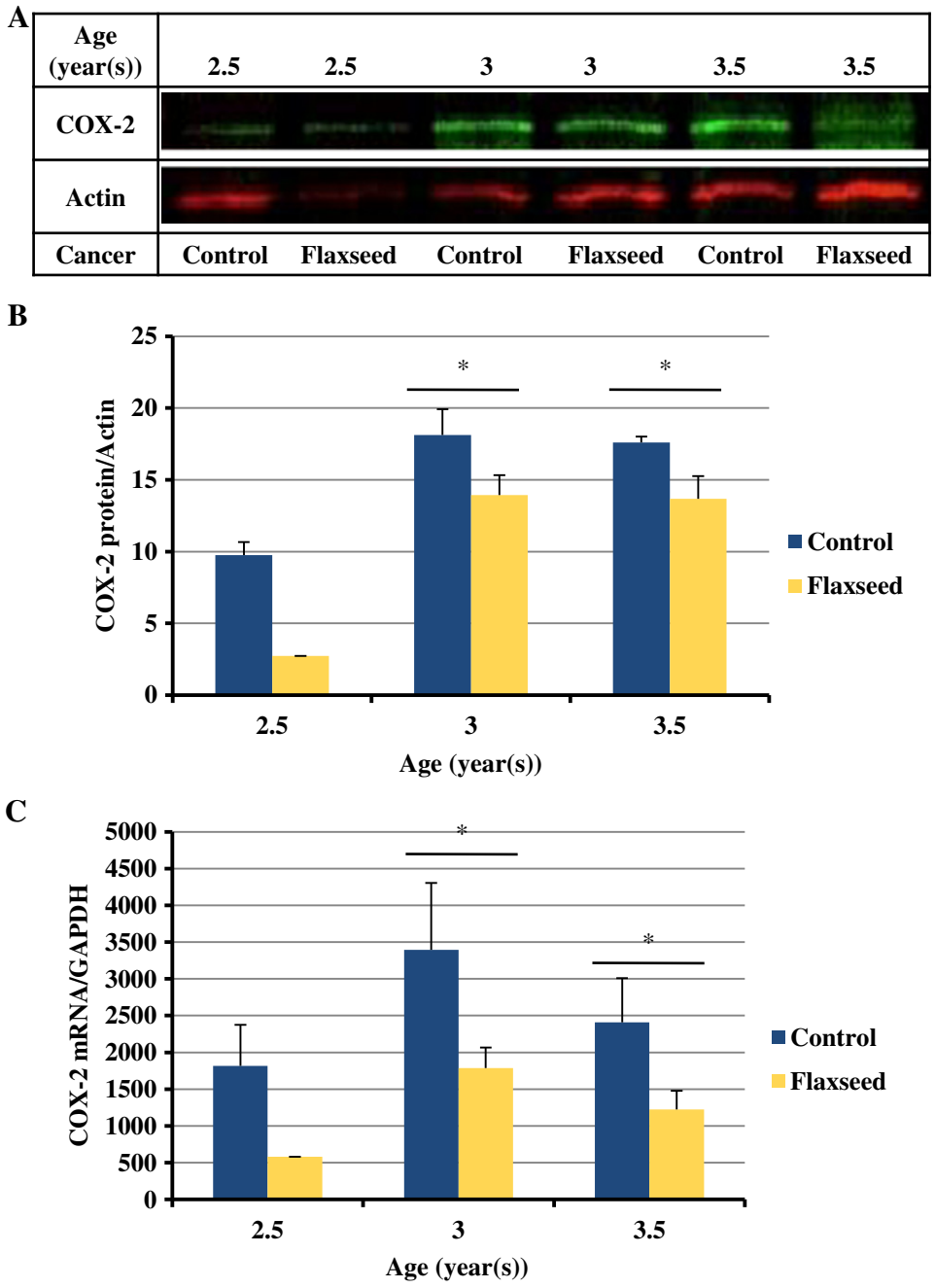

Fig. 5. COX-2 protein and mRNA expression in cancerous ovaries of hens fed flaxseed enriched diet versus cancerous ovaries of hens fed control diet. The 3 (n = 9) and 3.5 (n = 4) year old hens fed a flaxseed enriched diet had lower COX-2 protein (A&B) and mRNA (C) expression than 3 (n = 13) and 3.5 (n = 10) year old hens with ovarian cancer fed control diet, respectively; *, $P < 0.05$; Bars indicate standard error.

expression and induces apoptosis in hepatoma cells [41]. The present study confirms previous reports and demonstrates that COX-2 increases as a function of age, not ovarian cancer per se.

Taken together, our findings demonstrated that the long-term dietary intervention with flaxseed decreased the concentration of PGE_2; the expression of COX-2 in ovaries and incidence and severity of ovarian cancer in the laying hens. Regardless if the ovarian cancers originate from the ovarian surface epithelium, or result from carcinogenic transformation of the distal tubal epithelium, the inflammation associated with ovulation likely contributes to the development of cancers from both sites. Flaxseed reduces PGE_2, the most pro-inflammatory prostaglandin thus reduces the inflammation associated with ovulation. These findings are in consistent with the notion that high concentrations of PGE_2 contributes to initiation and/or progression of ovarian cancer and reducing PGE_2 concentrations in the ovary may prevent this highly lethal malignancy. Moreover, we demonstrated that the decreased COX-2 expression corresponds to the reduction in PGE_2 concentrations suggesting that blocking its expression might be a suitable target to reduce ovarian cancer. To our knowledge the present study provides the first insight into the efficacy of long-term consumption of flaxseed-enriched diet on incidence and severity of ovarian cancer, COX enzymes expression and prostaglandin E_2 concentration in ovarian tissue. These findings may provide the basis for clinical trials of

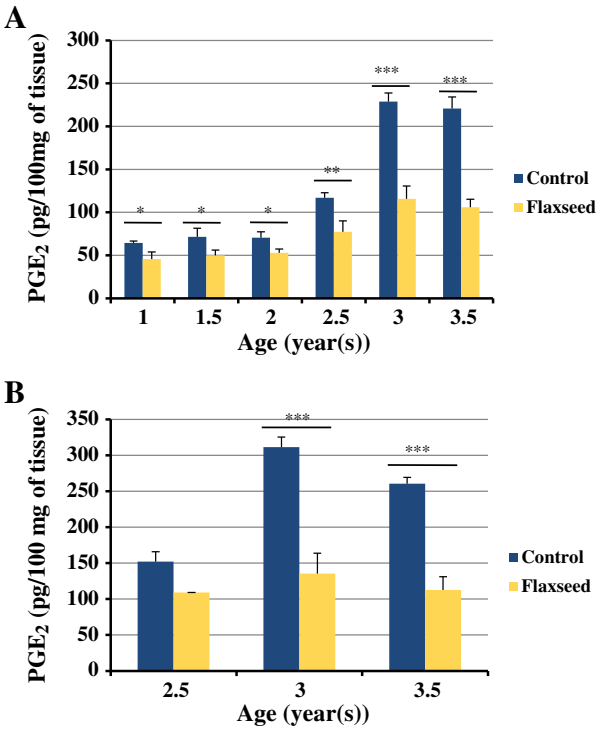

Fig. 6. Comparison of prostaglandin E_2 concentrations in ovaries of hens fed a flaxseed enriched diet and hens fed control diet. (A) There was a significant reduction in concentration of PGE_2 in normal ovaries of hens fed a flaxseed enriched diet compared to normal ovaries of hens fed control diet regardless of age. (B) PGE_2 concentration in cancerous ovaries of 3 ($n = 8$) and 3.5 ($n = 4$) year old hens fed a flaxseed enriched diet was significantly lower than PGE_2 concentration in cancerous ovaries 3 ($n = 8$) and 3.5 ($n = 8$) year old hens fed control diet. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; Bars indicate standard error.

dietary intervention targeting prostaglandin biosynthesis for the prevention and treatment of ovarian cancer.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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