



Effect of Egg yolk on Lipid profile, blood pressure and correlation with Atherosclerosis in Rats

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ABSTRACT

Egg yolk (EY) is a major source of triglycerides, phospholipids, cholesterol, fatty acids and vitamins among food products. The aim of this study was to investigate the effect of egg yolk on lipid profile, blood pressure and atherosclerosis in rats. The experiment was carried out using forty male albino rats (Sprague-Dawley strain). The rats were divided into two main groups at random: the first main normal rats (20) were divided into: Subgroup (1) as a negative control group, rats were fed the basal diet (-Ve). Rats in subgroups 2–4 were fed a basal diet supplemented with (3 %, 5 % and 10 % of egg yolk from the basal diet respectively). The second atherosclerotic rats; groups (n=20) were induced with atherosclerosis using an atherogenic diet and fed orally with high levels of vitamin D for five days. Following this period, groups (n= 20 rats) were divided into four groups, each with five rats. Group (5) as a positive control group (+Ve), Rats were fed an atherogenic diet. Atherosclerotic rats (6, 7, and 8) were fed an atherogenic diet supplemented with (3 %, 5 % and 10 % of egg yolk from the atherogenic diet respectively). The experimental period was four weeks, Blood samples were collected. At the end of the experiment, the results showed that using (5% and 10%) levels of EY in atherosclerosis and normal rats increased the mean values of serum sodium, total cholesterol, total lipids and triglycerides and decreased the mean value of potassium, but the level of 3% from EY doesn't increase that. This study recommends limiting egg yolk consumption to improve the lipid profile, blood pressure and atherosclerosis.

Keywords: Egg yolk, Atherosclerosis, Rats, Basal diet.

1. Introduction

The relationship between cholesterol or egg consumption and the onset of cardiovascular diseases (CVD) is unclear, with observational studies and trials finding inconsistent results. Eggs are a main source of dietary cholesterol, and their consumption has been associated with an increased risk of cardiovascular disease, all-cause mortality, hemorrhagic stroke, and diabetes; paradoxically, in meta-analysis eggs are associated with a decreased risk of hypertension, which is a major risk factor for cardiovascular disease (Kjeldsen, 2018).

Further, results are inconsistent between regions, and results are conflicting when studying specific CVD such as stroke or ischemic heart disease. Aside from cholesterol, eggs are a source of essential nutrients, vitamins, and high-quality proteins, which may be a reason for the inconsistencies, as overall diet quality may be important in the relationship between eggs and CVD. It has been demonstrated that higher dietary cholesterol can raise serum cholesterol, which is in turn associated with blood pressure. High levels of dietary cholesterol have been associated with increased systolic blood pressure in the INTERMAP study (Sakurai *et al.*, 2011) conducted in China, Japan, UK and the USA, but not in the Finnish Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) (Abdollahi *et al.*, 2019).

Recently, Mazidi *et al.* (2019) observed increased systolic and diastolic blood pressure in higher consumers of eggs but found no relationship with cardiovascular mortality. The complexity of the association between egg/cholesterol intake and CVD is reflected in confusing guidelines for egg and

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cholesterol consumption. As of 2015, guidelines from the American Heart Association do not include recommendations for cholesterol intake, but note that individuals should consume as little cholesterol as possible. French guidelines recommend limiting red meat and eggs to 500 g per week, but do not give guidelines for eggs or cholesterol specifically (Van Dooren and Kramer, 2012).

The purpose of this study was to investigate how the egg yolk affected lipid profiles, blood pressure, and atherosclerosis in rats.

2. Materials and Methods

2.1. Diet content

- Casein, all vitamins, minerals, cellulose and choline bitartrate were obtained from Elgomhoria Pharmaceutical Company, Cairo, Egypt.
- Egg yolk were purchased from Egyptian local market.

2.2. Induction of atherosclerosis

The first time succeeded in inducing atherosclerosis in rabbits by feeding cholesterol containing diet Development of atherosclerosis in rabbits usually takes at least 60 days of feeding an atherogenic diet. Rat is said to be resistant to such dietary manipulations for the development of atherosclerosis but will supplementation of very high doses of vitamin-D, along with an atherogenic diet success has been achieved in developing atherosclerosis in rats in a short period of 5 day by fed on atherogenic diet and orally fed on vitamin D3 (3,20,000 IU) in 1.5 ml of olive oil for five days (Chandler *et al.*, 1979). The atherogenic diet (AD) consisting 2 g of cholesterol and 8 of saturated fat and 100 mg calcium were added to 90 g of powdered standard basal diet and thoroughly mixed (Altman, 1973).

2.3. Experimental Design

Forty male rats were housed in well aerated cages under hygienic conditions and fed on basal diet for one week for adaptation in animal house in Agriculture Research Center in Cairo, Egypt. The basal diet were formulated according to Reeves *et al.*, (1993). The basal diet consisted of protein (14%), fat (5%), salt mixture (3.5%), vitamin mixture (1%), Choline Chloride (0.2%), cellulose (5%), Sucrose (10%) and the remainder were Corn starch. These constituents were thoroughly mixed together. Rats were divided into two main groups, the first normal rats and second atherosclerotic rats. Each group (5 rats).

2.3.1. The first normal rats divided into

- Group (1): Rats were fed on the basal diet during the experimental period as a negative control group (-Ve).
- Group (2): Rats were fed on basal diet supplemented with 3% of egg yolk from basal diet.
- Group (3): Rats were fed on basal diet supplemented with 5% of egg yolk from basal diet.
- Group (4): Rats were fed on basal diet supplemented with 10 % of egg yolk from basal diet.

2.3.2. The second atherosclerotic rats

Rats (n=20) were inducted with atherosclerosis by using the method describe by Chandler *et al.* (1979) and Altman, (1973) by feeding rats on atherogenic diet and high doses of vitamin D according to the methods described mention before. After this period blood samples were taken from all rats for measuring total cholesterol level to be sure that all rats have been suffering from Atherosclerosis. Rats (n= 20 rats) were divided into 4 groups, 5 rats each as follows:

- Group (5): Atherosclerotic Rats were fed on atherogenic diet as positive control group (+Ve).
- Group (6): Atherosclerotic Rats were fed on atherogenic diet supplemented with (3 % of egg yolk from atherogenic diet).
- Group (7): Atherosclerotic Rats were fed on atherogenic diet supplemented with (5 % of egg yolk from atherogenic diet).
- Group (8): Atherosclerotic Rats were fed on atherogenic diet supplemented with (10 % of egg yolk from atherogenic diet).

During the experimental period, water and diet will be introduced ad-Lib. under hygienic conditions. At the end of the feeding trial (Four weeks) rats were fasted over night before scarifying and blood was collected, then centrifuged to obtain serum for biochemical analysis.

2.4. Biological parameters

Feed intake (FI) were calculated daily. Body weight gain (BWG) and feed efficiency ratio (FER) were calculated according to Chapman *et al.* (1959).

2.5. Serum analyses

Serum cholesterol was determined according to Richmond (1973), triglyceride (Wahlefeld, 1974), serum total lipids (Albers *et al.*, 1983 and Fridewald *et al.*, 1972), serum sodium (Maruna, 1958) and serum potassium (Terri and Sesin, 1958).

2.6. Statistical analysis

The results were analyzed using Statistical Package for the Social Sciences (SPSS) for Windows, version 20 (SPSS Inc., Chicago, IL, USA). Collected data were presented as mean $\pm$ standard deviation (SD). Analysis of Variance (ANOVA) test were used for determining the significances among different groups according to Armitage and Berry, (1987). All differences were considered at level of significant P-values < 0.05.

3. Results and Discussion

Data presented in Table (1) showed the effect of EY with three levels on feed intake (g/day), body weight gain% and feed efficiency ratio in rats. The results in this study revealed that, atherosclerotic group (positive control group) showed increased in feed intake (g/day), body weight gain% and feed efficiency ratio, as compared to rats fed on basal diet (the negative control group). Data in this table revealed that, treating rats suffering from atherosclerosis with two levels (5% and 10%) from EY led to increase, as compared to the positive control group. Normal rats with tree levels (5% and 10%) from EY led to increase, as compared to the positive and negative control group. The group 3% EY is the best group in all treatment groups, because this group showed non-significant difference, as compared to the negative control group.

Table 1: Effect of egg yolk on body weight, BWG %, FI and FER in rats

Parameters	-ve control	+ve control	3% EY + Atherosclerosis	5% EY + Atherosclerosis	10% EY + Atherosclerosis	3% EY	5% EY	10% EY
BWG	16.66 $\pm$ 5.13 ^d	32.96 $\pm$ 5.6 ^b	33.69 $\pm$ 7.44 ^b	41.89 $\pm$ 1.66 ^a	44.19 $\pm$ 3 ^a	19.12 $\pm$ 1.2 ^d	24.32 $\pm$ 0.88 ^c	26.88 $\pm$ 5.4 ^c
FER	0.142 $\pm$ 0.41 ^d	0.188 $\pm$ 0.88 ^b	0.189 $\pm$ 1.078 ^b	0.213 $\pm$ 0.22 ^a	0.22 $\pm$ 0.36 ^a	0.143 $\pm$ 0.084 ^d	0.153 $\pm$ 0.056 ^c	0.158 $\pm$ 0.3 ^c
FI	9.5	12.4	12.8	13.89	14.20	9.74	10.83	11.36

Mean values are expressed as means $\pm$ SE.

Means with different superscript letters in the row are significantly different at P $\leq$ 0.05.

From this result, EY has a greater enhancement of weight gain and feed in rats. This increase FER of the diet which presented for each group. Bogoriani *et al.* (2019) showed that there was increased in body weight of rats in both treatments with a significant difference (P <0.05) compared to controls in weeks 3 and 4. According to Miranda *et al.* (2015) that egg-containing food full of nutrition. Eggs are easily digested and absorbed by the intestine so that the rapid body weight increased over the controls.

Egg yolk is a major source of cholesterol with an average egg containing 200 mg - 750 mg (Sunwoo and Gujral, 2015) and (Spence, 2010). Beside that Eggs are an excellent natural source of folate, riboflavin, selenium, choline, vitamin B12, and vitamins of A, D, E, and K of fat-soluble (Miranda *et al.*, 2015; Bogoriani *et al.*, 2019; Herron and Fernandez, 2004; Song and Kerver, 2000 and Valentine *et al.*, 2010).

As demonstrated in Table (2), the mean value $\pm$ SE of serum LDL, VLDL, cholesterol and triglycerides of the positive control group significantly increased p $\leq$ 0.05, as compared to the negative

control group. While there was a significant decreased in the serum of HDL when compared to the negative control group.

In the normal groups which feeding with three levels from EY showed that group 3% EY is the best group in all treatment groups, because this group showed non-significant difference, as compared to the negative control group. Non-significant difference in serum cholesterol was observed between the group treated with 3% EY in atherosclerosis rats and positive group. In the present study, groups treating with 5% and 10%, EY led to a significant increase in lipid profile (serum LDL, VLDL, cholesterol and triglycerides) as compared to basal diet group. A similar result was reported by Rouhani *et al.* (2018) published that the egg consumption increases total cholesterol, LDL-C and HDL-C.

Table 2: Effect of EY on Total cholesterol, Lipoproteins and Triglycerides of rats

Parameters	-ve control	+ve control	3% EY + Athero- sclerosis	5% EY + Athero- sclerosis	10% EY + Athero- sclerosis	3% EY	5% EY	10% EY
Cholesterol	60.64 ±2.59 ^c	210.42 ±3.25 ^b	213.9 ±3.9 ^b	235.66 ±1.71 ^a	238.96 ±1.82 ^a	63.39 ±2.36 ^c	99.13 ±1.87 ^d	114.53 ±2.35 ^e
Triglyceride	52.84 ±2.07 ^d	90.08 ±1.97 ^b	92.1 ±2.08 ^b	95.78 ±3.12 ^{ab}	100 ±3.29 ^a	55.81 ±4.8 ^d	75.2 ±1.85 ^c	80.36 ±0.55 ^c
HDL	46.46 ±1.34 ^a	25.66 ±1.68 ^{cd}	22.97 ±1.93 ^{de}	19.84 ±1.35 ^{ef}	18.03 ±1.79 ^f	43.52 ±1.72 ^a	33.11 ±1.4 ^b	29.4 ±0.6 ^{bc}
LDL	15.75 ±2.25 ^d	101.2 ±2.82 ^b	103.8 ±3.2 ^b	107.84 ±2.84 ^{ab}	113.13 ±2.68 ^a	17.72 ±2.18 ^d	59.55 ±2.34 ^c	67.79 ±4.13 ^c
VLDL	11.88 ±0.83 ^d	33.97 ±1.8 ^b	33.96 ±0.82 ^b	38.44 ±1.35 ^a	40.87 ±0.92 ^a	14.15 ±0.79 ^d	25.43 ±1.56 ^c	27.76 ±1.07 ^c

Mean values are expressed as means ± SE.

Means with different superscript letters in the row are significantly different at $P \leq 0.05$.

There is a risk of forming atherogenic risk factors, which factors are driving the increase in the formation of atherosclerosis. The increase in total cholesterol, LDL-C, triglycerides, and the ratio of total cholesterol / HDL-C and a decrease in HDL-C in treatment with egg yolk duck pellets and raw. Efforts to prevent hypercholesterolemia, should be limited to consume the yolk duck containing high cholesterol is below the quail egg yolk and above chicken (Mehdipour and Hamishehkar, 2018).

In another study, Li *et al.* (2020) concluded that medium and high egg intake per day may influence cardiovascular disease risks by increasing LDL-c and the LDL-c/HDL-c ratio, but low egg consumption may lead to lower LDL-c/HDL-c ratio and LDL-c in agreement with results (Wang *et al.*, 2019).

To avoid elevations in blood cholesterol and reduce CHD risk, the public has been advised to consume no more than 300 mg/d of cholesterol and limit consumption of eggs, which contain about 213 mg of cholesterol per egg (National Cholesterol Education Program, 1991 and Krauss *et al.*, 1996). However, eggs contain many other nutrients besides cholesterol, including unsaturated fats, essential amino acids, folate, and other B vitamins. In addition, consumption of eggs instead of carbohydrate-rich foods may raise high-density lipoprotein (HDL) cholesterol levels (McGee *et al.*, 1984 and Packard *et al.*, 1983) and decrease blood glycemic and insulinemic responses (Pelletier *et al.*, 1996).

As demonstrated in Table (3) it could be observed that the mean value of serum sodium (mg/dl) in the positive control group showed significant increase $P < 0.05$, as compared to the negative control group. While the mean value of serum Potassium (mg/dl) in the positive control group showed significant decrease $P < 0.05$, as compared to the negative control group. Non-significant difference in serum sodium was observed between the group treated with 3% EY in atherosclerosis rats and positive group. In the normal groups which feeding with three levels from EY showed non-significant difference, as compared to the negative control group.

In the present study, 5% and 10% from EY has increased in sodium and decreased in potassium serum as compared to basal diet group in this respected to Sakat *et al.* (2009), who reported that hypertension is one of the leading causes of disability, mortality and morbidity throughout population (Sakat *et al.*, 2009). It is the most common chronic illness among the world faces. Hypertension is the most common cardiovascular diseases and constituents a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, myocardium infarct, heart failure, renal

insufficiency, stroke and dissecting aneurysm of aorta. An elevated arterial pressure is an important public health issue in developed countries (Sharifi *et al.*, 2003).

Table 3: Effect of EY on serum sodium and potassium in rats

Parameters	-ve control	+ve control	3%EY + Athero- sclerosis	5% EY + Athero- sclerosis	10 % EY+ Athero- sclerosis	3% EY	5% EY	10% EY
Sodium	137.83 ±1.1 ^c	149.51 ±0.58 ^b	150.1 ±1.49 ^b	160.17 ±5.1 ^a	162.87 ±5.09 ^a	139.8 ±1.42 ^c	142.24 ±0.98 ^{bc}	143.5 ±0.5 ^{cb}
Potassium	4.56 ±0.23 ^a	2.3 ±0.23 ^c	2.27 ±0.23 ^c	2.97 ±0.23 ^{cd}	3.0 ±0.25 ^{cd}	4.3 ±0.17 ^a	3.83 ±0.2 ^b	3.5 ±0.26 ^{bc}

Mean values are expressed as means ± SE.

Means with different superscript letters in the row are significantly different at $P \leq 0.05$

Recently, Airaodion *et al.* (2020) reported that no significant difference was observed in the blood pressure of animals in both the control and experimental groups prior to treatment. However, the blood pressure of animals treated with egg yolk significantly increased after 7, 14 and 21 days of treatment respectively with 21 days treatment having the highest blood pressure. Egg yolk has been reported to contain high cholesterol concentration (Spence *et al.*, 2016). Cholesterol rich diets have been linked to dyslipidemia which is considered a major risk factor for hypertension (Ayele *et al.*, 2010 and Reaven and Ho, 1991). This might be responsible for the sustained increase in blood pressure of animals treated with egg yolk. This is in agreement with the report of Spence *et al.* (2016).

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