

Effect of chicken egg consumption on antioxidant status and lipid profile levels in students of Nnamdi Azikiwe University, Nnewi, Nigeria

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Abstract

Introduction: Chicken egg contains a number of biological substances which may play pivotal roles in human nutrition and health.

Aim: This is a case control study designed to assess the effect of chicken egg consumption on antioxidant status and lipid profile levels in students of Nnamdi Azikiwe University, Nnewi, Nigeria.

Materials and Methods: A total of thirty (30) participants comprising of fifteen (15) male and female students aged between eighteen (18) and twenty six (26) years were randomly recruited for the study. Five milliliters (5mls) of baseline samples (after an overnight fast) were collected from participants at day 0 as baseline samples and levels of antioxidants (TAC and MDA) and lipid profile were evaluated. Subsequently, in addition to their normal diet, each of the participants received one (1) egg before meal daily for a period of 30 days. After an overnight fast, 5mls of post research (post test) samples was collected on day 31 and the levels of antioxidants and lipid profile (TC, TG, LDL-C, HDL-C) were re-evaluated using standard laboratory methods. Also, the body mass index (BMI) of the participants was determined.

Results: the results showed that the consumption of one (1) chicken egg per day for a period of 30 days caused a significant increase ($p < 0.05$) in total antioxidant capacity (TAC) and serum high density lipoprotein cholesterol (HDL-C) levels; decrease in serum triglyceride (TG) and Malondialdehyde (MDA) levels ($p < 0.05$) with no significant alterations in the mean serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels ($p > 0.05$) respectively after (day 31) egg consumption compared to baseline (day 0) values. Also, gender comparisons revealed no significant alterations in the mean TAC, MDA, TC, LDL-C and HDL-C levels ($p > 0.05$) respectively while the mean serum TG level was significantly higher in female participants than in the males after egg consumption ($p < 0.05$).

Conclusion: this study revealed that the consumption of one chicken egg per day for a period of thirty days has beneficial effects on the antioxidant status and lipid profile levels in healthy individuals.

Keywords: Egg, Chicken, Oxidative stress, Antioxidants, Malondialdehyde (MDA), Total antioxidant capacity (TAC), Lipid profile.

Introduction

Eggs are a principal food for human consumption practically for the children and elderly, it is delicious, easy to digest, and contains most of the nutrients needed by human based on recommended daily allowance.¹ Egg yolk is a rich source of both nutritive and non-nutritive compounds important to human health.² Eggs are an inexpensive and highly nutritious food, providing 18 vitamins and minerals, the composition of which can be affected by several factors such as hen diet, age, strain as well as environmental factors.³ Average composition of one egg, weighing about 60g is as follows: Yolk 17.4g (29%), Albumin 36.9g (61.5%), and Shell 5.6g (9.5%). Yolk contains all fat soluble vitamins and most of the water soluble vitamins in addition to fats.⁴ Nevertheless, although different compositions have been reported by several authors,⁵ on average, the macronutrient content of eggs include low carbohydrates and about 12 g per 100 g of protein and lipids, most of which are monounsaturated⁶ and supply the diet with several essential nutrients. Some of these nutrients, such as zinc, selenium, retinol and tocopherols, are deficient in people consuming a western diet, and given its antioxidant activity, can protect humans from many degenerative processes, including CVD.⁷ There is also scientific evidence that eggs contain other biologically active compounds that

may have a beneficial role in the therapy and prevention of chronic and infectious diseases. The presence of compounds with antimicrobial, immune-modulator, antioxidant, anti-cancer or anti-hypertensive properties have been reported in eggs.⁸ Lysozyme, ovo-mucoid, ovo-inhibitor and cystatin are biologically active proteins in egg albumen, and their activity prolongs the shelf life of table eggs.⁹ Some of these protective substances are isolated and produced on an industrial scale as lysozymes and avidin. Additionally, eggs are an important source of lecithin and are one of the few food sources that contain high concentrations of choline.¹⁰ Lecithin, as a polyunsaturated phosphatidylcholine, is a functional and structural component of all biological membranes, which acts in the rate-limiting step of the activation of membrane enzymes such as superoxide dismutase. Other interesting egg components from the nutritional point of view are the carotenoids. Carotenoids are natural pigments in hen egg yolks that confer its yellow color, which can range from very pale yellow to dark brilliant orange. Egg carotenoids represent less than 1% of yolk lipids, and are mainly composed of carotene and xanthophylls (lutein, cryptoxanthin and zeaxanthin).¹¹ The total concentration of lutein and zeaxanthin is 10 times greater than of cryptoxanthin and carotene, combined⁹ and are not

endogenously synthesized by the human body and tissue levels therefore depend on dietary intake.

Oxidative stress is the damage to cells caused by oxidation, which causes a large increase in the cellular reduction potential. It causes destruction of cells by the production of reactive oxygen species (ROS). ROS are chemically reactive molecules containing oxygen. In low levels, they get countered by the cell antioxidants.¹² Malondialdehyde (MDA) is a biomarker for oxidative stress. MDA is the end-product of the radical-initiated oxidative decomposition of polyunsaturated fatty acids. MDA levels are predictive for the occurrence of cardiovascular events (myocardial infarction, stroke), metabolic diseases (diabetes mellitus).¹³ Total antioxidant capacity (TAC) is the measure of the amount of free radicals scavenged by a test solution,¹⁴ being used to evaluate the antioxidant capacity of biological samples.¹⁵

An antioxidant can be defined as “any substance that delays, prevents or removes oxidative damage to a target molecule”¹⁶ or “any substance that directly scavenges reactive oxygen species (ROS) or indirectly acts to up-regulate antioxidant defenses or inhibit ROS production.”¹⁷ Vitamin E (tocopherol) is one of such antioxidants. Some lipophilic antioxidants such as vitamin E, carotenoids, selenium, iodine and others can be transferred from feed into egg yolk to produce antioxidant-enriched eggs.¹⁸

Furthermore, Kishimoto *et al.* observed significant decreases in the levels of MDA and MDA-LDL/LDL-C ratio while plasma TAC value significantly increased with a negative correlation existing between the change in MDA-LDL and changes in TAC following egg consumption.¹⁹ In a study carried out by Nargis *et al.* the serum total cholesterol and LDL-C were significantly decreased while no significant difference in value was observed in the levels of HDL-C and TG after egg consumption.⁴ This result was consistent with the study of Techakriengkrai *et al.*,²⁰ but differed from some other studies which documented no significant changes in TC and LDL-C.²¹ Kishimoto *et al.* suggested that the ameliorative effects of egg on serum MDA-LDL level and LDL oxidizability may be attributed to the antioxidants contained in chicken egg.¹⁹ However, the bioactivity of egg antioxidants as observed by Nimalaratne and Wu¹⁸ can be affected by food processing, storage and gastrointestinal digestion. Egg proteins are high quality proteins and are used as a golden standard for measuring the quality of other food proteins²² and in addition to its nutritional value; egg components have various biological activities which may render important health benefits.²³ It is a nutrient dense food and also contains a number of antioxidants. Intake of antioxidants through diet is known to be important in reducing oxidative damage in cells and improving human health.¹⁸ Therefore, the present study investigated the effect of chicken egg consumption on antioxidant status and lipid profile levels in students of Nnamdi Azikiwe University, Nnewi, Nigeria.

Materials and Methods

Study Design

This is a case-control study designed to assess the effect of chicken egg consumption on serum lipid profile and antioxidant status in apparently healthy students of College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. A total of thirty (30) participants comprising of fifteen (15) male and female students aged between eighteen (18) and twenty six (26) years were randomly recruited for the study. The protocols for the study were properly explained to the intending participants and thereafter, written consents were obtained from participants prior to the commencement of the study. Each participant was advised to abstain from egg intake for a period of three weeks. Afterwards, 5mls each of baseline samples (after an overnight fast) was collected from the participants at day 0 as baseline samples, and levels of antioxidants (total antioxidant capacity (TAC) and Malondialdehyde) and lipid profile (TC, TG, LDL-C, HDL-C) were evaluated. Subsequently, in addition to their normal diet, each of the participants received one (1) egg before meal daily for a period of 30 days. After an overnight fast, 5mls of post research (post test) samples was collected on day 31 and the levels of antioxidants and lipid profile were re-evaluated. Antioxidants (TAC and MDA) and lipid profile levels (TC, TG, LDL-C, HDL-C) were determined using standard laboratory methods. Also, a structured questionnaire was used to obtain relevant information such as age, height, sex, demographic factors, dietary patterns, physical activities, medical history, lifestyle and history of egg intake, while participants' weight were obtained using weighing scale before and after egg consumption.

Inclusion Criteria

Apparent healthy male and female participants aged between 18 and 26 years who consented to the study were included for this study.

Exclusion Criteria

Individuals consuming eggs, Diabetic and hypertensive individuals, and those diagnosed with cardiovascular disease, alcoholics and smokers or those outside the age bracket of 18-26 years were excluded from the present study.

Ethical Consideration

The ethical approval for this study was sought and obtained from the Ethics Committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi, Anambra State, Nigeria.

Estimation of total Antioxidant Capacity (TAC)

TAC activity was estimated by Ferric Reducing Ability of Plasma (FRAP) method as described by Benzie and Strain.²⁴

Estimation of Malondialdehyde (MDA)

MDA level was estimated by the colorimetric method described by Gutteridge and Wilkins.²⁵

Estimation of Total Cholesterol (TC)

Total Cholesterol level was estimated using enzymatic method as described by Roeschlau *et al.*²⁶

Estimation of Triglycerides

Triglyceride level was estimated with the enzymatic method as described by Tietz.²⁷

Estimation of High Density Lipoprotein Cholesterol (HDL-C)

HDL-C level was estimated using the method described by Burstein *et al.*²⁸

Estimation of low Density Lipoprotein Cholesterol (LDL-C)

LDL-C level was estimated using the enzymatic method described by Assman *et al.*²⁹

Statistical Analysis

The data obtained was statistically analyzed using Statistical package for Social Sciences (SPSS) Version 23.0. Students' t-test and Pearson correlation coefficient were used to compare means. The results were expressed as mean±SD and confidence limits was chosen at 95% ($p < 0.05$). $p < 0.05$ was considered statistically significant.

Results

There was no significant difference in mean BMI (24.16±2.75), Age (23.47±1.70), Height (1.71±0.10) and Weight (70.27±10.41) in participants studied when compared with values obtained before and after egg consumption respectively ($p > 0.05$). See table 1.

The mean serum MDA (2.37 ±0.71) was significantly

lower after egg consumption compared to the baseline value (=2.83 ±0.68), ($p = 0.014$) with no significant difference in the mean serum levels of TAC when compared before and after egg consumption ($P > 0.05$). Also, there were no significant differences observed in the mean serum levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) in the participants studied when compared before and after egg consumption respectively ($p > 0.05$). However, the mean serum level of triglyceride (TG) was significantly lower in post egg consumption than in baseline (1.09±0.15 Vs 1.64±0.45; $p = 0.000$), while the mean serum level of high density lipoprotein cholesterol (HDL-C) was significantly higher post egg consumption than before egg consumption (1.44±0.19 Vs 1.20±0.27; $p = 0.000$). See table 2.

The mean serum level of TAC (938.88±113.58) in the female participants was significantly higher compared to that of the male participants (780.17±99.82) before egg consumption ($p > 0.05$). However, there was no significant difference in the mean serum level of MDA in the female participants when compared to that of the male participants before egg consumption ($p > 0.05$). Furthermore, there was no significant difference in the mean serum levels of MDA and TAC in female participants compared to that in male participants after egg consumption ($p > 0.05$). See table 3.

Table 1: Anthropometric parameters in groups studied (Mean ±SD)

Variables	Age (year)	Weight (kg)	Height (m)	BMI (kg/m ²)
Baseline (n=30)	23.47±1.70	71.03±10.23	1.71±0.10	23.90±2.68
Post consumption (n=30)	23.47±1.70	70.27±10.41	1.71±0.10	24.16±2.75
t-value	0.030	0.000	0.000	0.009
p-value	0.711	1.000	1.000	0.775

*Statistically significant at $p < 0.05$.

Table 2: Serum levels of antioxidants (TAC and MDA) and lipid profile in groups studied before and after egg consumption (Mean ±SD)

Variables	TAC (µmol/L)	MDA (nmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Baseline (n=30)	804.23 ±104.00	2.83 ±0.68	4.79 ±0.75	1.64 ±0.45	1.20 ±0.27	2.71 ±0.82
Post consumption (n=30)	854.94 ±131.99	2.37 ±0.71	4.46 ±0.86	1.09 ±0.15	1.44 ±0.19	2.53 ±0.77
t-value	1.948	0.015	0.748	14.599	0.922	0.311
p-value	0.114	0.014	0.120	0.000	0.000	0.377

*Statistically significant at $p < 0.05$.

Table 3: Gender comparison of serum levels of antioxidants (TAC and MDA) before and after egg consumption (Mean±SD).

Variables	TAC (µmol/L)	MDA (nmol/L)
Baseline (day 0)		
Female (n=15)	938.88±113.58	2.41±0.37
Male (n=15)	780.17±99.82	2.32±0.98
t-value	1.127	6.497
p-value	0.000	0.733
Post consumption (day 31)		
Female (n=15)	810.45±111.59	3.02±0.76
Male (n=15)	803.17±103.55	2.64±0.98
t-value	0.000	1.876
p-value	0.857	0.144

*Statistically significant at $p < 0.05$.

The mean serum level of TG (1.15 ± 0.12) was significantly higher in the female participants compared to that of the male participants (1.05 ± 0.16) before egg consumption ($p=0.057$). Also, there was no significant difference in the mean serum levels of TC, HDL-C and LDL-C in the female participants compared to the values obtained in the male participants before egg consumption ($p>0.05$).

Furthermore, the mean serum level of TG (1.81 ± 0.44) was significantly higher in the female participants compared to that of the male participants (1.46 ± 0.41) after egg consumption ($p=0.035$), but no significant differences were observed in the mean serum levels of TC, HDL-C and LDL-C in the female participants compared to that of the male participants after egg consumption respectively ($p>0.05$). See table 4.

There was a positive correlation between MDA and TG ($r=0.025$), while negative correlations were observed between MDA and weight ($r=-0.035$), TC and weight ($r=-0.008$), and between HDL-C and age ($r=-0.033$) respectively in parameters studied before egg consumption ($p>0.05$). See table 5.

There was a positive correlation between TAC and HDL-C ($r=0.015$), while negative correlations were observed between MDA and BMI ($r=-0.008$), HDL-C and BMI ($r=-0.027$), and between HDL-C and age ($r=-0.025$) respectively in parameters studied after egg consumption ($p>0.05$). See table 6.

Discussion

Eggs play an important role in the human diet and nutrition as an affordable nutrient-rich food commodity that contains highly digestible proteins, lipids, minerals, and vitamins.³⁰ Diet is an important modifiable factor that can impact CVD risk and atherosclerosis progression.³¹

In this study, the mean serum level of HDL-C was significantly increased after egg consumption compared with the baseline value. An increase in HDL-C has been previously shown to coincide with increased HDL lipid and antioxidant composition.³² This finding is in line with the study of Kishimoto *et al.*,¹⁹ who reported that HDL-C values were significantly increased by an average of 6.5% after 4 weeks of egg consumption in healthy individuals, stating that the significant increase in HDL-C may possibly be due to the

cholesterol and phospholipids present in egg yolk which are incorporated into HDL. Also, Missimer *et al.* had earlier proved in their study that consumption of two eggs per day was shown to increase plasma HDL-C without elevating other known CVD risk factors in young healthy population.³³ HDL-C plays an important antioxidative function and has the potential to protect LDL from oxidation by free radicals.³² It is also responsible for reverse cholesterol transport from extrahepatic tissues to the liver in order to clear cholesterol from the body, primarily by utilizing cholesterol as a precursor for bile acid synthesis.^{34,35} Furthermore, several other previous similar studies are in keeping with the present finding.^{36,37}

The present study revealed a significant decrease in the mean serum level of triglyceride (TG) after egg consumption compared to the values obtained before egg consumption. This is in contrast with the reports of some previous similar studies who documented no effect of egg consumption in the mean serum TG level after egg consumption.^{4,38}

However, in this work, there was no significant difference in the mean serum levels of total cholesterol (TC) after egg consumption when compared to the baseline value. This is in consonance with the finding of Kishimoto *et al.* who showed no alteration in serum TC after 4 weeks of egg consumption in male healthy individuals.¹⁹ Similarly, some other authors had earlier reported similar findings with the present study,^{39,40} but the work of Nargis *et al.* held a different view with the current report.⁴ Previously, Flynn *et al.* have shown that dietary cholesterol provided by eggs does not affect the cholesterol concentration.⁴¹

Interestingly, there was no significant difference in the mean serum level of LDL-C post egg consumption compared with values obtained before egg consumption. This corroborate the work of Rueda *et al.*⁴² and Clayton *et al.*⁴³ which showed no effect of egg consumption on LDL-C. LDL transports cholesterol in the blood stream to cells and plays pivotal role in the development of cardiovascular diseases particularly atherosclerosis. This result may therefore imply that the consumption of egg could further reduce the oxidation of LDL-C and perhaps help improve cardio-metabolic health functions in healthy consumers. The presence of unsaturated fatty acid contents in egg may be the underlying mechanism.

Table 4: Gender comparison of serum lipid profile levels before and after egg consumption (Mean±SD)

Variables	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Baseline (day 0)				
Female (n=15)	4.61±0.93	1.15±0.12	2.87±1.92	2.87±0.99
Male (n=15)	4.30±0.46	1.05±0.16	1.21±0.31	2.53±0.55
t-value	1.880	1.681	0.664	1.021
p-value	0.275	0.057	0.848	0.262
Post consumption (day 31)				
Female (n=15)	4.63±0.44	1.81±0.44	1.46±0.20	2.47±0.56
Male (n=15)	4.97±1.14	1.46±0.41	1.44±0.19	2.60±0.96
t-value	6.128	0.499	0.293	3.524
p-value	0.307	0.035	0.738	0.645

*Statistically significant at $p<0.05$.

Table 5: Levels of association between parameters studied before egg consumption (n=30)

Parameters	Pearson r coefficient	F-value
MDA Vs TG	0.025	0.894
MDA Vs Weight	-0.035	0.855
TC Vs Weight	-0.008	0.965
HDL-C Vs Age	-0.033	0.940

*Statistically significant at $p < 0.05$.

Table 6: Levels of association between parameters studied after egg consumption (n=30)

Parameters	Pearson r coefficient	F-value
TAC Vs HDL-C	0.015	0.938
MDA Vs BMI	-0.008	0.965
HDL-C Vs BMI	-0.027	0.888
HDL-C Vs Age	-0.025	0.894

*Statistically significant at $p < 0.05$.

The present study also shows that the mean serum level of malondialdehyde (MDA) was significantly decreased after egg consumption compared to values observed before egg consumption. MDA is an important biomarker of oxidative stress due to increased lipid peroxidation. Oxidative stress induces the generation of free radicals which interacts with biological molecules including the constituents of cell membrane resulting in lipid peroxidation.⁴⁴ The free radicals induce damage to cells by passing the unpaired electron resulting in oxidation of cell components and molecules.⁴⁵ Therefore MDA as a marker of oxidative stress tends to increase in conditions of increased lipid peroxidation. However, compounds with antioxidant properties should be able to reduce the concentration of free radicals and hence, cause a reduction in MDA level. The decrease in MDA level following the consumption of chicken egg in this study may imply that chicken egg contains antioxidants which are thought to play a protective role against oxidative damage. Antioxidant systems have potentials to work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage.⁴⁶ This confirms the report of Nahariah *et al.* that chicken egg contains antioxidants.⁴⁷ The present result is in agreement with previous similar studies.¹⁹

In contrast, there was a significant increase in the serum levels of total antioxidant capacity (TAC) after egg consumption compared to baseline or before egg consumption. Intake of antioxidants through diet is known to be important in reducing oxidative damage in cells and improving human health.¹⁸ This is in consonance with previous similar studies.¹⁹ The gender comparison in this study revealed a significantly higher level of TG with no significant alterations in the mean serum levels of TC, HDL-C and LDL-C in the female participants than in male participants post egg consumption respectively. Also, there was no significant difference in the mean serum levels of MDA and TAC in female participants compared to the values observed in male participants after egg consumption respectively.

Interestingly, there was a positive correlation between TAC and HDL-C ($r=0.015$) levels while negative correlations

were observed between MDA and BMI ($r= -0.008$), HDL-C and BMI ($r= -0.027$), and between HDL-C and age ($r= -0.025$) respectively in parameters studied after egg consumption.

Conclusion

This study has shown that the consumption of egg caused a significant increase in TAC and HDL-C levels, decrease in TG and MDA levels with no significant alterations in TC and LDL-C levels respectively after egg consumption. Also, no significant alterations were seen in parameters studied when compared between the female and male participants except for TG level which was significantly higher in female participants. Therefore, this study revealed that the consumption of one chicken egg per day for a period of thirty days has beneficial effects on the antioxidant status and lipid profile levels in healthy individuals.

Conflict of Interest: None.

References

1. Youssef AA, Mohammed AA, Mohamed AK, Mohamed MS. Fatty acid and cholesterol profiles and hypocholesterolemic, atherogenic, and thrombogenic indices of table eggs in the retail market. *J Lipids Health Dis* 2015;14:136.
2. Attia YA, Al-Harhi MA, Shiboob. Evaluation of quality and nutrient contents of table eggs from different sources in the retail market. *Int J Anim Sci* 2014;13:369.
3. Fraeye I, Bruneel C, Lemahieu C, Buyse J, Muylaert K, Foubert I. Dietary enrichment of eggs with omega-3 fatty acids: A review. *Food Res Int* 2012;48:961–9.
4. Nargis S, Majumder M, Debnath BC, Hossain MS. Effect of Egg Consumption on Serum Lipid Profile in Young Adults. *Bangladesh J Med Biochem* 2015;8(1):5-9.
5. Samman S, Kung FP, Carter LM, Foster MJ, Ahmad Z, Phuyal JL. Fatty acid composition of certified organic, conventional and omega-3 eggs. *J Food Chem* 2009;116:911–4.
6. Kassis N, Drake SR, Beamer SK, Matak KE, Jaczynski J. Development of nutraceutical egg products with omega-3-rich oils. *LWT-Food Sci Technol* 2010;43:777–83.
7. Natoli S, Markovic T, Lim D, Noakes M, Kostner K. Unscrambling the research: Eggs, serum cholesterol and coronary heart disease. *Nutr Diet* 2007;64:105–11.
8. Abeyrathne EDNS, Lee HY, Ahn DU. Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. *J Poult Sci* 2013;92:3292–9.

9. Rakonjac S, Bogosavljevic-Boskovic S, Pavlovski Z, Skrbic Z, Doskovic V, Petrovic MD, et al. Laying hen rearing systems: Laying hen rearing Systems: A review of Chemicals composition and hygienic conditions of eggs. *World Poult Sci J* 2014;70:151–63.
10. Herron KL, Fernandez ML. Are the current dietary guidelines regarding egg consumption appropriate? *J Nutr* 2004;134:187–90.
11. Skrivan M, Englamaierová M. The deposition of carotenoids and α -tocopherol in hen eggs produced under a combination of sequential feeding and grazing. *Anim Feed Sci Technol* 2014;190:79–86.
12. Yin H, Xu L, Porter NA. Free radical lipid peroxidation: Mechanisms and analysis. *Chem Rev* 2011;111:5944–72.
13. Grune T, Berger MM. Markers of oxidative stress in ICU clinical settings: present and future. *Curr Opin Clin Nutr Metab Care* 2007;10(6):712–7.
14. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *J Free Rad Biol Med* 2000;29:1106–14.
15. Marques SS, Magalhães LM, Tóth IV, Segundo MA. Insights on antioxidant assays for biological samples based on the reduction of copper complexes—the importance of analytical conditions. *Int J Molec Sci* 2014;15(11):387–402.
16. Halliwell B. Biochemistry of oxidative stress. *Biochem Soc Transl* 2007; 35:1147–50.
17. Khlebnikov AI, Schepetkin IA, Domina NG, Kirpotina LN, Quinn MT. Improved quantitative structure-activity relationship models to predict antioxidant activity of avonoids in chemical, enzymatic, and cellular systems. *J Bioorganic Med Chem* 2007;15:1749–70.
18. Nimalaratne C, Wu J. Hen Egg as an Antioxidant Food Commodity: A Review. *Nutrients* 2015;7:8274–93.
19. Kishimoto Y, Taguchi C, Suzuki-Sugihara N, Saita E, Usuda M, Wang W, et al. The effect of consumption of egg on serum lipids and antioxidant status in healthy subjects. *J Nutr Sci Vitaminol* 2016;462:361–365.
20. Techakriengkrai T, Klangjareonchai T, Pakpeank-itwattana V, Sritara P, Roongpisuthipong C. The effects of ingestion of egg and low density lipoprotein (LDL) oxidation on serum lipid profiles in hypercholesterolemic women. *Songklanakarinn J Sci Technol* 2012;34(2):173–8.
21. Tanchoco CC, Infante LN, Rodriguez MP, Aquino MGC, Orense CL. The Effect of Egg Consumption on Lipid Profile Among Selected 30-60 Year-Old Filipino Adults. *Philippine J Sci* 2011;140(1):51–8.
22. Seuss-baum I. Nutritional Evaluation of Egg Compounds. In: Huopalahti R., López-Fandiño R., Anton M., Schade R. (eds) *Bioactive Egg Compounds*. Springer, Berlin, Heidelberg, 2007:114–44.
23. Miranda JM, Anton X, Redondo-Valbuena C, Roca-Saavedra P, Rodriguez JA, Lamas A, et al. Egg and egg-derived foods: Effects on human health and use as functional foods. *Nutrients* 2015;7:706–29.
24. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power, the FRAP Assay. *Analyt Biochem* 1996; 239(1):70–6.
25. Gutteridge JM, Wilkins S. Copper dependent hydroxyl radical damage to ascorbic acid; formation of thiobarbituric acid reactive products. *Fed Euro Biochem Soc Letters* 1982;137:327–30.
26. Roeschlau P, Bernt E, Gruber JW. Enzymatic procedure for cholesterol determination. *J Clin Chem Clin Biochem* 1974;12:403.
27. Tietz NW, Saunders WB. & Co. *Clinical Guide to Laboratory Tests*; 3rd Edition, Philadelphia, 1995:578–580.
28. Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from serum by precipitation with polyanions. *Scand J Clin Lab Invest* 1980;40:583–95.
29. Assman G, Jabs HU, Kohnert U, Nolte W, Schriewer H. LDL-C determination in blood serum following precipitation of LDL with polyvinyl sulphate. *Analytica Chimica Acta*, 1984;140:77–83.
30. Fisinin VI, Papazyan TT, Surai PF. Producing specialist poultry products to meet human nutrition requirements: selenium enriched eggs. *World's Poult Sci J* 2008;64:85–98.
31. Griffiths K, Aggarwal BB, Singh RB, Buttar HS, Wilson D, De Meester F. Food Antioxidants and Their Anti-Inflammatory Properties: A Potential Role in Cardiovascular Diseases and Cancer Prevention. *Dis* 2016;4:28.
32. DiMarco DM, Missimer A, Murillo AG, Lemos BS, Malysheva OV, Caudill MA, et al. Intake of up to 3 Eggs/Day Increases HDL Cholesterol and Plasma Choline While Plasma Trimethylamine-N-oxide is Unchanged in a Healthy Population. *Lipids* 2017;52(3):255–63.
33. Missimer A, DiMarco DM, Andersen CJ, Murillo AG, Vergara-Jimenez M, Fernandez ML. Consuming Two Eggs per Day, as Compared to an Oatmeal Breakfast, Decreases Plasma Ghrelin while Maintaining the LDL/HDL Ratio. *Nutrients* 2017;9(2):89.
34. Rader DJ, Alexander ET, Weibel GL, Billheimer J, Rothblat GH. The role of reverse cholesterol transport in animals and humans and relationship to atherosclerosis. *J Lipid Res* 2009;50(Suppl):S189–S94.
35. Rosenson RS, Brewer HB Jr, Davidson WS, Fayad ZA, Fuster V, Goldstein J, et al. Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circ* 2012;125(15):1905–19.
36. Sawrey-Kubicek L, Zhu C, Stevens A, Randolph J, Sacchi R, Steinberg F, et al. Whole Egg Increases High Density Lipoprotein-Cholesterol (HDL-C) Levels in Overweight Postmenopausal Women. *FASEB J* 2017;31(1):suppl 631.1.
37. Lemos BS, Medina-Vera I, Blesso CN, Fernandez ML. Intake of 3 Eggs per Day When compared to a Choline Bitartrate Supplement, down regulates Cholesterol Synthesis without Changing the LDL/HDL Ratio. *Nutrients* 2018;10(258):2–12.
38. Aljohia H, Dopler-Nelsona M, Cifuentesb M, WilsonTA. The consumption of 12 Eggs per week for 1 year does not alter fasting serummarkers of cardiovascular disease in older adults with early macular degeneration. *J Nutr Intermediary Metab* 2019;15:35–41.
39. Njike V, Faridi Z, Dutta S, Gonzalez-Simon AL, Katz DL. Daily egg consumption in hyperlipidemic adults—Effects on endothelial function and cardiovascular risk. *Nutr J* 2010;9:28.
40. Blesso CN, Andersen CJ, Barona J, Volek JS, Fernandez ML. Whole egg consumption improves lipoprotein profiles and insulin sensitivity to a greater extent than yolk-free egg substitute in individuals with metabolic syndrome. *Metab Clin Exp* 2013;62:400–10.
41. Flynn MA, Nolph GB, Flynn TC, Kahrs R, Krause G. Effect of dietary egg on human serum cholesterol and triglycerides. *Am J Clin Nutr* 1979;32(5):1051–7.
42. Rueda JM, Khosla P. Impact of Breakfasts (with or without eggs) on Body Weight Regulation and Blood Lipids in University Students over a 14-Week Semester. *Nutrients* 2013;5:5097–13.
43. Clayton ZS, Scholar KR, Shelechi M, Hernandez LM, Barber AM, Petrisko YJ, et al. Influence of Resistance Training Combined with Daily Consumption of an Egg-based or Bagel-based breakfast on risk factors for chronic diseases in healthy untrained Individuals. *J Am Coll Nutr* 2015;34:113–9.
44. Memisogullari R, Bakan E. Levels of ceruloplasmin, transferrin, and lipid peroxidation in the serum of patients with type 2 diabetes mellitus. *J Diabetes Complications* 2004;18(4):193–7.
45. Bansal AK, Bilaspuri GS. Impacts of Oxidative Stress and

- Antioxidants on Semen Functions. *Veterinary Med Int* 2011, <https://doi.org/10.4061/2011/686137>.
46. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 2010;4(8):118–26.
 47. Nahariah, Legowo AM, Abustam E, Hintono A, Bintoro P, Pramono YB. Endogenous Antioxidant Activity in the Egg Whites of Various Types of Local Poultry Eggs in South Sulawesi, Indonesia. *Int J Poult Sci* 2014;13(1):21-5.