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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 study examined how egg yolk and cod liver oil affected osteoporosis in rats induced by dexamethasone. Nutritional analysis showed that intake of egg yolk or cod liver oil increased body weight gain and feed efficiency ratio values. Biochemical analysis showed that bone mass loss was evident in the control +ve group, as evidenced by heightened levels of serum ALP, osteocalcin concentration (OC), urinary Ca, creatinine, uric acid, urinary nitrogen, as well as urinary pyridinoline (Pyr) and deoxypyridinoline (D-Pyr). Bone mineral density and concentration (BMD&BMC), Ca, and P levels were decreased in the femur bone, indicating dexamethasone-induced osteoporosis. The intake of egg yolk or cod liver oil could maintain the levels of serum Ca, P, and OC. The intake of cod liver oil in osteoporotic rats increased excretion of Ca and Pyr, decreased urinary P, and showed no significant excretion of D-Pyr. The intake of egg yolk plus cod liver oil showed no significant changes in liver and renal function, except for elevated creatinine levels, and helped maintain normal levels of bone loss. Daily oral administration of egg yolk or cod liver oil, or both, for sixty days lowered the incidence of side effects of dexamethasone-induced osteoporosis.

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

1. Introduction

Osteoporosis is a condition that results in weakened bones, making them more susceptible to fractures and breaks. It may affect people of all ages and is considered a significant public health concern, particularly in older adults. While there are medications available to treat osteoporosis, many people are turning to dietary interventions to help prevent or ameliorate the condition. An approach involves the consumption of egg yolk and cod liver oil, which are rich sources of nutrients such as vitamin D, vitamin K, and omega-3 fatty acids that are thought to play a role in bone health. In this context, researchers have conducted a study to investigate the potential ameliorative effects of dietary intake of egg yolk and cod liver oil on dexamethasone-induced osteoporosis in rats. The findings of this study could have important implications for the development of dietary interventions for the prevention and treatment of osteoporosis [1, 2].

Osteoporosis is a chronic skeletal disease characterized by reduced bone density, microarchitectural deterioration, increased fragility, and delicacy, resulting in weakened bone strength and a higher risk of fractures. This can lead to disability, severe morbidity, and increased mortality [3,4]. Generalized osteoporosis is classified as primary or secondary, with primary osteoporosis resulting from postmenopausal or senile conditions, while the consumption of glucocorticoids is the most common cause of secondary osteoporosis [5].

One of the glucocorticoids used in clinical practice is dexamethasone, which is indicated for the treatment of various inflammatory conditions, autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus, lung disorders such as

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bronchial asthma and chronic obstructive pulmonary disorders, and organ transplantation [6].

Glucocorticoid therapy has many complications, including myopathy, glaucoma, diabetes, obesity, and cardiovascular disease. Excess glucocorticoids hinder bone formation and increase bone resorption by decreasing the differentiation and maturation of osteoblasts, resulting in a decrease in their number and function. Glucocorticoids also induce apoptosis of bone marrow-derived mesenchymal stem cells, osteoblasts, and osteocytes. Osteoporosis and osteopenia are global health problems due to the loss of bone mass, which results in brittle bone structure, recurrent fractures, slow healing, and extreme morbidity. Bone loss caused by pharmacological glucocorticoids depends on the dose and duration of treatment [7,8].

Several prescription therapies have been developed to prevent bone loss and increase bone density, such as antiresorptive drugs like calcitonin, estrogen, and bisphosphonates, and parathyroid hormone medication. However, these treatments are expensive, have poor long-term compliance, and can have side effects [4,9]. This study aims to evaluate the effect of dietary intake of egg yolk and cod liver oil on dexamethasone-induced osteoporosis in rats.

2. Materials and Methods

2.1. Diet composition

The rats were fed a basal diet that was formulated based on Reeves et al. method [10]. The cod liver oil used in the study was purchased from SevenSeas Co. and administered to the rats at a dosage of 0.5 g/kg daily through a stomach tube for 60 days [11]. The egg yolks were separated from the hens' eggs and freeze-dried. These egg yolks were then suspended in 1 ml of 5% polyoxyethylenesorbitan mono-oleate and given to the rats at a dosage of 0.500 mg/rat orally through a stomach tube for 60 days.

2.2. Experimental animals

Thirty-five healthy female Albino rats, weighing an average of 180 ± 5 grams, were housed under standard conditions at a temperature of 25°C, with 40–50% humidity, and a 12/12 h light/dark cycle. The rats had ad libitum access to basal diet and water. The experiment conformed to institutional standards and the regulations of the Institute of Laboratory Animal Resources had been followed during all the experiment phases [12].

2.3. Experimental design

After one week of acclimatization, the rats (n=7) were randomly divided into:

- Control –ve: Normal rats fed basal diet and received sterile physiologic saline.
- Control +ve: Rats were induced osteoporosis with 0.6 mg/kg of dexamethasone subcutaneously every three days for 60 days and fed basal diet.
- Egg yolk group: Rats with induced osteoporosis fed basal diet plus egg yolk dosage of 0.500 mg/rat orally through a stomach tube for 60 days.
- Cod liver oil group: Rats with induced osteoporosis fed basal diet plus a cod liver oil dosage of 0.5 g/kg daily through a stomach tube for 60 days.
- Egg yolk plus cod liver oil group: Rats with induced osteoporosis fed basal diet plus egg yolk dosage of 0.500 mg/rat orally and cod liver oil dosage of 0.5 g/kg daily through a stomach tube for 60 days.

Food intake and weekly weight gain were recorded daily to estimate body weight gain (BWG), food intake (FI), and feed efficiency ratio (FER) at the end of the experimental period, following Chapman et al. method [13] for dietary assessment.

2.4. Blood samples and organ collections

At the end of the experimental period, the rats were deprived of food for 24 hours and kept in metabolic cages to collect urine samples, which were acidified with 2 mL of 1 mol/L HCl. Blood samples were collected from the rats after being anesthetized with ketamine hydrochloride (35 mg/kg, i. m) and then euthanized by cervical dislocation, and both serum and urine samples were stored at −20°C for later analysis. The femurs were dissected, wrapped in wet gauze, and stored at -20°C.

2.5. Biochemical parameters

Calcium and phosphorus levels in serum and urine were measured spectrophotometrically according to Gindler and King's method [14] and Goodwin's method [15], respectively. Serum osteocalcin concentration (OC), alkaline phosphatase (ALP), alanine transaminase, and aspartate transaminase (ALT&AST), creatinine, uric acid, and urinary nitrogen were estimated spectrophotometrically using the methods of Craciun et al. [16], Roy [17], Reitman and Frankel [18], Husdan and Rapoport [19], Barham and Trinder [20], and Eastham [21], respectively. Urinary levels of pyridinoline (Pyr) and deoxypyridinoline (D-pyr) were estimated according to Takahashi et al. method [21]. The femurs were dried in a muffle furnace at 700 ^{o}C for seven hours after being defatted and soaked in acetone to obtain ash. Bone mineral density

(BMD), bone mineral concentration (BMC), calcium, and phosphorus were determined in the femur bone according to Lochmüller et al. method [23].

2.6. Statistical analysis:

The data were analyzed using parametric statistics (ANOVA test), followed by post hoc Tukey's test to compare the results between various treatment groups using SPSS. The data were presented as mean ± standard deviation (SD), and $p<0.05$ was considered statistically significant.

3. Results and Discussion

3.1. Nutritional parameters

After being treated orally for 60 days, the control +ve group exhibited a significant decrease in BWG, FI, and FER when compared to the control -ve group. The consumption of egg yolk or cod liver oil led to increased values of BWG and FER compared to the control +ve group, but still fell short of the values observed in the control -ve group. Meanwhile, the intake of both egg yolk and cod liver oil resulted in an elevation of BWG levels when compared to the control +ve group, and no significant differences in FI and FER were observed when compared to the control -ve group (Table 1).

Table 1
Measurements of body weight gain (BWG), total food intake (TFI) and feed efficiency ratio (FER) of controls (-ve& +ve) and different groups treated with egg yolk and cod liver oil

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}
Egg yolk	76.75 ± 6.11b^{c}	8318.7 ± 1.11^{ab}	0.009 ± 5.5^{d}
Cod liver oil	79.88 ± 7.11^{bc}	8318.9 ± 1.22^{ab}	0.01 ± 5.83^{c}
Egg yolk + cod liver oil	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 parameters

Various studies have previously shown that among different corticosteroids used in laboratory animal models, dexamethasone is considered the most potent inducer of osteoporosis. Moreover, the dose, duration, and long-term effects of the drug have been noted to produce osteoporosis [24,25]. Our findings revealed that dexamethasone induced osteoporosis accompanied by growth retardation, which was characterized by lower BWG, FI, and FER. These results are consistent with other studies results [26,27,28,29]. The ability of glucocorticoids to activate or reduce growth hormone, insulin-like growth factor 1 receptors, and insulin-like growth factor-1 signaling by chondrocytes has been reported by Wong et al. [30]. In a previous study, Mazziotti and Giustina [31] reported that glucocorticoids inhibit the synthesis of prostaglandin E2 and the pulsatile secretion of growth hormone by elevating the somatostatin tone from the anterior pituitary gland.

Eggs have been previously noted to possess high nutritional value due to their egg proteins and related ingredients, such as peptides, protein hydrolysates, and amino acids, in addition to several biological activities, such as antioxidant activity. Marine oils, such as cod liver oil, have also been shown to be beneficial for rheumatoid arthritis by minimizing inflammation, discomfort, tenderness, and stiffness. The results of this study agreed with Kovacs-Nolan et al. and Trofimiuk and Braszko [32,33]. Cod liver oil is a popular dietary supplement due to its high levels of omega-3 fatty acids, eicosapentaenoic acid, and docosahexaenoic acid, as well as relatively high levels of carotenoids, vitamin A, and vitamin D. Furthermore, cod-liver oil contains bile acids, mainly cholic acid and taurine, that are necessary for the utilization of fat-soluble vitamins. This oil provides the essential vitamins required for conjugating bile acids for intestinal fat-soluble vitamin absorption, as reported by Wilton and Rajakumar [34,35].

Table 2 shows that serum Ca levels were reduced, while serum P and OC levels were significantly elevated in the osteoporotic control +ve group. However, intake of egg yolk or cod liver oil increased the Ca levels compared to the control +ve group and did not result in significant changes in P and OC levels compared to the control -ve group. Intake of both egg yolk and cod liver oil did not result in significant changes in Ca, P, and OC levels compared to the control -ve 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}
Egg yolk	10.70±1.13^{b}	5.21±0.34^{b}	10.99±1.43^{bc}
Cod liver oil	10.66±1.14^{b}	4.99±0.25^{bc}	11.41±1.66^{b}
Egg + cod liver oil	11.45±1.75^{ab}	5.21±0.33^{b}	10.88±1.35^{bc}

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).

Previous studies have suggested that glucocorticoids indirectly affect calcium metabolism by reducing intestinal calcium absorption, increasing

renal calcium excretion, suppressing growth hormones, and diminishing calcitonin secretion [36]. Also, this study showed reduced serum Ca levels in the osteoporotic control +ve group.

Studies by Ji et al. and Forsmo et al. [37,38] had reported that egg yolk soluble protein can increase collagen staining, elevate ALP activity, and increase calcium content. Cod liver oil, which is a traditional source of vitamin D, is recommended for bone health. Our results suggest that the combination of egg yolk and cod liver oil may act as favorable agents for the prevention and treatment of osteoporosis.

The osteoporotic control +ve group exhibited significantly elevated levels of serum ALP, ALT, AST, creatinine, uric acid and urinary nitrogen in comparison to the control -ve group (Table 3). However, rats that received egg yolk or cod liver oil did not show any significant increase in serum ALT, AST and uric acid, but displayed a significant increase in serum ALP, creatinine and urinary nitrogen compared to the control -ve group. In contrast, intake of egg yolk plus cod liver oil did not show significant values of serum ALP, ALT, AST, uric acid and urinary nitrogen, but exhibited elevated values of creatinine compared to control -ve group.

Table 3
Changes of serum ALP, ALT and AST enzymes, creatinine, uric acid and urinary nitrogen 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}
Egg yolk	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}
Cod liver oil	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 cod liver oil	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 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)

The obtained results are consistent with Nagui and Khalil's study [39], which demonstrated that egg-yolk and cod liver oil could improve hepatic and renal function impairment in rats. Cod liver oil's omega-3 polyunsaturated fatty acids hinder the production of inflammatory mediators needed for osteoclastogenesis, including TNF-α and IL-6. ALP enzyme, secreted by osteoblasts, is released into the bloodstream and correlates with osteoblast count, making it a useful bone formation marker.

Previous studies have reported an increase in bone-formation indicators such as alkaline phosphatase and osteocalcin after treatment with ω-3 fatty acids found in cod liver oil. High fish oil intake was found to be progressively correlated with bone mineral density by Högström et al. and Farina et al. [40,41]. Omega-3 was also reported to reduce osteoclast count and elevate alkaline phosphatase in plasma [42]. Furthermore, cod liver oil's omega-3 fatty acids and vitamin A contents reduced oxidative stress and augmented antioxidant activity in other animal models. Vitamin A and associated carotenoids have an antioxidant role conferred by the hydrophobic chain of polyene units, which can reduce singlet oxygen, neutralize thiyl radicals, combine with peroxyl radicals, stimulate circulating neutrophils, and reduce plasma lipoperoxides. Cod liver oil restores hepatic cytochrome c oxidase enzyme activity in rats [33,43].

Table 4 shows that the control +ve group exhibited significantly higher levels of urinary Ca, P, Pyr, and D-Pyr excretion compared to the control -ve group. Consumption of egg yolk by osteoporotic rats led to an increase in the excretion of Ca and Pyr, while no significant changes were observed in the excretion of P and D-Pyr in urine compared to the control -ve group. Conversely, consumption of cod liver oil by osteoporotic rats increased the excretion of Ca and Pyr, decreased the urinary excretion of P, and showed no significant changes in D-Pyr excretion compared to the control -ve group. In addition, the consumption of egg yolk and cod liver oil together did not result in any significant changes in urinary Ca, P, Pyr, and D-Pyr levels compared to the control -ve group.

Table 4
Changes of urinary Ca, P, Pyr and D- pyr in experimental groups

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}
Egg yolk	6.10±0.66^{b}	8.07±1.21^{bc}	65.17±6.77^{b}	81.41±9.61^{b}
Cod liver oil	6.11±0.71^{b}	7.96±1.11^{c}	66.22±6.14^{b}	83.19±8.96^{b}
Egg plus cod liver oil	5.80±0.55^{bc}	8.44±1.10^{bc}	59.67±5.44^{bc}	84.22±8.77^{b}

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)

Currently, Pyr and D-Pyr levels in urine are regarded as markers of bone resorption caused by collagen breakdown during bone degradation, which is released into the bloodstream and excreted in urine [44,45]. The results of this study are consistent with the findings of Govindarajan et al. [46], who observed that long-term dexamethasone treatment reduced the bone mineral density, calcium content, and femur length of adrenalectomized rats. Our study's findings suggest that consuming ω-3 fatty acids found in cod liver oil has a positive effect on bone metabolism by increasing calcium absorption through elevated calcium ATPase enzyme activity and reducing urinary calcium excretion. Furthermore, the antioxidant peptides in egg yolk help prevent oxidative damage by scavenging free radicals, chelating pro-oxidative transition metal ions, and reducing hydroperoxides [47,48,49].

According to Table 5, the femur bone of the osteoporotic control +ve group exhibited significantly lower BMD, BMC, Ca, P, and ash concentration than the control -ve group. However, consuming egg yolk or cod liver oil by osteoporotic rats resulted in an increase in BMD, BMC, Ca, P, and ash concentration compared to the control +ve group. Furthermore, consuming egg yolk and cod liver oil together elevated these parameters and reached the concentration levels of the control -ve group.

Table 5
Changes of femur BMD, BMC, Ca, P and ash in experimental groups

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}
Egg yolk	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}
Cod liver oil	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 cod liver oil	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 presented as mean ± SD, and significant differences among mean values in each column were indicated by different superscript letters (a, b, c, d)

The suppression of BMD, BMC, Ca, and P concentration is a strong indicator of decreased bone strength and increased susceptibility to bone fractures due to an imbalance in bone metabolism that causes an increase in bone resorption over bone formation. Egg yolk is a source of various essential nutrients and biologically active compounds, including growth-promoting factors such as yolk soluble protein that promotes bone growth, increases growth plates, and initiates bone morphogenetic proteins that regulate osteoblast differentiation and bone formation. Egg yolk also functions as a generator of antioxidants, containing free aromatic amino acids such as tryptophan and tyrosine [50]. Previous research has shown that the effect of ω-3 fatty acids in cod liver oil on osteoclastogenesis is related to the enhancement of osteoblastogenesis by reducing parathyroid hormone activity and increasing osteoblastic bone formation markers [51].

Conclusion

In conclusion, the study examined the effects of dietary intake of egg yolk and cod liver oil on dexamethasone-induced osteoporosis in rats. The findings showed that intake of egg yolk or cod liver oil maintained the levels of serum calcium, phosphorus, and osteocalcin compared to the control group. Additionally, the study found that the intake of egg yolk and cod liver oil helped maintain normal levels of bone loss and lowered the incidence of side effects of dexamethasone-induced osteoporosis. Therefore, the study concluded that fortifying food products with egg yolk and cod liver oil could be a useful approach for patients undergoing long-term treatment with dexamethasone. These findings may have significant implications for the development of

dietary interventions for the prevention and treatment of osteoporosis.

Conflicts of interest

There is no conflict to declare.

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