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Ameliorative Effects of Dietary Intake of Egg Yolk and Cod Liver Oil on Dexamethasone Induced Osteoporosis in Rats

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Abstract

This research investigated the impact of egg yolk (EY) and cod liver oil (CLO) on osteoporosis in rats caused by dexamethasone. Nutritional investigation indicated that the use of EYor CLO enhanced body weight growth and improved feed efficiency ratio values. The biochemical study indicated significant bone mass loss in the control positive group, as seen by elevated blood alkaline phosphatase (ALP), osteocalcin (OC) levels, and increased urine calcium, creatinine, uric acid, nitrogen, along with urinary pyridinoline (Pyr) and deoxypyridinoline (D-Pyr). Bone mineral density and concentration (BMD&BMC), together with calcium and phosphorus levels, were reduced in the femur, indicating dexamethasone-induced osteoporosis. The use of EY or CLO may sustain blood levels of calcium, phosphorus, and osteocalcin. The administration of CLO in osteoporotic rats increased the excretion of Ca and Pyr, reduced urine P, and exhibited no significant excretion of D-Pyr. The use of EY with CLO showed no notable alterations in hepatic and renal function, other than increased creatinine levels, and assisted in preserving normal bone loss levels. The daily oral dosage of EYor CLO, or both, for sixty days, reduced the occurrence of adverse symptoms associated with dexamethasone-induced osteoporosis.

Keywords: Cod liver oil; Dexamethasone; Egg yolk;Osteoporosis

1. Introduction

Osteoporosis is a chronic condition marked by diminished bone density, increasing the risk of fractures and breaks. It is more prevalent among older persons; however, it may impact individuals of any age, making it a significant public health issue. While several drugs are available to treat osteoporosis, many people are investigating nutritional approaches as preventative or therapeutic options. One technique is the consumption of EY and CLO, both rich in vital nutrients such as omega-3 fatty acids, vitamin K, and vitamin D, which are thought to promote bone health. Researchers have done a study to ascertain if these dietary ingredients might possibly mitigate dexamethasone-induced osteoporosis in rats. The results in this context may facilitate the formulation of dietary recommendations for the treatment and prevention of osteoporosis. Osteoporosis is characterized by decreased bone density, degradation of bone microarchitecture, heightened brittleness, and an elevated risk of fractures. This bone disorder may lead to considerable disability, morbidity, and death [1, 2]. Osteoporosis is categorized into two types: primary and secondary. Primary osteoporosis often results from postmenopausal alterations or senescence, while secondary osteoporosis is frequently associated with glucocorticoid use, with dexamethasone being a prevalent factor [3, 4]. Dexamethasone is a glucocorticoid commonly employed in clinical practice for the management of diverse inflammatory conditions, autoimmune disorders such as systemic lupus erythematosus and rheumatoid arthritis, respiratory ailments including chronic obstructive pulmonary disease and asthma, as well as in organ transplantation protocols. Glucocorticoid treatment may result in several consequences, such as myopathy, glaucoma, diabetes, obesity, and cardiovascular problems. Excessive glucocorticoids impede osteoblast development and maturation, leading to diminished bone formation and increased bone resorption, significantly decreasing their quantity and functioning. Furthermore, osteocytes, osteoblasts, and mesenchymal stem cells originating from bone marrow may also experience apoptosis upon exposure to glucocorticoids [6]. The worldwide incidence of osteoporosis and osteopenia presents substantial health issues owing to the concomitant

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reduction in bone density, leading to brittle bones, recurrent fractures, extended recovery periods, and major morbidity. The degree of bone loss resulting from pharmacological glucocorticoids depends on the dose and length of therapy. A variety of prescription medications have been used to improve bone density and prevent bone loss. [7, 8]. This includes antiresorptive medicines such as calcitonin, estrogen, bisphosphonates, and parathyroid hormone therapies. Nonetheless, these alternatives may be expensive and may lead to inadequate long-term compliance owing to adverse consequences [4, 9]. This research is to assess the effects of dietary consumption of EY and CLO on dexamethasone-induced osteoporosis in rats.

2. MATERIALS AND METHODS

Dietary Composition

The rats were given a basic diet created according to the approach of Reeves et al. [10]. CLO was obtained from Seven Seas Co. and delivered at a daily dose of 0.5 g/kg via a stomach tube for a duration of 60 days. Egg yolks were obtained from hens' eggs and freeze-dried, thereafter suspended in 1 ml of 5% polyoxyethylene sorbitan mono-oleate for oral administration at a dose of 0.500 mg per rat via a stomach tube for the same time [11].

Experimental Animals

Thirty-five healthy female albino rats were maintained in a controlled environment including a 12-hour light/dark cycle, a temperature of 25°C, and humidity levels of 40–50%. The rats had an average weight of 180 ± 5 grams and were provided with unlimited access to water and a baseline diet throughout the experiment, in accordance with institutional norms established by the Institute of Laboratory Animal Resources [12].

Experimental Design

The rats were acclimatized for one week and then randomized into five groups (n=7):

- Control -ve: Normal rats were given alone the standard food and sterile physiological saline.
- Control positive: Rats were induced with osteoporosis with subcutaneous injections of 0.6 mg/kg dexamethasone every three days for a duration of 60 days while maintained on a basal diet.
- Osteoporosis-induced rats were administered a basic diet supplemented with EY (0.500 mg/rat) orally via a stomach tube for a duration of 60 days.
- CLO Group: Rats with osteoporosis were administered a baseline diet supplemented with CLO (0.5 g/kg) daily via a stomach tube for a duration of 60 days.
- EY Plus CLO Group: Osteoporosis-induced rats were administered EY (0.500 mg/rat) orally and CLO (0.5 g/kg) daily via a gastric tube for a duration of 60 days.

Feed efficiency ratio (FER), food intake (FI), and body weight gain (BWG) were calculated at the conclusion of the experimental period by daily monitoring of food intake and weekly assessments of weight gain, according to the methods established by Chapman et al. [13].

Hematological Specimens and Organ Repositories

At the conclusion of the experimental period, rats were subjected to a 24-hour fast and placed in metabolic cages for the collection of urine samples, which were then acidified with 2 mL of 1 mol/L HCl. Blood samples were collected subsequent to the anesthetization of the rats with ketamine hydrochloride (35 mg/kg intramuscularly), followed by killing by cervical dislocation. Serum and urine specimens were obtained and thereafter preserved at −20°C for further examination. The femurs were excised, wrapped in moist gauze, and preserved at −20°C.

Biochemical Metrics

Serum and urine calcium and phosphorus concentrations were quantitatively assessed by spectrophotometry, employing the methodologies established by Gindler & King [14] and Goodwin [15], respectively. Serum levels of osteocalcin (OC), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatinine, uric acid, and urinary nitrogen were evaluated utilizing methodologies outlined by Craciun et al. [16], Roy [17], Reitman & Frankel [18], Husdan & Rapoport [19], Barham & Trinder [20], and Eastham [21]. The concentrations of urine pyridinoline (Pyr) and deoxypyridinoline (D-pyr) were measured according to the technique established by Takahashi et al. [22]. The femurs were dried in a muffle furnace at 700°C for seven hours after defatting with acetone to yield ash. Bone mineral density (BMD), bone mineral concentration (BMC), calcium, and phosphorus levels were assessed in femoral bones using the approach of Lochmüller et al. [23].

Statistical Examination

Data analysis was conducted using parametric statistics (ANOVA test). The SPSS program was used to compare data across treatment groups, and the post-hoc Tukey's test was performed. The results

are expressed as mean ± standard deviation (SD), with statistical significance evaluated at $P < 0.05$.

3. Results and Discussion

Nutritional Metrics

Following 60 days of oral therapy, the control positive group demonstrated substantial decreases in body weight gain, food intake, and feed efficiency relative to the control negative group. The consumption of either EY or CLO led to elevated BWG levels compared to the control positive group; however, these values were still inferior to those recorded in the control negative group. Both EY and CLO consumption significantly increased BWG levels. In comparison to the control positive group, no substantial variations in food intake (FI) or feed efficiency ratio (FER) were seen relative to the control negative group (refer to Table 1).

Table 1. Assessments of body weight gain (BWG), total food intake (TFI), and feed efficiency ratio (FER) for control groups (-ve & +ve) and various groups administered with EY and CLO.

Groups	BWG (g)	TFI (g)	FER
Control -ve	91.17 ± 5.11^{a}	8320.5 ± 2.11^{a}	0.011 ± 2.42^{ab}
Control +ve	52.33 ± 4.61^{d}	8315.6 ± 1.03^{c}	0.006 ± 4.48^{e}
EY	76.75 ± 6.11b^{c}	8318.7 ± 1.11^{ab}	0.009 ± 5.5^{d}
CLO	79.88 ± 7.11^{bc}	8318.9 ± 1.22^{ab}	0.01 ± 5.83^{c}
EY+ CLO	88.71 ± 8.25^{b}	8319.8 ± 1.14^{a}	0.011 ± 7.24^{a}

The mean values were presented as mean ± SD, and significant differences among mean values in each column were indicated by different superscript letters (a, b, c, d). FER = BWG/FI.

3.2. Biochemical metrics

Numerous investigations have shown that, among corticosteroids used in experimental animal models, dexamethasone is acknowledged as the most powerful inducer of osteoporosis. The dose, duration, and long-term effects of this medication have been shown to substantially contribute to the development of osteoporosis [24, 25]. Our data demonstrate that dexamethasone promotes osteoporosis and results in growth retardation, as shown by diminished feed efficiency ratio (FER), food intake (FI), and body weight gain (BWG). These discoveries correspond with findings from further research investigations [26-29]. The capacity of glucocorticoids to influence growth hormone activity, insulin-like growth factor 1 (IGF-1) receptor signaling, and IGF-1 pathways in chondrocytes has been established by Wong et al. [30]. Furthermore, Mazziotti and Giustina [31] indicated that glucocorticoids suppress the production of prostaglandin E2 and interfere with the pulsatile release of growth hormone by elevating somatostatin levels from the anterior pituitary gland. Eggs are esteemed for their substantial nutritional value, encompassing proteins, peptides, hydrolyzed proteins, and amino acids, along with several biological attributes, including antioxidant characteristics. Likewise, marine oils such as cod liver oil have shown efficacy in alleviating rheumatoid arthritis by reducing inflammation, pain, soreness, and rigidity. These results corresponded with the research conducted by Kovacs-Nolan et al. and Trofimiuk and Braszko [32, 33]. CLO is a popular nutritional supplement because to its high concentration of Omega-3 fatty acids, including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), along with substantial quantities of carotenoids, vitamin A, and vitamin D. Moreover, it includes bile acids like cholic acid and taurine, which are essential for the absorption of fat-soluble vitamins [34, 35].

Table 2 demonstrates that blood calcium levels were decreased, although serum phosphorus and osteocalcin levels were markedly increased in the osteoporotic control group. Nevertheless, the consumption of EY or CLO resulted in elevated calcium levels relative to the osteoporotic control group, whereas no significant changes were seen in phosphorus and osteocalcin levels when compared to the control group. Moreover, the intake of both EY and CLO did not produce significant variations in calcium, phosphorus, or osteocalcin levels relative to the control group.

Table 2. Changes of serum Ca, P, and OC in experimental groups

Groups	Ca (mg/dl)	P (mg/dl)	OC (µg/l)
Control -ve	12.10±2.11^{a}	5.77±0.35^{b}	10.14±1.34^{bc}
Control+ve	8.33±1.20^{c}	6.96±0.77^{a}	13.22±1.17^{a}
EY	10.70±1.13^{b}	5.21±0.34^{b}	10.99±1.43^{bc}
CLO	10.66±1.14^{b}	4.99±0.25^{bc}	11.41±1.66^{b}
Egg + CLO	11.45±1.75^{ab}	5.21±0.33^{b}	10.88±1.35^{bc}

The mean values were expressed as mean ± SD, with significant variations among the mean values in each column denoted by distinct superscript letters (a, b, c, d).

Prior studies have shown that glucocorticoids may indirectly affect calcium metabolism by increasing renal calcium excretion, diminishing intestinal calcium absorption, inhibiting growth hormone levels, and decreasing calcitonin production. The research noted markedly reduced blood calcium levels in the osteoporotic control group. Research conducted by Ji et al. [37] and Forsmo et al. [38] indicates that EY-soluble protein may augment collagen staining, boost alkaline phosphatase (ALP) activity, and improve calcium content. CLO, acknowledged as a conventional source of vitamin D, is often advised for enhancing bone health. Our findings indicate that the combination of CLO and EY may function as effective medicines for the treatment and prevention of osteoporosis. The

osteoporotic positive control group had significantly increased blood levels of alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), uric acid, creatinine, and urine nitrogen compared to the negative control group. Rats treated with either EY or CLO did not show substantial elevations in blood ALT, AST, or uric acid levels. Nonetheless, there was a significant increase in serum alkaline phosphatase (ALP), creatinine, and urine nitrogen relative to the negative control group. The simultaneous treatment of EY and CLO did not cause significant alterations in serum ALP, ALT, AST, uric acid, or urinary nitrogen levels; however, it did lead to increased creatinine levels relative to the negative control group.

Table 3 Alterations in blood ALP, ALT, and AST enzymes, as well as creatinine, uric acid, and urine nitrogen levels in experimental groups

Groups	ALP(U/L)	ALT(U/L)	AST(U/L)	Creatinine (mg/dl)	Uric acid (mg/dl)	Urinary nitrogen(mg/dl)
Control -ve	135.77±10.11^{c}	28.81±2.1^{bc}	35.77±4.96^{bc}	0.59±0.01^{d}	1.55±0.40^{bc}	65.77±6.41^{c}
Control +ve	307.41±25.22^{a}	55.96±4.33^{a}	62.41±6.27^{a}	1.12±0.22^{a}	3.22±0.77^{a}	98.11±9.22^{a}
EY	151.33±12.15^{b}	31.44±3.22^{b}	37.22±5.33^{b}	0.77±0.05^{bc}	1.77±0.35^{b}	73.16±7.30^{b}
CLO	155.71±13.22^{b}	30.51±3.41^{b}	38.35±4.91^{b}	0.80±0.07^{b}	1.80±0.42^{b}	74.69±7.11^{b}
Egg plus CLO	140.31±14.27^{bc}	32.11±3.61^{b}	35.76±5.14^{bc}	0.78±0.01^{bc}	1.66±0.33^{bc}	70.22±6.11^{bc}

The average values were expressed as mean ± SD, with significant variances among the mean values in each column denoted by distinct superscript letters
(a, b, c, d).

The findings of this study corresponded with the conclusions of Nagui and Khalil [39], which indicated that both EY and CLO may improve hepatic and renal function in rats. The omega-3 polyunsaturated fatty acids found in CLO are recognized for their ability to suppress the synthesis of inflammatory mediators critical for osteoclastogenesis, including TNF-α and IL-6. Moreover, alkaline phosphatase (ALP), an enzyme produced by osteoblasts, is an important indicator of bone production because of its association with osteoblast quantity. Prior research has shown that omega-3 fatty acids included in cod liver oil may elevate levels of bone formation indicators, including alkaline phosphatase and osteocalcin. Studies by Högström et al. [40] and Farina et al. [41] have shown a positive correlation between increased fish oil intake and improved bone mineral density. Furthermore, omega-3 fatty acids have been shown to elevate plasma ALP levels while reducing the quantity of osteoclasts. Furthermore, the omega-3 fatty acids and vitamin A included in cod liver oil have been shown to diminish oxidative stress and improve antioxidant activity in many animal models. Vitamin A and its related carotenoids have antioxidant capabilities via their hydrophobic polyene chain, which neutralizes reactive oxygen species and mitigates oxidative damage [33, 43].

Table 4 demonstrates that the osteoporotic positive (+ve) control group had substantially higher levels of urine calcium (Ca), phosphorus (P), pyridinoline (Pyr), and deoxypyridinoline (D-Pyr) in comparison to the negative (-ve) control group. Conversely, the intake of EY by osteoporotic rats led to heightened excretion of Ca and Pyr, with no notable alterations in urine P and D-Pyr levels relative to the control group. CLO ingestion resulted in heightened excretion of Ca and Pyr, with a reduction in urinary P excretion, with no notable alterations in D-Pyr levels compared to the control group. Moreover, the concurrent consumption of EY and CLO did not provide significant variations in urine Ca, P, Pyr, or D-Pyr levels relative to the control group.

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Table 4. Variations in urine calcium, phosphorus, pyridinoline, and deoxypyridinoline in experimental

Groups	Ca (mg/dl)	P (mg/dl)	Pyr (µmol/mol)	D-Pyr (µmol/mol)
Control -ve	5.11±0.43^{c}	9.22±1.14^{b}	57.88±5.33^{c}	85.61±8.55^{b}
Control +ve	9.22±1.17^{a}	11.77±1.55^{a}	99.14±8.41^{a}	132.71±13.51^{a}
EY	6.10±0.66^{b}	8.07±1.21^{bc}	65.17±6.77^{b}	81.41±9.61^{b}
CLO	6.11±0.71^{b}	7.96±1.11^{c}	66.22±6.14^{b}	83.19±8.96^{b}
Egg plus CLO	5.80±0.55^{bc}	8.44±1.10^{bc}	59.67±5.44^{bc}	84.22±8.77^{b}

The mean values were expressed as mean ± SD, with significant variations among the mean values in each column denoted by distinct superscript letters (a, b, c, d).

Recent studies demonstrate that urinary pyridinoline (Pyr) and deoxypyridinoline (D-Pyr) serve as biomarkers for bone resorption, indicating collagen degradation during bone deterioration, which subsequently enters the circulation and is eliminated in urine [44, 45]. Our findings correspond with those of Govindarajan et al. [46], who indicated that extended dexamethasone treatment led to reductions in femur length, calcium content, and bone mineral density in adrenalectomized rats. This research indicates that omega-3 fatty acids from cod liver oil favorably affect bone metabolism by improving calcium absorption via heightened calcium ATPase enzyme activity and reducing urine calcium excretion. Furthermore, the antioxidant peptides included in EY assist in alleviating oxidative damage by chelating pro-oxidative transition metal ions, scavenging free radicals, and diminishing hydroperoxides [47-49].

Table 5 indicates that the femur bones of the osteoporotic control group had markedly lower values for bone mineral content (BMC), bone mineral density (BMD), calcium (Ca), phosphorus (P), and ash concentration in comparison to the control group. Nonetheless, the administration of either EY or CLO to osteoporotic rats led to increased BMD, BMC, calcium, phosphorus, and ash concentration compared to the osteoporotic control group. Moreover, the simultaneous consumption of EY and CLO resulted in these parameters being increased to values akin to those seen in the control group.

Table 5. Alterations in femoral bone mineral density (BMD), bone mineral content (BMC), calcium (Ca), phosphorus (P), and ash in experimental cohorts

Groups	BMD (g/cm2)	BMC (g)	Ca (mg/g dry weight)	P (mg/g dry weight)	Ash (g)
Control -ve	0.20 ± 0.04^{a}	0.13 ± 0.02^{a}	105.77 ± 10.11^{a}	61.77 ± 6.78^{a}	0.80 ± 0.04^{a}
Control +ve	0.10 ± 0.01^{e}	0.06 ± 0.003^{e}	55.96 ± 6.22^{d}	36.77 ± 3.44^{d}	0.60 ± 0.02^{d}
EY	0.16 ± 0.03^{cd}	0.10 ± 0.01^{cd}	91.25 ± 8.41^{c}	50.96 ± 4.50^{bc}	0.74 ± 0.03^{c}
CLO	0.17 ± 0.02^{c}	0.11 ± 0.02^{bc}	90.33 ± 7.11^{bc}	49.82 ± 4.77^{c}	0.72 ± 0.04^{bc}
Egg plus CLO	0.19 ± 0.03^{ab}	0.12 ± 0.04^{ab}	96.41 ± 8.11^{ab}	55.77 ± 5.98^{ab}	0.76 ± 0.05^{ab}

The mean values were expressed as mean ± SD, with significant variations among the mean values in each column denoted by distinct superscript letters (a, b, c, d).

The decrease in bone mineral content (BMC), bone mineral density (BMD), calcium (Ca), and phosphorus (P) levels is a critical indicator of reduced bone strength and increased fracture risk, primarily due to an imbalance in bone metabolism favoring resorption over formation. Egg yolk is rich in essential nutrients and biologically active compounds, including growth-promoting factors such as yolk soluble protein, which facilitates bone growth, enhances growth plate development, and activates bone morphogenetic proteins that regulate osteoblast differentiation and bone formation. Furthermore, EY serves as a source of antioxidants, including free aromatic amino acids such as tryptophan and tyrosine [50]. Research indicates that omega-3 fatty acids included in cod liver oil favorably affect osteoclastogenesis by promoting osteoblastogenesis. This impact is facilitated by a decrease in parathyroid hormone activity and an elevation in markers linked to osteoblastic bone growth [51]. The results from several research corroborate these claims. Omega-3 polyunsaturated fatty acids (PUFAs) have shown the ability to enhance bone characteristics, such as bone mineral density (BMD), by facilitating osteoblast development and concurrently reducing osteoclast production. The advantageous impacts of omega-3 supplementation on bone health are ascribed to their capacity to regulate inflammatory cytokines and improve calcium absorption by augmenting the activity of calcium ATPase enzymes. The incorporation of EY and CLO into the diet may provide a synergistic strategy to boost bone health by delivering essential nutrients that facilitate bone formation and diminish resorption, hence improving skeletal strength and lowering fracture risk.

Limitation of study and future prospectives

The study has several limitations that should be acknowledged. Firstly, the research was conducted exclusively on female albino rats, which may limit the generalizability of the findings to other populations, including male rats and humans. The effects observed in this specific animal model may not fully replicate the complex interactions present in human physiology, particularly concerning osteoporosis and dietary interventions. Secondly, the duration of the study was limited to 60 days, which may not be sufficient to assess long-term effects and potential side effects of egg yolk and cod liver oil consumption on bone health. Future studies should consider longer observation periods to evaluate sustained impacts on bone density and overall health. Additionally, the study did not explore the underlying mechanisms by which egg yolk and cod liver oil exert their beneficial effects, leaving a gap in understanding their biochemical pathways.

Prospective challenges include the need for further research to validate these findings in diverse populations and to elucidate the mechanisms of action involved. Moreover, translating these results into clinical recommendations will require rigorous human trials to establish safety, efficacy, and optimal dosages of egg yolk and cod liver oil for osteoporosis management. Finally, potential dietary restrictions or allergies related to these foods could limit their applicability in broader dietary guidelines for osteoporosis prevention and treatment.

4. Conclusion

This research examined the effects of dietary supplementation with evening primrose oil (EPO) and cod liver oil (CLO) on dexamethasone-induced osteoporosis in rats. The results indicated that the intake of either EY or CLO preserved blood concentrations of calcium, phosphorus, and osteocalcin relative to the control group. Moreover, the research demonstrated that these dietary treatments aided in maintaining normal bone density and mitigating the adverse consequences linked to dexamethasone-induced osteoporosis. The results indicate that enhancing food items with EY and CLO may be an advantageous approach for individuals receiving prolonged dexamethasone therapy. This strategy might have considerable ramifications for formulating dietary interventions to prevent and cure osteoporosis, underscoring the potential contribution of these nutrient-dense foods in improving bone health and alleviating the negative impacts of glucocorticoid treatment

Conflicts of interest

There is no conflict to declare.

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