SciMedCentral

Journal of Pharmacology & Clinical Toxicology

Research Article

Egg Yolk Feeding Induces Hyperlipidemia with a Concomitant Increase in Oxidative Stress in Liver Tissue and Erythrocyte Susceptibility to Hemolysis in Rats

Shafiul Alam, Himel Dutta, Hriday Debnath, Probad Azmain, Shoriful Islam Joy, Taslima Nahar, Borhan Uddin, Mohammad Azizur Rahman, and Shahdat Hossain*

Department of Biochemistry and Molecular Biology, Jahangirnagar University, Bangladesh

***Corresponding author**
Shahdat Hossain, Laboratory of Alternative Medicine and Behavioral Neurosciences, Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh

Submitted: 11 September 2023

Accepted: 20 October 2023

Published: 22 October 2023

ISSN: 2333-7079

Keywords
- Egg yolk
- Hyperlipidemia
- Oxidative stress
- lipid peroxide
- Hemolysis
- Cardiovascular disease
- Rat

Abstract

Objectives: The common belief is that egg yolk consumption is associated with hyperlipidemia, but some recent studies have reported that egg consumption may help reduce hypercholesterolemia and obesity, and is not associated with incidences of cardiovascular disease. In this study, we investigated whether feeding chicken egg yolk affects the lipid profile of rats. In addition, we measured, if, yolk supplementation affected the degree of red blood cell hemolysis and oxidative stress, which are several parameters associated with the short survival of circulating red blood cells.

It is widely believed that egg yolk consumption is associated with hyperlipidemia. However, some recent studies have reported that egg consumption may help reduce hypercholesterolemia and obesity and is not associated with the occurrence of cardiovascular disease. In this study, we investigated whether feeding chicken egg yolk affects the lipid profile of rats. In addition, we measured whether yolk complementation affects the degree of red blood cell hemolysis and oxidative stress, which are several parameters associated with the short survival of circulating red blood cells.

Methods: Rats were placed into three groups according to a random selection: Control group (CO), was fed with regular diet; EY1 group, was fed a diet with 20% egg yolk, and EY2 group, was fed a diet with 40% egg yolk for 10 consecutive weeks. Weight was measured every other day. Reactive oxygen species (ROS) were ascertained using fluorescent dyes 2'and 7'dichlorodihydrofluorescein diacetate (DCFDA). The concentration of lipid peroxide (LPO) was measured by determining the concentration of thiobarbituric acid-reactive substances (TBARS). Comparing RBC hemoglobin liberation estimates to the hemoglobin standard determined hemolysis. All biochemical parameters were determined using standard laboratory procedures.

The rats were randomly divided into three groups: the control group (CO) was fed normal chow; Group EY1 received a 20% egg yolk diet for 10 consecutive weeks and group EY2 received a 40% egg yolk diet. Weight was measured every other day. Reactive oxygen species (ROS) were detected using the fluorescent dyes 2'- and 7'-dichlorodihydrofluorescein diacetate (DCFDA). The concentration of lipid peroxide (LPO) was measured by determining the concentration of thiobarbituric acid reactive substances (TBARS). Hemolysis was determined by comparing estimates of erythrocyte hemoglobin release to the hemoglobin standard. All biochemical parameters were determined using standard laboratory procedures.

Results: Body weight, plasma total cholesterol (TC), and LDL-C levels were significantly increased in rats fed egg yolk compared to control rats, but HDL-C levels were unchanged in yolk-fed rats. In EY1 and EY2 rats, liver TC increased by 24.8% and 88.8%, respectively, and TG increased by 54.3% and 84.3%, respectively. LDL-C/HDL-C in both the plasma and liver of EY1 and EY2 rats increased significantly. Oxidative stress (OS) levels and RBC hemolytic tendencies were higher in yolk-fed rats.

Body weight, plasma total cholesterol (TC), and LDL-C levels were significantly increased in egg yolk-fed rats compared to control rats, but HDL-C levels remained unchanged in egg yolk-fed rats. In EY1 and EY2 rats, liver TC increased by 24.8% and 88.8% and TG by 54.3% and 84.3%, respectively. LDL-C/HDL-C increased significantly in both plasma and liver of EY1 and EY2 rats. In rats fed egg yolk, levels of oxidative stress (OS) and hemolytic tendencies of erythrocytes were higher.

Conclusion: Egg yolk feeding not only increased body weight, which is conducive to obesity, but it worsened hyperlipidemia, oxidative status, and hemolysis in rats. Augmented hemolysis may occur by enhanced incorporation of plasma cholesterol into erythrocyte membranes, leading to decreased membrane fluidity and disturbances of oxygen transport across the membrane.

Egg yolk feeding not only increased body weight, which favors obesity, but also worsened hyperlipidemia, oxidative status and hemolysis in rats. Increased hemolysis can occur due to increased incorporation of plasma cholesterol into the erythrocyte membranes, leading to reduced membrane fluidity and disruption of oxygen transport across the membrane.

Cite this article: Alam S, Dutta H, Debnath H, Azmain P, Joy SI, et al. (2023) Egg Yolk Feeding Induces Hyperlipidemia with a Concomitant Increase in Oxidative Stress in Liver Tissue and Erythrocyte Susceptibility to Hemolysis in Rats. J Pharmacol Clin Toxicol 11(2):1177.

INTRODUCTION

Demand for eggs is increasing day by day because of their high availability and low price. Egg yolks had a bad reputation in the past due to the high levels of cholesterol that they contained. Medical professionals and health officials strongly advise their patients and the general public to reduce egg yolk consumption. This is especially important for patients who have been diagnosed with excessive cholesterol, high blood pressure, or cardiac issues [1]. Egg yolk ingestion affects postprandial oxidative stress, endothelial dysfunction, and vascular inflammation for at least four hours after eating a high-fat, cholesterol-rich meal [2]. Dietary cholesterol and egg yolks both contribute to an increase in fasting levels of LDL cholesterol of around 10%. The consumption of egg yolk was found to promote carotid atherosclerosis in a manner that was comparable to the impact of smoking in a trial that involved 1206 individuals who were participating in vascular preventive clinics [3]. Ireland-Boston Diet-Heart and Western Electric studies indicated that dietary cholesterol increases cardiovascular disease (CVD) risk. The intake of dietary cholesterol and eggs was found to dramatically increase the risk of coronary disease in British research on health-conscious persons, and in animal models, the consumption of dietary cholesterol was proven to be responsible for atherosclerosis [4, 5]. Egg yolk contains phosphatidylcholine, which contributes to the synthesis of trimethylamine n-oxide (TMAO), an agent that has been shown in animal studies to promote atherosclerosis. In individuals who were sent for coronary angiography, eating two egg yolks each day for three years was connected with a 2.5-fold increase in the possibility of having a stroke, passing away, or having a myocardial infarction [2]. The mean level of serum total cholesterol rose by 13% with the consumption of egg yolk among this mass; this rise was due to LDL cholesterol increasing by 21% and serum total triglycerides increasing by 17% [6].

The demand for eggs is increasing day by day due to high availability and low price. Egg yolks have had a bad rap in the past due to their high cholesterol content. Medical professionals and public health officials are urging their patients and the public to reduce egg yolk consumption. This is especially important for patients who have been diagnosed with high cholesterol, high blood pressure or heart problems [1]. Egg yolk ingestion affects postprandial oxidative stress, endothelial dysfunction, and vascular inflammation for at least four hours after eating a high-fat, high-cholesterol meal [2]. Dietary cholesterol and egg yolk both contribute to an approximately 10% increase in fasting LDL cholesterol levels. Egg yolk consumption has been found to promote carotid artery atherosclerosis in a manner comparable to the effects of smoking, as noted in a study of 1206 people attending vascular prevention clinics [3]. Studies by Ireland-Boston Diet-Heart and Western Electric showed that dietary cholesterol increases the risk of cardiovascular disease (CVD). British studies of health-conscious individuals have found that dietary cholesterol and egg intake dramatically increases the risk of cardiovascular disease, and animal models have shown that dietary cholesterol consumption is responsible for atherosclerosis [4,5]. Egg yolk contains phosphatidylcholine, which contributes to the synthesis of trimethylamine-n-oxide (TMAO), a compound that has been shown in animal studies to promote atherosclerosis. In people sent for coronary angiography, eating two egg yolks per day for three years was associated with a 2.5-fold increased risk of having a stroke, death, or myocardial infarction [2]. The average total serum cholesterol level increased by 13% with the consumption of egg yolk in this mass; This increase was due to a 21% increase in LDL-cholesterol and a 17% increase in serum total triglycerides [6].

There are many different and controversial findings on egg yolk, and the majority of studies work with serum cholesterol, HDL, LDL, VLDL, and triglycerides (TG). Recently, some studies have demonstrated that eating egg yolk can have therapeutic effects on hyperlipidemia. These benefits include improving the activities of lipoproteins, lowering TG and TC levels, and modulating the pathways involved in lipid metabolism [7-9]. Other research indicated that eating three eggs per day was associated with a substantial improvement in plasma levels of HDL-C, apolipoprotein A-I (8%), and apolipoprotein E. Another clinical investigation that was based on half a million individuals in China found that a moderate level of egg intake (up to 1 egg per day) was strongly correlated with a decreased the CVD. Despite the fact that these positive benefits were identified, the molecular mechanism that is based on lipid metabolites is yet unclear [10].

There are many different and controversial findings about egg yolk, and most studies work with serum cholesterol, HDL, LDL, VLDL, and triglycerides (TG). Recently, some studies have shown that egg yolk consumption can have therapeutic effects in hyperlipidemia. These benefits include enhancing the activities of lipoproteins, lowering TG and TC levels, and modulating pathways involved in lipid metabolism [7–9]. Other research suggested that eating three eggs per day was associated with a significant improvement in plasma levels of HDL-C, apolipoprotein A-I (8%), and apolipoprotein E. Another clinical study, based on half a million people in China, found that moderate egg intake (up to 1 egg per day) was highly correlated with a reduction in cardiovascular disease. Despite the fact that these positive benefits have been identified, the molecular mechanism based on lipid metabolites is still unclear [10].

The potential risk of high cholesterol intake from egg yolks is not considered significant. Currently, there are serious questions about the relationship between dietary egg yolks and cardiovascular disease. As for cholesterol, much debate has focused on the lack of a clear consensus on whether eating eggs permanently raises serum cholesterol and whether it adversely affects the vascular responsiveness associated with CVD. There are also limited studies that have observed the relationship between egg yolk, lipid peroxidation (LPO) of the liver, and hemolysis. Hemolysis is the process in which the membrane of red blood cells (RBCs) is ruptured, which results in the hemoglobin and other components of the RBC's interior being released into the surrounding fluid [11]. Oxidative stress is responsible for hemolysis and tissue injury. Hemolysis also can manifest as anemia, jaundice, cholelithiasis, or isolated reticulocytotic [12,13].

Therefore, we planned to investigate the effects of feeding different amounts of egg yolks and observed plasma and liver cholesterol, total lipid, HDL-C and LDL-C levels, blood hemolysis, lipid peroxidation, and reactive oxygen species in the liver.

The potential risk of high cholesterol intake from egg yolk is not considered significant. There are currently serious questions about the link between dietary egg yolks and cardiovascular disease. Regarding cholesterol, much debate has centered on the lack of a clear consensus on whether egg consumption permanently increases serum cholesterol levels and whether it impairs vascular responsiveness associated with cardiovascular disease. There are also limited studies that have observed the association between egg yolk, liver lipid peroxidation (LPO) and hemolysis. Hemolysis is the process by which the membrane of red blood cells (RBCs) ruptures, resulting in hemoglobin and other components of the interior of red blood cells being released into the surrounding fluid [11]. Oxidative stress is responsible for hemolysis and tissue damage. Hemolysis can also manifest itself as anemia, jaundice, cholelithiasis, or isolated reticulocytosis [12, 13]. Therefore, we wanted to investigate the effects of feeding different amounts of egg yolk and observed plasma and liver cholesterol, total lipid, HDL-C and LDL-C levels, blood hemolysis, lipid peroxidation and reactive oxygen species in the liver.

MATERIALS AND METHODS

Collection and Preparation of Egg Yolk

Eggs were collected from a firm, in Savar, Dhaka, Bangladesh. The eggs were cleaned properly with water to remove any dirt. Then boiled properly and again the boiled eggs were washed with distilled water and the yolk is separated from the white part carefully. After that, we dried the yolk in the sun and demolished them, and kept them in an incubator at 50◦C for 2-3 days to remove the water substance properly (Figure 1). When the egg yolk had reached the appropriate level of dryness, we ground it, placed it in plastic bags that were hermetically sealed with Ziplocs, and chilled the bags to a temperature of -20°C.

The eggs were picked up by a company in Savar, Dhaka, Bangladesh. The eggs were thoroughly cleaned with water to remove any dirt. Then it is properly cooked and again the boiled eggs are washed with distilled water and the egg yolk is carefully separated from the egg white. After that, we dried the yolk in the sun, crushed it and kept it in an incubator at 50 °C for two to three days to properly remove the water substance (Figure 1). When the yolk reached the appropriate degree of dryness, we ground it, placed it in plastic bags hermetically sealed with Ziplocs, and cooled the bags to a temperature of -20°C.

Figure 1 Boiled egg and separated egg yolk.

Estimation of the Total Amount of Lipid

The total lipid content of egg yolk was determined using the Folch, et al. [14], technique, and the total cholesterol content was determined using a kit. For the purpose of this test, blended yolk powder was utilised, and the quantity of total lipid contained in the yolk was expressed as a percentage.

The total lipid content of egg yolk was determined using the method of Folch et al. certainly. [14], technique and total cholesterol were determined using a kit. Mixed egg yolk powder was used for this test and the amount of total lipid contained in the egg yolk was expressed as a percentage.

Animals

Inbred male 8-week-old Long Evans rats were used for the experiment. These were housed in an animal room that was air-conditioned for 12 hours, both light and dark, at a regulable temperature (24 ± 3ºC) and humidity (55 ± 15%). All animals were performed in compliance with the methods stated in the 'Guidelines for Animal Experimentation of Jahangirnagar University' and with the agreement of Jahangirnagar University's ethical approval committee.

Inbred male Long Evans rats 8 weeks old were used for the experiment. They were housed in an animal room that was both light and dark air-conditioned for 12 hours and had adjustable temperature (24°C) and humidity (55°C - 15%). All animals were performed in accordance with the methods specified in the "Policy on Animal Experimentation of Jahangirnagar University" and with the approval of the Ethics Approval Committee of Jahangirnagar University.

In vivo Experimental Design

The 18 male rats that were utilized in this experiment were arbitrarily split up into three groups, each of which comprised 6 rats: (1) Control group (CO); (2) Egg yolk group-1 (EY1); and (3) Egg yolk group-2 (EY2). The rats had unrestricted access to a diet (containing the food composition in (Table 1), as the only available food source. Every rat had no limitations on the supplied tap water. Intake of both food and liquids was monitored at regular intervals, and the quantities that were ingested were computed afterward. The experimental rats were housed for 10 weeks. The experimental design is shown below in Figure 2.

The 18 male rats used in this experiment were randomly divided into three groups each containing 6 rats: (1) control group (CO); (2) egg yolk group-1 (EY1); and (3) egg yolk group-2 (EY2). The rats had unrestricted access to a diet (which contained the dietary composition in (Table 1), as the only available food source. Each rat had no restrictions on the tap water supplied. The intake of food and liquids was monitored at regular intervals and the amounts consumed were then calculated. The experimental rats were kept for 10 weeks. The experimental design is shown in Figure 2 below.

Table 1: The composition of the 100-g diet for experimental rats.

Composition	CO	EY1	EY2
Casein	20.0	20.0	20.0
L-Cysteine	0.30	0.30	0.30
Starch	65	45	25
Cellulose	7	7	7
Oil	5	5	5
NaCl	2.36	2.36	2.36
αTocopherol	0.05	0.05	0.05
Cholic acid	0.25	0.25	0.25
*Vitamin +Minerals	.04	.04	.04
Egg Yolk powder	0.0	20	40

CO=Control rats. EY1=(Egg yolk 20%-fed) Egg Yolk group-1 rats. EY2=(Egg yolk 40%-fed) Egg Yolk group-2 rats. *Vitamins + mineral contains: (g/100 gm) Vit A=52500 KIU; Vit=E (50%), 5.0; VitB1- VitB6=1.25; 2.5; 2.5; 2.5;1.25; Vit B12(0.1%)=0.25; Vit C=5.0; Folic acid=0.125; Biotin=0.01; VitD3=300 KIU; Vit K3 (10%)=2.0; Co=0.01; Fe=0.10; KI=0.01; Zn=1.0; Mg=1.0; l-Lys=2.0; dl-Met=1; Cu=0.01.

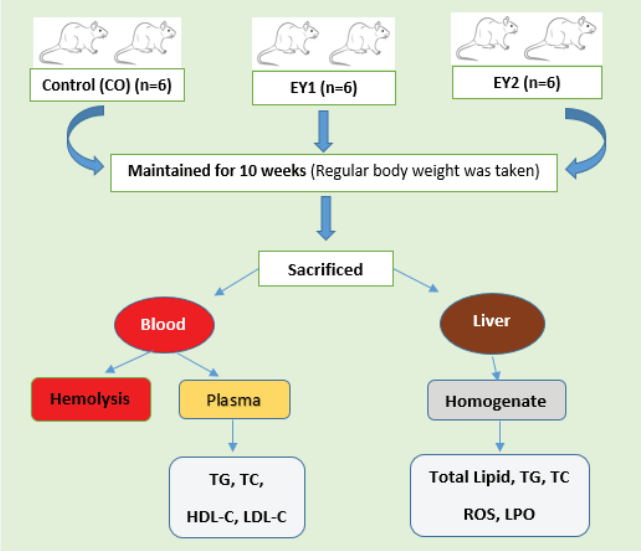

Figure 2 Experimental setup (In Vivo). Plasma and liver homogenates of each experimental group of rats were used for the analyses. LPO=lipid peroxide; TG=triglycerides; TC=total cholesterol; LDL-C = low density lipoprotein cholesterol; HDL-C = high density lipoprotein cholesterol; ROS=Reactive Oxygen Species.

Hemolysis Assay

After administering pentobarbital to put the rat into a deep slumber, blood was drawn using a heparin-treated syringe from the inferior vena cava. After that, erythrocytes were removed from the blood, as previously explained by Hashimoto et al. [15]. The fresh erythrocytes that came out of this were then put through hemolysis. The erythrocyte hemolysis level was measured of erythrocyte hemolysis was measured in the same way that Hossain, et al. [8], has described before. In short, 2% erythrocyte suspensions were mixed with freshly made Fenton's reagent [H_2O_2 (45mM) + $FeSO_4$ (2mM)] for 1 hour at 37°C (5mg/mL). After the appropriate time had elapsed in the incubation process, the erythrocytes were extracted by centrifuging the samples at 300g for 10 minutes. The quantity of hemoglobin (Hb) in the supernatant was then compared to a hemoglobin standard at 540 nm to determine the degree of hemolysis.

After administration of pentobarbital to put the rat into a deep sleep, blood was collected from the inferior vena cava with a heparinized syringe. Thereafter, erythrocytes were removed from the blood as previously described by Hashimoto et al. explained. [15]. The resulting fresh erythrocytes were then subjected to hemolysis. The degree of erythrocyte hemolysis was measured in the same way as Hossain et al. [8], has already described. Briefly, 2% erythrocyte suspensions were mixed with freshly prepared Fenton's reagent [H2O2 (45 mM) + FeSO4 (2 mM)] for 1 hour at 37°C (5 mg/ml). After the appropriate incubation time had elapsed, the erythrocytes were extracted by centrifuging the samples at 300 g for 10 minutes. The amount of hemoglobin (Hb) in the supernatant was then compared to a hemoglobin standard at 540 nm to determine the degree of hemolysis.

Plasma & Liver Lipid Profile Assay

Extraction of Total lipid from Liver Tissue Homogenate for Lipid Profile Test: The procedure that has been reported in the past by Folch et al. [14], was utilized in order to isolate total lipids from liver tissue homogenate. After thoroughly combining the liver homogenate with 3 mL of a chloroform-methanol solution with a ratio of 2:1, the mixture was left to stand for three days while being kept mixing. After that, the solution was filtered, the methanol was removed, and the chloroform was removed by heating the mixture to 80°C for five minutes. After that, the weight of the pulped lipid was determined. After that, 2 mL of water was added, and a suspension was created with the help of a bath sonicator. After that, an analysis of the TC and TG levels in the liver was performed using this solution.

In the past, by Folch et al. [14], was used to isolate total lipids from liver tissue homogenate. After thorough combination of the liver homogenate with 3 ml of a 2:1 chloroform-methanol solution, the mixture was allowed to stand for three days with constant mixing. Thereafter, the solution was filtered, the methanol removed and the chloroform removed by heating the mixture at 80°C for 5 minutes. Thereafter, the weight of the ground lipid was determined. Then 2 ml of water were added and a suspension was produced with the aid of an ultrasonic bath. An analysis of the TC and TG levels in the liver was then carried out with this solution.

Estimation of Plasma and Liver TC, TG, HDL-C & LDL-C: Following the preparation of the liver for the lipid profile test, the TC, TG, and HDL-C levels in the plasma were determined using a kit that is available for purchase (Randox). A colorimeter set to 530 nm was used to test the absorbance, and the results showed that it was directly proportional to the concentration. The TC, TG, HDL-C, and LDL-C values in plasma and liver were represented as mg/dL. According to the Fried Ewald formula published in 1992, the levels of low-density lipoprotein cholesterol (LDL-C) were determined as follows: LDL-C = TC – [(TG/5) - HDL-C [16].

After preparing the liver for the lipid profile test, plasma TC, TG and HDL-C levels were determined using a commercially available kit (Randox). A colorimeter set at 530 nm was used to test absorbance and the results showed that it was directly

proportional to concentration. The TC, TG, HDL-C and LDL-C values in plasma and liver were expressed in mg/dl. According to the Fried-Ewald formula published in 1992, low-density lipoprotein cholesterol (LDL-C) levels were determined as follows: LDL-C = TC [(TG/5) - HDL-C [16].

Assay of Lipid Peroxidation (LPO) in Liver Tissue: Thiobarbituric acid reactive substance (TBARS) was estimated from liver tissue homogenates to measure LPO levels in each group, as described [17]. Rat liver homogenates (0.1 mL) were combined with sodium dodecyl sulfate (8.1% w/v), thiobarbituric acid (0.4% w/v) in acetic acid (20% v/v and pH 3.5), and 0.1 mL of deionized water. Every tube was sealed properly and heated for an hour at 95°C. The test tubes were cooled in tap water, then 2 mL of n-butanol-pyridine (15:1) (v/v) was added and the tubes were vigorously shaken for 10 min. The tubes were then centrifuged at room temperature at 1200 g for 10 minutes. TBARS levels were calculated as moles of malondialdehyde (MDA) per mg of protein, and the absorbance of the top organic layer was analysed at 532 nm. Comparisons against a standard 1,1,3,3-tetra ethoxy propane (TEP) formulation were used to determine MDA levels. The Lowry technique was used to determine the amount of protein present in the homogenates [18].

Thiobarbituric acid reactive substance (TBARS) was estimated from liver tissue homogenates to measure LPO levels in each group as described [17]. Rat liver homogenates (0.1 mL) were combined with sodium dodecyl sulfate (8.1% w/v), thiobarbituric acid (0.4% w/v) in acetic acid (20% v/v and pH 3.5) and 0.1 mL deionized water. Each tube was properly capped and heated at 95°C for one hour. The test tubes were cooled in tap water, then 2 ml of n-butanol-pyridine (15:1) (v/v) was added and the tubes shaken vigorously for 10 minutes. The tubes were then centrifuged at 1200 g for 10 minutes at room temperature. TBARS values were calculated as moles of malondialdehyde (MDA) per mg protein and the absorbance of the upper organic layer analyzed at 532 nm. To determine the MDA values, comparisons were made with a standard formulation of 1,1,3,3-tetraethoxypropane (TEP). The Lowry technique was used to determine the amount of protein present in the homogenates [18].

Assay of Lipid Reactive Oxygen Species (ROS) in Liver Tissue: A method for measuring ROS baseline levels was developed by Montoliu et al. [19]. Tissue homogenate was freshly prepared, diluted with 100 mM potassium phosphate buffer (pH 7.4), and incubated in methanol with 5 M dichlorofluorescein diacetate for 15 minutes at 37°C. The dye-containing samples were spun at 12,500 g for 10 minutes at 4°C. After removing the pellet from the 37°C incubator, 5 ml of 100 mM phosphate buffer (pH 7.4) was mixed, and the mixture was stirred at ice-cold temperatures for 60 minutes. The fluorescence was analysed using a spectrofluorometer at excitation (488 nm) and emission (525 nm) wavelengths. The cuvette's container was maintained at 37°C. The concentration of ROS was determined by treating a dichlorofluorescein standard curve in methanol (0-100 nM) [20] .

A method for measuring baseline ROS levels was proposed by Montoliu et al. developed. [19]. Tissue homogenate was freshly prepared, diluted with 100 mM potassium phosphate buffer (pH 7.4) and incubated in methanol with 5 M dichlorofluorescein diacetate for 15 minutes at 37°C. The dye-containing samples were spun at 12,500g for 10 minutes at 4°C. After removing the pellet from the 37°C incubator, 5 ml of 100 mM phosphate buffer (pH 7.4) was mixed and the mixture was stirred at ice-cold temperatures for 60 minutes. Fluorescence was analyzed with a spectrofluorometer at excitation (488 nm) and emission wavelengths (525 nm). The well of the cuvette was kept at 37°C. The ROS concentration was determined by treating a dichlorofluorescein standard curve in methanol (0–100 nM) [20].

Statistical Analyses

The results were given as mean SEM, which stands for "standard error of the mean." One-way ANOVA was used to look at changes between groups in vitro tests. After ANOVA, Fisher's protected least square differences (PLSD) test was used to compare groups after the fact. Simple regression analysis was used to figure out how strong the link was. The statistical programms used were IBM SPSS Modeller 16.0 and GraphPad PRISM® version 9). $P < 0.05$ was supposed statistically significant.

The results were expressed as the mean SEM, which represents the standard error of the mean. One-way ANOVA was used to examine changes between groups in vitro tests. After ANOVA, Fisher's Protected Least Square Differences (PLSD) test was used to compare groups retrospectively. A simple regression analysis was used to determine how strong the connection was. The statistical programs used were IBM SPSS Modeller 16.0 and GraphPad PRISM version 9). P<0.05 was considered statistically significant.

RESULT & DISCUSSION

Total lipid and Cholesterol in Yolk

We measured total lipid and cholesterol from one dried yolk and found 54.2% lipid and 4.7% cholesterol.

We measured the total lipid and cholesterol content of a dried egg yolk and found 54.2% lipid and 4.7% cholesterol.

Effects of Feeding Egg Yolk on Body Weight and Liver Weight

Overweight and obesity have quickly become a serious public health issue in emerging nations [7]. In this experiment, after consuming egg yolk the body weight and liver weight of all group rats were analysed. We measured the approximate daily dietary intake and regular body weight. CO, EY1, and EY2 daily intake of 18.7 ± 0.8 g, 17.8 ± 0.8 g, and 17.4 ± 0.4 g of diet respectively, but body weight has increased by 106 g, 209 g, and 220.3 g on average. After being sacrificed we also measured the liver weight and found that the liver weight increased 50.5% in EY1 and 68.5% in EY2 rats, which is significant ($P < 0.05$) compared with CO rats (Figure 3). This data indicates the dietary administration of egg yolk has increased body weight and also liver weight

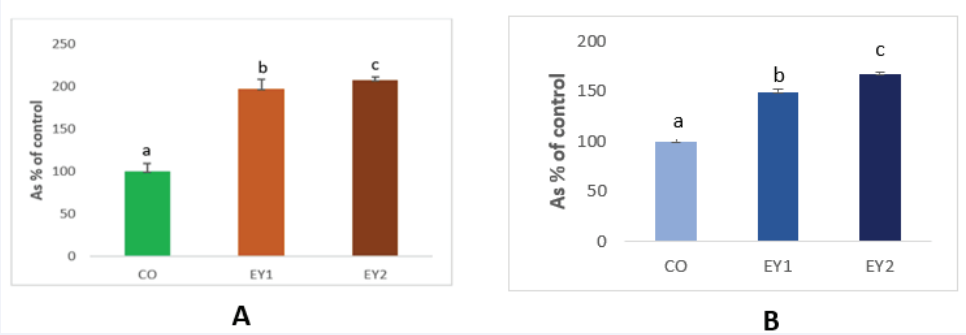

Figure 3 Effect of feeding egg yolk in body weight (A) and liver weight (B). The data were presented as a mean standard error of the mean.

significantly. Thus, egg yolk could be an agent that is responsible for gaining body weight, obesity, and fatty liver.

Overweight and obesity have quickly become a serious public health problem in emerging economies [7]. In this experiment, body weight and liver weight of all the rats in the group were analyzed after eating egg yolk. We measured approximate daily food intake and normal body weight. Daily dietary CO, EY1 and EY2 intakes were 18.70.8 g, 17.80.8 g and 17.40.4 g, respectively, but body weight increased by 106 g, 209 g and 220.3 g on average. After sacrifice, we also measured liver weights and found that liver weights increased by 50.5% in EY1 rats and 68.5% in EY2 rats, which is significant compared to CO rats ($P<0.05$) (Figure 3). These data suggest that dietary administration of egg yolk significantly increased body weight and also liver weight. Thus, egg yolk could be a trigger for weight gain, obesity, and fatty liver.

For the examination of the data, One-way analysis of variance (ANOVA) was used, and Fisher's PLSD was used for post hoc analysis. CO=Control; EY1=Egg yolk group1, EY2= Egg yolk group2. After feeding yolk in both groups body weight and liver weight significantly increased. Here, $^{a, b, c}$ are used to express the significant difference where ($P < 0.05$).

Effect of feeding egg yolk on body weight (A) and liver weight (B). Data were presented as the mean standard error of the mean. One-way analysis of variance (ANOVA) was used to examine the data, and Fisher's PLSD was used for post hoc analysis. CO=control; EY1=yolk group1, EY2=yolk group2. After feeding egg yolk, body weight and liver weight increased significantly in both groups. Here a, b, c are used to express the significant difference, where ($P<0.05$).

Effect of Feeding Egg Yolk on Plasma Lipid Profile

The high cholesterol content in eggs, notably egg yolks (EY), was linked to many disorders associated with hyperlipidemia [21]. Hypercholesterolemia, which usually occurs due to high dietary intake of cholesterol, may cause many life-threatening complications; particularly it raises the possibility of heart disease, heart attack, and stroke. Although there are many anti-hypercholesterolemic drugs, most of them are expensive, exhibit side effects, and cause recurrent hypercholesterolemia when the drugs are withdrawn [22]. Administration of 20% EY in EY1 rats and 40% EY in EY2 rats, Plasma total cholesterol increased by 16.5% and 30% respectively compared with the CO rats (Table 2). The levels of plasma TG were increased, after yolk feeding, we found an 8.2% increment in EY1 and 11.9% in EY2 rats (Table 2).

The levels of plasma HDL-C have also increased in a little amount which is not significant but LDL-C has increased significantly. We measured HDL-C in CO=27.5 mg/dl, EY1=28.9 mg/dl and in EY2=30.1 mg/dl and LDL-C in CO=56.9 mg/dl, EY1=78.4 mg/dl and in EY2=81 mg/dl (Table 2). LDL-C is known as bad cholesterol. The higher plasma HDL-C level indicates that excess cholesterol was transported back to the liver from peripheral tissues for breakdown. Expulsion of free cholesterol or its conversion into bile acids via the bile duct [23]. This process occurs when the liver hydrolyzes LDL-C ester and HDL-C ester into free cholesterol. High LDL-C and low HDL-C levels suggest an imbalance in the transfer of cholesterol from the liver to extra-hepatic tissues and back to the liver. According to Hongu et al. [24], elevated TC, LDL-C, and TG levels are risk factors for CVD, hyperlipidemia, and dyslipidemias. Very low-density lipoproteins (VLDL) are the carriers of dietary lipids in normal lipid metabolism. Lipoprotein lipase breaks down VLDLs into free fatty acids (FFA), which are then used as fuel by peripheral tissues [25]. However, if lipid intake exceeds energy expenditure, the excess will be stored as fat in the body's adipose cells. Finally, if these unneeded fats are retained, plasma fatty acids will surpass the threshold, resulting in hyperlipidemia, dyslipidemia, and other CVD [26]. In our study, we found 68.7% and 306.5% increments of LDL-C/HDL-C ratio after feeding 20% and 40% EY respectively (Table 2). Therefore, it can be concluded that the high level of egg yolk consumption will increase plasma LDL-

Table 2: Effects of dietary administration of egg yolk on plasma lipid profile.

Parameter (Plasma)	CO	EY1	EY2
TC (mg/dL)	91.9±3.1^{a}	107.1±4.1^{b}	119.5±5.2^{b}
TG (mg/dL)	37.8±2.5	40.9±2.6	42.3±2.9
HDL-C (mg/dL)	27.5±1.3	28.9±1.2	30.1± 3.0
LDL-C (mg/dL)	56.9 ± 3.8^{a}	78.4± 4.9^{b}	81.0 ± 2.3^{c}
LDL-C/HDL-C	29.4±3.4^{a}	49.6±3.8	119.5±5.2^{b}

cholesterol significantly but not HDL-cholesterol at a significant amount which resultant an elevation of LDL-C/HDL-C ratio may have a devastating effect on health.

High cholesterol in eggs, particularly the yolk (EY), has been implicated in many hyperlipidemia-related disorders [21]. Hypercholesterolemia, which usually occurs due to high dietary cholesterol intake, can cause many life-threatening complications; In particular, it increases the possibility of heart disease, heart attack and stroke. Although there are many antihypercholesterolemic drugs, most of them are expensive, have side effects, and cause recurrent hypercholesterolemic episodes when the drugs are stopped [22]. Administration of 20% EY to EY1 rats and 40% EY to EY2 rats increased plasma total cholesterol by 16.5% and 30%, respectively, compared to CO rats (Table 2). Plasma TG levels were increased, after feeding egg yolk we found an increase of 8.2% in EY1 and 11.9% in EY2 rats (Table 2). Plasma HDL-C levels also increased slightly, which is not significant, but LDL-C increased significantly. We measured HDL-C in CO=27.5 mg/dl, EY1=28.9 mg/dl and in EY2=30.1 mg/dl and LDL-C in CO=56.9 mg/dl, EY1=78.4 mg/dl and in EY2=81 mg/dl (Table 2). LDL-C is known as bad cholesterol. The higher plasma HDL-C level indicates that excess cholesterol from peripheral tissues has been transported back to the liver for breakdown. Excretion of free cholesterol or its conversion into bile acids via the bile duct [23]. This process occurs when the liver hydrolyzes LDL-C esters and HDL-C esters into free cholesterol. High LDL-C and low HDL-C levels indicate an imbalance in the transfer of cholesterol from the liver to extrahepatic tissues and back to the liver. According to Hongu et al. [24], elevated TC, LDL-C and TG levels are risk factors for cardiovascular disease, hyperlipidemia and dyslipidemia. Very low density lipoproteins (VLDL) are the carriers of dietary lipids in normal lipid metabolism. Lipoprotein lipase breaks down VLDLs into free fatty acids (FFA), which are then used as fuel by peripheral tissues [25]. However, when lipid intake exceeds energy expenditure, the excess is stored as fat in the body's fat cells. When these unnecessary fats are eventually retained, plasma fatty acids exceed threshold, leading to hyperlipidemia, dyslipidemia, and other cardiovascular diseases [26]. In our study, we found an increase in the LDL-C/HDL-C ratio of 68.7% and 306.5% after feeding 20% and 40% EY, respectively (Table 2). Therefore, it can be concluded that high consumption of egg yolk significantly increases plasma LDL-cholesterol but not HDL-cholesterol by a significant amount, leading to an increase in the LDL-C/HDL-C ratio can have devastating health effects.

Mean standard error of the mean, One-way ANOVA, and Fisher's PLSD were used to do post hoc comparisons of the data. HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low-Density Lipoprotein-Cholesterol, TC = Total-cholesterol, TG = Triglycerides. Values in the same row that don't have the same superscript are very different ($P < 0.05$).

Standard error of the mean, one-way ANOVA, and Fisher's PLSD were used for post-hoc comparisons of the data. HDL-C = High Density Lipoprotein Cholesterol, LDL-C = Low Density Lipoprotein Cholesterol, TC = Total Cholesterol, TG = Triglycerides. Values in the same row that do not have the same superscript are very different ($P < 0.05$).

Effects of Dietary Egg Yolk on Hepatic TC, TG, HDL-C, and LDL-C Levels

Hepatic TC and TG levels are effective markers of hyperlipidemia since the liver is the primary location for cholesterol production. As the levels of these parameters rise, the risk of atherosclerosis rises in lockstep [27]. In the liver, HMG-CoA is converted to mevalonate the microsomal enzyme HMG-CoA reductase when the conversion process is improved, the TC level rises as well. As a result, inhibiting this enzyme is the first rate-limiting step in the production of cholesterol [28]. In addition, hepatic steatosis is a disease characterised by the formation of TG fat vacuoles inside liver cells. The liver fat content is a reflection of the equilibrium between FFA flow through de novo lipogenesis, fatty acid oxidation, lipolysis and the release of VLDL-C into the circulation. Elevated FFA levels have been associated with metabolic diseases [29]. Hu bscher postulated that if FFA transport to the liver was increased and fatty acid metabolism in the liver was inhibited, a net increment of TG would occur in the liver. Fatty acid inflow into the liver exceeds FFA outflow, leading to metabolic diseases such as dyslipidemia and hyperlipidemia [30]. After feeding 20% yolk in EY1 rats hepatic total lipid, TC, and TG increased 9.1%, 24.8%, and 54.3%, and feeding 40% yolk in EY2 has increased 39.1%, 88.8%, and 84.3% respectively (Table 3). In our study, we found a clear indication of the accumulation of TC and TG in the hepatocytes of egg yolk rat liver. Excessive fat in the liver will also increase the cholesterol and triglyceride in the plasma [31].

Stroke risk is increased by carotid plaques, which are in turn related to high LDL-C/HDL-C ratios; the risk of carotid plaques increases by 65% for every unit increase in LDL-C/HDL-C [32]. We found the level of HDL-C from CO, EY1, and EY2 was 24.3 mg/dl, 24.5 mg/dl, and 25.6 mg/dl. The increment was not significant. On the other hand, the hepatic LDL-C was 51.1 mg/dl, 59.2 mg/dl, and 117.9 mg/dl where the increment in EY2 rats was significant, as compared to those of the CO and EY1rats (Table 3). The LDL-C and HDL-C ratio has increased by 40.3% and 244.4% in EY1 and EY2 rats respectively (Table 3). Therefore, it is confirmed that the high level of EY consumption will increase the LDL-C/HDL-C ratio which may damage hepatocytes. The significant increase of atherogenic lipid levels in both plasma

Table 3: Effect of dietary administration of egg yolk on liver lipid profile

Parameter (Liver)	CO	EY1	EY2
Total Lipid (%)	11 ± 0.4^{a}	12±1.3	15.3 ± 1^{b}
TC (mg/dL)	100.9 ± 3.2^{a}	125.9 ± 8.6^{b}	190.5 ± 8.8^{c}
TG (mg/dL)	127.5 ± 5.5^{a}	196.7 ± 13.9^{b}	235 ± 15.2^{c}
HDL-C (mg/dL)	24.3± 1.6	24.5±1.4	25.6± 1.4
LDL-C (mg/dL)	51.1 ± 3.3^{a}	59.2 ± 9.8^{a}	117.9 ± 4.9^{c}
LDL-C/HDL-C	26.8 ± 2.9^{a}	37.6 ± 4.8^{a}	92.3 ± 3.1^{c}

Mean standard error of the mean, One-way ANOVA, and Fisher's PLSD were used to do post hoc comparisons of the data. HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low-Density Lipoprotein-Cholesterol, TC = Total-cholesterol, TG = Triglycerides. Values in the same row that don't have the same superscript are very different ($P < 0.05$).

and liver by consuming yolk, suggests that it might influence hyperlipidemia and hypercholesterolemia-mediated changes in the cardiovascular risk factors, especially in EY2 rats. However, the results showed that high consumption of EY increases TG in the liver, thus increasing the risk of hyperlipidemia. The results of this study imply that a diet heavy in EY might lead to metabolic illnesses such as hyperlipidemia due to the buildup of TG in the liver.

Liver TC and TG levels are effective markers for hyperlipidemia since the liver is the main site of cholesterol production. With increasing values of these parameters, the arteriosclerosis risk increases in step [27]. In the liver, HMG-CoA is converted to mevalonate by the microsomal enzyme HMG-CoA reductase. As the conversion process improves, so does the TC level. Therefore, inhibition of this enzyme is the first rate-limiting step in the production of cholesterol [28]. In addition, hepatic steatosis is a disease characterized by the formation of TG fat vacuoles in liver cells. Liver fat content reflects the balance between FFA flux through de novo lipogenesis, fatty acid oxidation, lipolysis, and the release of VLDL-C into the circulation. Elevated FFA levels have been associated with metabolic diseases [29]. Schreiner postulated that if FFA transport to the liver was increased and fatty acid metabolism in the liver was inhibited, there would be a net increase in TG in the liver. The fatty acid inflow into the liver exceeds the FFA outflow, leading to metabolic diseases such as dyslipidemia and hyperlipidemia [30]. After feeding 20% egg yolk in EY1 rats, total hepatic lipids, TC and TG increased by 9.1%, 24.8% and 54.3%, respectively, and feeding 40% egg yolk in EY2 rats increased by 39.1%, 88.8% and 84.3%, respectively (Table 3). In our study, we found clear evidence for the accumulation of TC and TG in the hepatocytes of the egg yolk rat liver. Excess fat in the liver also increases plasma cholesterol and triglyceride levels [31]. The risk of stroke is increased by carotid plaques, which in turn are associated with high LDL-C/HDL-C ratios; The risk of carotid plaques increases with every 65% increase in LDL-C/HDL-C [32]. We found that the HDL-C level of CO, EY1 and EY2 was 24.3 mg/dl, 24.5 mg/dl and 25.6 mg/dl. The increase was not significant. On the other hand, the hepatic LDL-C was 51.1 mg/dl, 59.2 mg/dl and 117.9 mg/dl, with the increase being significant in EY2 rats compared to those of CO and EY1 rats (Table 3). The LDL-C and HDL-C ratio increased by 40.3% and 244.4% in EY1 and EY2 rats, respectively (Table 3). Therefore, it is confirmed that high EY consumption increases the LDL-C/HDL-C ratio, which can lead to hepatocyte damage. The significant increase in atherogenic lipid levels in both plasma and liver from egg yolk consumption suggests that it may influence hyperlipidemia- and hypercholesterolemia-mediated changes in cardiovascular risk factors, particularly in EY2 rats. However, the results showed that high consumption of EY increases TG in the liver and thus increases the risk of hyperlipidemia. The results of this study suggest that an EY-rich diet can lead to metabolic diseases such as hyperlipidemia due to the accumulation of TG in the liver.

Effects of Dietary Administration of Egg Yolk on Liver ROS, LPO

Reactive oxygen species (ROS) induce oxidative damage and hemolysis. Some diseases, such as thalassemia, glucose-6-phosphate dehydrogenase failure, and sickle cell anemia, are caused by free radicals. Their main target is the red blood cells (RBCs). Due to their high levels of polyunsaturated fatty acids (linoleic, arachidonic acids) and O2 transportation linked to redox-active hemoglobin molecules, the RBCs in this state are in short supply [33]. The removal of membrane protein content by oxidation alters the shape of RBCs in an unfavourable way, disrupting microcirculation [34]. ROS is very much harmful to blood because of causing hemolysis. In this study, after consuming EY, ROS level in the hepatic tissues was significantly increased in both groups (EY1 and EY2). In EY1 it has increased by 105.8% and in EY2 it has increased by 178.6% (Table 4, Figure 3). These results confirmed that 20% and 40% egg yolk in daily food could increase liver oxidative stress which will influence the hemolysis and destruction of normal cells.

Several studies reported that oxidative stress and the damage it causes may be a link between ongoing liver damage and hepatic fibrosis. Oxidative stress, which leads to lipid peroxidation (LPO), is an important factor in how nonalcoholic steatohepatitis and liver cancer start and progress [35]. Thus, an increase in LPO levels in the liver tissues has a damaging effect on cells and tissues. In this study, we also analysed the LPO from hepatocytes of all group rats and found 102.9%, and 170.6% increments in EY1, and EY2 rats respectively (Table 4, Figure 3). The findings confirmed that 20% and 40% egg yolk increase lipid peroxidation in liver tissues.

Reactive oxygen species (ROS) induce oxidative damage and hemolysis. Some diseases such as thalassemia, glucose-6-phosphate dehydrogenase failure and sickle cell anemia are caused by free radicals. Their main target is the red blood cells (RBCs). Due to their high content of polyunsaturated fatty acids (linoleic acid, arachidonic acid) and O2 transport associated with redox-active hemoglobin molecules, erythrocytes are in short supply in this state [33]. Removal of membrane protein content by oxidation unfavorably changes the shape of erythrocytes and disrupts microcirculation [34]. ROS are very harmful to the blood as they cause hemolysis. In this study, after taking EY, the level of ROS in liver tissue was significantly increased in both groups (EY1 and EY2). It has increased by 105.8% in EY1 and by 178.6% in EY2 (Table 4, Figure 3). These results confirmed that 20% and 40% egg yolks in the daily diet could increase oxidative stress in the liver affecting hemolysis and destruction of normal cells. Several studies reported that oxidative stress and the damage it causes may be a link between persistent liver damage and liver fibrosis. Oxidative stress leading to lipid peroxidation (LPO) is

Table 4: Effects of yolk feeding on liver ROS, LPO & RBC hemolysis

Parameter	CO	EY1	EY2
ROS (nmol /mg Protein)	20.6 ±2.6^{a}	42.4±3.9^{b}	57.4±6.2^{c}
LPO (nmol /mg Protein)	3.4 ±0.7^{a}	6.9±0.5^{b}	9.2±0.4^{c}
Hemolysis Hb (µg/ml)	78.5±1.9^{a}	96.8±1.1^{b}	104.2±0.8^{c}

Results are expressed as mean ± SEM, and the data were analysed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Values in the same row with different alphabets are significantly different at $P < 0.05$. ROS= Reactive oxygen species, LPO=lipid peroxidation

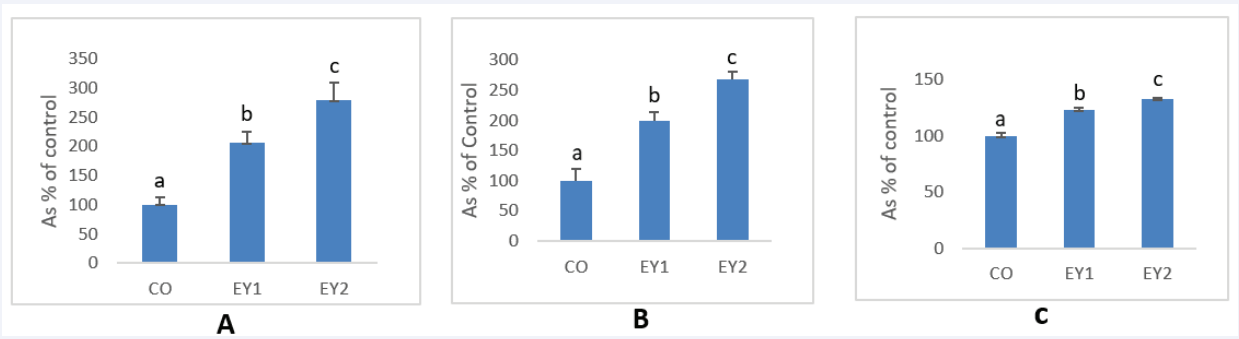

Figure 4 Liver ROS (A), Liver LPO (B), and RBC hemolysis (C) in EY1 and EY2 groups as a percent of CO. Data were analysed by one-way ANOVA followed by Fisher's PLSD for post hoc comparison. Here, CO = Rats of the control group, EY1 = Egg yolk group-1 rats, and EY2 = Egg yolk group-2 rats. [a, b, c] denotes the significant values. EY1 and EY2 values showed a percent of CO.

an important factor in the onset and progression of nonalcoholic steatohepatitis and liver cancer [35]. Thus, an increase in LPO levels in liver tissue has a damaging effect on cells and tissues. In this study we also analyzed the LPO from hepatocytes of all groups of rats and found an increase of 102.9% and 170.6% in EY1 and EY2 rats, respectively (Table 4, Figure 3). The results confirmed that 20% and 40% egg yolks increase lipid peroxidation in liver tissue.

Egg Yolk Feeding and RBC Hemolysis

During hemolysis, hemoglobin is released from erythrocyte into the plasma. Under a number of clinical circumstances, hemolysis is shown including autoimmunity to an RBC surface antigen, rupture of RBCs, thalassemia, malaria infection, and sickle cell disease [36]. Erythrocytes transport oxygen across the body, distribute nutrients, and help the body get rid of a wide range of potentially toxic xenobiotics [37]. RBCs are especially susceptible to oxidative stress because of the large levels of oxygen and hemoglobin in their cells and the high levels of polyunsaturated fatty acids. Hemolysis, transportation, radical scavenging, and biotransformation are all affected by oxidative stress on RBCs. Oxidants harm erythrocyte heme iron, globin chain, and other vital biological components [33]. Our findings showed that feeding 20% and 40% EY has significantly increased ROS and LPO in both groups. Excessive production of ROS may increase RBC hemolysis. We have also analysed the RBC hemolysis and found that hemolysis increased significantly in EY1 and EY2 group rats also (Table 4, Figure 4). After yolk feeding hemolysis has increased 23.2% and 32.7% in some respect of EY1 and EY2 group rats compared with the CO rats (Table 4, Figure 4). This result indicates that the dietary administration of a high level of egg yolk may be an agent for hemolysis and a high level of EY consumption may threaten RBC.

During hemolysis, hemoglobin is released from erythrocytes into the plasma. Hemolysis is demonstrated in a variety of clinical circumstances, including autoimmunity to an erythrocyte surface antigen, rupture of erythrocytes, thalassemia, malaria infection, and sickle cell anemia [36]. Red blood cells carry oxygen throughout the body, distribute nutrients, and help the body eliminate a variety of potentially toxic xenobiotics [37]. Erythrocytes are particularly vulnerable to oxidative stress due to the high oxygen and hemoglobin content in their cells and the high content of polyunsaturated fatty acids. Hemolysis, transport, scavengers, and biotransformation are all affected by oxidative stress on erythrocytes. Oxidizing agents damage erythrocyte heme-iron, the globin chain, and other vital biological components [33]. Our results showed that feeding 20% and 40% EY significantly increased ROS and LPO in both groups. Excessive production of ROS can increase erythrocyte hemolysis. We also analyzed RBC hemolysis and found that hemolysis also increased significantly in rats of groups EY1 and EY2 (Table 4, Figure 4). After feeding egg yolk, hemolysis increased by 23.2% and 32.7% in the EY1 and EY2 groups compared to the CO rats, respectively (Table 4, Figure 4). This result indicates that dietary administration of a large amount of egg yolk can be a trigger for hemolysis and that high consumption of EY can endanger erythrocytes.

CONCLUSION

Our results suggest that a high level of dietary egg yolk is not good for health, though it contains vitamins, and minerals and other benefits. A high amount of egg yolk, especially more than 20% in daily food may be responsible for obesity, fatty liver, and oxidative stress which leads to hemolysis and lipid peroxidation injurious to health.

However, further studies with egg yolk are essential to clarify the effects of egg yolk on hyperlipidemia, other cardiovascular risk factors, and their association with cardiovascular disease incidences in human subjects.

Our results suggest that a high proportion of egg yolk in the diet is not good for health, even though it contains vitamins, minerals and other benefits. A high amount of egg yolk, especially more than 20% in the daily diet, can be responsible for obesity, fatty liver and oxidative stress leading to harmful hemolysis and lipid peroxidation. However, further studies of egg yolk are

essential to clarify the effects of egg yolk on hyperlipidemia, other cardiovascular risk factors and their association with the occurrence of cardiovascular disease in humans.

REFERENCES

1. Xiao Nanhai, Zhao Yan, Yao Yao, Wu Na, Xu Mingsheng, Du Huaying, Tu Yonggang. Biological activities of egg yolk lipids: A review. J Agric Food Chem. 2020; 68: 1948-1957.
2. David Spence J. Dietary cholesterol and egg yolk should be avoided by patients at risk of vascular disease. J Transl Int Med. 2016; 4: 20-24.
3. Spence J David, Jenkins David JA, Davignon Jean. Egg yolk consumption and carotid plaque. Atherosclerosis. 2012; 224: 469-473.
4. Mann Jim I, Appleby Paul N, Key Timothy J, Thorogood Margaret. Dietary determinants of ischaemic heart disease in health conscious individuals. Heart. 1997; 78: 450-455.
5. Gresham GA, Leat WMF, Howard AN, Jennings IW. Pathological changes in pigs reared on semi-synthetic diets containing no fat, beef tallow and maize oil. Br J Exp Pathol.1964; 45: 128.
6. Khanam Masuma Akter, Qiu Chengxuan, Lindeboom Wietze, Streatfield Peter Kim, Kabir Zarina Nahar, Wahlin Åke. The metabolic syndrome: prevalence, associated factors, and impact on survival among older persons in rural Bangladesh. PLoS One. 2011; 6: e20259.
7. Who Joint, Consultation FAO Expert. Diet, nutrition and the prevention of chronic diseases. World Health Organ Tech Rep Ser. 2003; 916: 1-149.
8. Hossain Shahdat, Bhowmick Sujan, Islam Saiful, Rozario Liza, Jahan Sabrin, Hassan Mehedi, et al. Oral administration of ganoderma lucidum to lead-exposed rats protects erythrocytes against hemolysis: implicates to anti-anemia. Evid Based Complement Alternat Med. 2015.
9. Röschlau v P, Bernt E, Gruber W. Enzymatische bestimmung des gesamt-cholesterins im serum. 1974.
10. Yu Zhihui, Wang Ning, Ahn Dong U, Ma Meihu. Long term egg yolk consumption alters lipid metabolism and attenuates hyperlipidemia in mice fed a high-fat diet based on lipidomics analysis. Eur J Lipid Sci Technol. 2019; 121: 1800496.
11. Ercal NH, Gurer-Orhan, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. Curr Top Med Chem. 2001; 1: 529-539.
12. Halliwell Barry. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? The lancet. 1994; 344: 721-724.
13. Chen Xingyong, Du Yeye, Boni Grace F, Liu Xue, Kuang Jinlong, Geng Zhaoyu. Consuming egg yolk decreases body weight and increases serum HDL and brain expression of TrkB in male SD rats. J Sci Food Agric. 2019; 99: 3879-3885.
14. Marzan Sarkar, Mahmudul Hasan, Bhowmick Sujan, Hussain Jakir, Haque Mozammel, Khan Md Asaduzzaman, et al. Evaluation of the Anti-oxidative, Erythrocyte Membrane Stabilizing Effect and Nutritional Status of Neolamarckia cadamba Fruit. Am J Food Nutrit. 2019; 7: 6-12.
15. Hashimoto Michio, Hossain Shahdat, Katakura Masanori, Al Mamun Abdullah, Shido Osamu. The binding of Aβ1–42 to lipid rafts of RBC is enhanced by dietary docosahexaenoic acid in rats: Implicates to Alzheimer's disease. Biochim Biophys Acta. 2015; 1848: 1402-1409.
16. Cohn Jeffrey S, McNamara Judith R, Schaefer Ernst J. Lipoprotein cholesterol concentrations in the plasma of human subjects as measured in the fed and fasted states. Clin Chem. 1988; 34: 2456-2459.
17. Ohkawa Hiroshi, Ohishi Nobuko, Yagi Kunio. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979; 95: 351-358.
18. Lowry Oliver H, Rosebrough Nira J, Farr A Lewis, Randall Rose J. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193: 265-275.
19. Montoliu Carmina, Vallés Soraya, Renau-Piqueras Jaime, Guerri Consuelo. Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain: effect of chronic alcohol consumption. J Neurochem. 1994. 63: 1855-1862.
20. Hossain Md Shahdat, Hashimoto Michio, Gamoh Shuji, Masumura Sumio. Antioxidative effects of docosahexaenoic acid in the cerebrum versus cerebellum and brainstem of aged hypercholesterolemic rats. J Neurochem. 1999; 72: 1133-1138.
21. Beynen Anton C, Katan Martijn B. Effect of egg yolk feeding on the concentration and composition of serum lipoproteins in man. Atherosclerosis. 1985; 54: 157-166.
22. Mahley Robert W, Bersot Thomas P. Drug therapy for hypercholesterolemia and dyslipidemia. The pharmacological basis of therapeutics. 2006; 933-966.
23. Nelson David L, Lehninger Albert L, Cox Michael M. Lehninger principles of biochemistry. 2008: Macmillan.
24. Hongu Nobuko, Kitts David D, Zawistowski Jerzy, Dossett Cynthia M, Kopeć Aneta, Pope Benjamin T, Buchowski Maciej S. Pigmented rice bran and plant sterol combination reduces serum lipids in overweight and obese adults. J Am Coll Nutrit. 2014; 33: 231-238.
25. Barter Philip. The role of HDL-cholesterol in preventing atherosclerotic disease. European Heart J Supplements. 2005; 7: F4-F8.
26. Lee Yeon Ri, Kim Chae Eun, Kang Mi Young, Nam Seok Hyun. Cholesterol-lowering and antioxidant status-improving efficacy of germinated giant embryonic rice (Oryza sativa L.) in high cholesterol-fed rats. Ann Nutr Metab. 2008; 51: 519-526.
27. Lennernäs Hans, Fager Gunnar. Pharmacodynamics and pharmacokinetics of the HMG-CoA reductase inhibitors: similarities and differences. Clin pharmacokinet. 1997; 32: 403-425.
28. O'Meara Niall MG, Devery Rosaleen AM, Owens Daphne, Collins Patrick B, Johnson Alan H, Tomkin Gerald H. Cholesterol metabolism in alloxan-induced diabetic rabbits. Diabetes. 1990; 39: 626-633.
29. Reddy Janardan K, Sambasiva Rao M. Lipid metabolism and liver inflammation. II. Fatty liver disease and fatty acid oxidation. Am J Physiol Gastrointestinal Liver Physiol. 2006; 290: G852-G858.
30. Fabbrini Elisa, Sullivan Shelby, Klein Samuel. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatol. 2010; 51: 679-689.
31. Weusten-Van der Wouw MP, Katan MB, Viani R, Huggett AC, Liardon R, Lund-Larsen PG, et al. Identity of the cholesterol-raising factor from boiled coffee and its effects on liver function enzymes. J lipid Res. 1994; 35: 721-733.
32. Geng Yang, Liu Yan, Chen Yan, Zhang Zhenwen, Wang Liping, Li Xiaobo, Xia Binlan, et al. Association of LDLc to HDLc ratio with carotid plaques in a community-based population with a high stroke risk: A cross-sectional study in China. Clin Biochem. 2021; 88: 43-48.
33. Sarkar Marzan, Bhowmick Sujan, Hussain Jakir, Hasan Mahmudul, Hossain Shahdat. Hot water extract of Moringa oleifera leaves protects erythrocytes from hemolysis and major organs from oxidative stress in vitro. J Basic Applied Res Biomed. 2017; 3: 120-126.
34. Yang Hsin-Ling, Chen Ssu-Ching, Chang Nai-Wen, Chang Jia-Ming, Lee

Mei-Ling, Tsai Pei-Chuan, et al. Protection from oxidative damage using Bidens pilosa extracts in normal human erythrocytes. Food Chem Toxicol. 2006; 44: 1513-1521.

35. Nair Jagadeesan, Srivatanakul Petcharin, Haas Claudia, Jedpiyawongse Adisorn, Khuhaprema Thiravud, Seitz Helmut K, et al. High urinary excretion of lipid peroxidation-derived DNA damage in patients with cancer-prone liver diseases. Mutat Res. 2010; 683: 23-28.

36. Giardina Bruno, Messana Irene, Scatena Roberto, Castagnola Massimo. The multiple functions of hemoglobin. Crit Rev Biochem Mol Biol. 1995; 30: 165-196.

37. Sivilotti Marco LA. Oxidant stress and haemolysis of the human erythrocyte. Toxicological reviews. 2004; 23: 169-188.es