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Incorporation of Nutritionally Important Fatty Acids into Eggs and Evaluation of "Bio-Omega-3" Eggs in Humans with Moderate Hypercholesterolemia

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Abstract: This study focuses on impact of consuming "Bio-omega-3" eggs on serum lipids profile of humans with a view to improve the nutrient intake of consumers in a palatable and acceptable commodity. Twenty four statin-treated volunteers with moderate hypercholesterolemia ranging in age from 25-40 years old having no diagnosed heart disease were selected from the community. Average increment in HDL-cholesterol (by 10.27%) and decrease in serum lipids concentration (by 14.18%) without significantly altering the circulating total cholesterol levels was found in human subjects after consumption of omega-3 fatty acids-enriched eggs as compared to control eggs. LDL-cholesterol, total protein, albumin and glucose concentration remained unchanged after consumption of experimental eggs ($p<0.05$). The results of the present study demonstrated that eggs that are enriched with omega-3 fatty acids may offer the public an alternative. With such modifications, the omega-3 fatty acids-enriched eggs may be more healthful than the conventional eggs.

Key words: Eggs, omega-3 fatty acids, humans, hypercholesterolemia, serum lipids

INTRODUCTION

Coronary heart disease is a major cause of mortality and morbidity, both in developed and developing countries. It accounts for approximately one third and one-quarter of all deaths among men and women, respectively (Sacks *et al*., 1996; Tasawar *et al*., 2011). Risk factors for incidence of cardiovascular diseases include hypertension, obesity, platelet adhesiveness, whole blood viscosity and elevated blood lipids. Serum parameters such as total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides collectively constitute blood lipids profile and their levels are particularly responsive to type and amount of dietary fat present in diet (Wilai and Donpichit, 2004; Dauqan *et al*., 2011).

Modern diets with increased consumption of highly saturated lipid contents fall well short of meeting healthy eating guidelines and are deficient in certain long chain polyunsaturated fatty acids. One of the emerging trends in human nutrition is that appropriately selected foods and their components can reduce disease burden (Milner and Craig, 2000). The biological effects of dietary omega-3 fatty acids in modulating numerous physiological functions have stimulated research to ensure adequate dietary supply of these fatty acids for humans and animals. The research efforts have been initiated to seek alternative food sources comprising the short- and long chain omega-3 fatty acids. In recent years, the recommendations have been made to increase the omega-3 fatty acids consumption and bioavailability by modifying the food composition through processing (Metcalf *et al*., 2003).

Chicken eggs are rich source of essential amino acids, fatty acids, minerals and vitamins. The lipids of egg yolk are exclusively made up of triglycerides (60-62%), phospholipids (30-33%) and less than 5% cholesterol. Saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids comprise 30-35, 40-45 and 20-25%, respectively of egg yolk lipids composition (Seuss-Baum, 2007; Shapira, 2008). However, a significant barrier to increase eggs consumption is associated with a rise in blood cholesterol levels (Smuts *et al*., 2003). The lipids of chicken eggs have been an area of primary consumer concern due to their relationship with coronary heart diseases, atherosclerosis, increasing stroke rate (Khelani *et al*., 2008), high proportion of gallstones, enhancing depression rate and as a consequence is deleterious to human health and life expectancy (Simopoulos and Salem, 1992). In the past decade, science has provided new information on the importance of the balance between omega-3 and omega-6 fatty acids in health and disease. The ability to enrich the eggs with polyunsaturated fatty acids has explored new awareness for a healthful food supply (Simopoulos, 2006).

Therefore, the objectives were to improve the nutritional properties of the chicken egg by the supplementation of extruded flaxseed in the laying hen diet and comparison

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evaluation of control and omega-3 fatty acids enriched experimental eggs on serum profile of human subjects with moderate hypercholesterolemia.

MATERIALS AND METHODS

Subjects: The eligibility in the present study program required willingness and ability to adhere with research program protocol. Twenty four statin-treated volunteers with mild hypercholesterolemia (serum cholesterol <5.2 mmol/L); ranging in age from 25-40 years old having no diagnosed heart disease were provided an hard copy of informed consent form after expressed an interest as potential participants in the program. The selected hypercholesterolemic patients were under physician-directed treatment with statin drug therapy for blood cholesterol reduction for more than two months. They were randomly allocated to two groups (Group A = 12 and Group B = 12). The participants were asked to complete surveys which include questions about dietary intake, physical activities, stress management and any life threatening illness that would prevent accurate physical and biochemical assessments. At the end of survey, the participants were asked to make an appointment for physical assessment on baseline and after three week of each study interval. The complete data of participant regarding contact information and method of recruitment was recorded. The participants were given free hand for refuse or answer any questions that were part of the survey. All the information provided by the participants, either on paper or in person, was kept confidential.

Diets and human serum bio-chemical profile assay: The volunteers were randomly assigned to 2 experimental treatments: a low-fat, self-selected diet with 6 eggs per week (control treatment); and a low-fat, self-selected diet with 6 eggs per week (omega-3 fatty acids-enriched eggs). This study was designed as a 3-wk randomized, two treatments, with a 2-week washout period between treatments and a two-week baseline period (a low-fat, self-selected diet with no eggs) before treatments. The patients were advised to avoid consumption of fish products or foods concentrated with polyunsaturated fatty acids two weeks prior to the study.

The blood drawn for day 0 and day 22 after an overnight 12 h fast was analyzed for serum parameters. The total cholesterol was determined by liquid cholesterol CHOD-POP method (Allain *et al*., 1974). The high-density lipoprotein concentration was analyzed by using HDL-cholesterol kits (Assmann 1979). Low density lipoprotein concentration was assay by following the mathematical expression described by McNamara *et al*. (1990). The triglycerides concentration was determined by liquid triglycerides GPO-PAP method (Annoni *et al*., 1982). The blood glucose, total protein and albumin concentration was measured by the method described by Thomas and Labor (1992), Josephson and Gyllensward (1975) and Webster (1974), respectively.

Statistical analyses: The statistical analyses for study parameters were conducted with Minitab® (Ver. 8.2.0) software and experimental results were expressed as means of triplicate measurements.

Experimental eggs: The eggs used in this study were produced by the Department of Poultry Science at the University of Agriculture, Faisalabad, Pakistan. Regular eggs were produced by feeding hens a standard diet. Omega-3 fatty acid-enriched eggs were produced utilizing standard diet substituted with 20% full-fat flaxseed meal extruded at barrel temperature 138.4-138.8°C.

RESULTS AND DISCUSSION

Eggs used in this study contained an average of 220 mg cholesterol per control egg and 208 mg per omega-3 fatty-acids-enriched egg. Consumption of both Regular Eggs and omega-3 Eggs resulted in higher cholesterol intake. Feeding laying hens with extruded flaxseed supplemented diet rich in omega-3 fatty acids resulted in accumulation of alpha-linolenic (510 mg/egg) and docosahexaenoic (80 mg/egg) acids in egg yolk mostly at the expense of monounsaturated fatty acids.

Effect on total cholesterol: Total cholesterol in plasma represents the total amount of circulating cholesterol in blood which can be used to assess the risk level of cardiovascular disease incidence. The total cholesterol level significantly differed among human subjects fed on different experimental eggs (Table 1). The highest serum total cholesterol in human subjects was observed when subjected to control experimental eggs. The human subjects group fed on omega-3 fatty acids-enriched experimental eggs possessed average serum total cholesterol 180.52±3.80 mg/dL. The rise in serum total cholesterol (5.7%) in human subjects fed on control experimental eggs is in close agreement with the studies documented in early research reports. Yannakopoulos *et al*. (1999) reported about plasma total cholesterol reduction in human serum after consumption of omega-3 modified experimental eggs. However, some studies have also demonstrated a non-significant change in serum total cholesterol level after consumption of DHA-enriched experimental eggs (Lewis *et al*., 2000; Gillingham *et al*., 2005).

The studies have demonstrated that the consumption of long chain omega-3 fatty acids-enriched modified eggs have beneficial effects on plasma total cholesterol as omega-3 fatty acids have cardioprotective benefits through changes in lipids and lipoproteins. In addition, omega-3 fatty acids especially alpha-linolenic, eicosapentaenoic and docosahexaenoic acids contribute benefits through their anti-inflammatory and anti-thrombotic effects. The increase in long chain

Table 1: Effects of experimental eggs on serum parameters in human subjects

Serum parameter	No. egg (reference)	Control eggs	[1]omega-3 fatty acids-enriched eggs
Total cholesterol (mg/ dL)	176.63±3.56	187.45±4.72***	180.52±3.80
HDL-cholesterol (mg/dL)	43.56±2.32	45.68±2.24	51.29±2.18***
LDL-cholesterol (mg/dL)	106.22±3.24	108.45±3.13	110.38±2.10
Triglycerides (mg/dL)	80.46±3.55	84.64±2.72	72.67±2.44***
Total protein (G/dL)	6.74±1.18	6.93±1.34	7.04±1.17
Albumin (G/dL)	3.92±1.34	4.16±1.28	4.23±1.39
Glucose (mg/dL)	84.63±4.72	96.38±3.92	87.28±4.64

***Means differ significantly from reference (p = 0.05);
[1]Obtained from laying hens fed on experimental diet supplemented with 20% extruded flaxseed meal

omega-3 fatty acids possesses inversely relationship with total cholesterol level and incidence of cardiovascular disease (Mozaffarian, 2005; Alshatwi and Alrefai, 2007). The saturated fatty acids from control experimental eggs increased the level of total cholesterol in human serum. The results of this study demonstrated that long chain omega-3 fatty acids-enriched experimental eggs can be incorporated in mild hypercholesterolmic human's diet without significant increase in plasma total cholesterol concentration.

Effect on HDL-cholesterol: The HDL-cholesterol comprises of a cholesterol core surrounded by an outer shell of phospholipids and apolipoproteins. HDL-cholesterol is also known as “good cholesterol” due to fighting against incidence of cardiovascular diseases through reverse cholesterol transport mechanism as extracting cholesterol particles from artery walls and disposing of them in bile through liver function (Lewis *et al*., 2000; von Eckardstein *et al*., 2001). The higher concentrations of HDL-cholesterol in blood stream are desirable while low levels are indicator of coronary heart disease (Poli *et al*., 2008). The level of HDL-cholesterol in human subjects varied significantly after three weeks consumption of omega-3 fatty acids-enriched experimental eggs (Table 1).

The results revealed an increasing HDL-cholesterol concentration when human subjects were fed omega-3 fatty acids-enriched experimental eggs. Yannakopoulos *et al*. (1999); Narahari *et al*. (2004) and Bovet *et al*. (2007) found rise in HDL-cholesterol level after consumption omega-3 fatty acids-enriched experimental eggs. Ferrier *et al*. (1995) and Legrand *et al*. (2010) reported non-significant changes in HDL-cholesterol level after control eggs consumption. The considerable changes in HDL-cholesterol levels are known about the effects of fatty acids present in the diet. The saturated fatty acids decrease HDL-cholesterol, whereas unsaturated fatty acids increase HDL-cholesterol level in serum (Omar and Tandon, 2009).

Effect on LDL-cholesterol: Low-density lipoprotein cholesterol is also known as “bad cholesterol” because of association with increased risk of cardiovascular diseases. LDL-cholesterol deposits cholesterol on artery walls and causes formation of a hard and thick substance known as cholesterol plaque. The LDL-cholesterol is a principal atherogenic component of total cholesterol and principal component of non-HDL cholesterol (Sacks *et al*., 1996; Tasawar *et al*., 2011). The LDL-cholesterol varied non-significantly in human subject groups who were subjected to consumption of experimental eggs (Table 1). Lewis *et al*. (2000) and Bovet *et al*. (2007) showed non-significant changes in LDL-cholesterol in human subjects after intake of omega-3 fatty acids-enriched eggs. However, a non-significant increase in LDL-cholesterol concentration may be attributed to very long-chain omega-3 fatty acids which partly increase conversion of VLDL to LDL (Huff *et al*., 1989; Chan *et al*., 2003).

Effect on triglycerides: A trend in decrease of serum triglycerides after consumption of experimental eggs has been depicted in Fig. 1. Consumption of 1320 mg cholesterol from control eggs plus regular home diet resulted in a significant increase in triglycerides concentration. In contrast, subjects who received 1248 mg cholesterol from omega-3 fatty acids-enriched eggs and regular meals showed significant decrease in triglycerides concentration. The serum triglycerides among human subjects decreased from 14.18-15.48% after consumption of omega-3 fatty acids-enriched experimental eggs as compared to control eggs. Oh *et al*. (1991); Sim and Jiang (1994) and Lewis *et al*. (2000) recorded a decrease in serum lipids after consumption of omega-3 fatty acids-enriched eggs. The consumption of omega-3 fatty acids-enriched eggs for two weeks also showed a noticeable decline (by 35%) of serum lipid levels (Ferrier *et al*., 1992). The difference in the magnitude of changes in triglycerides concentrations between the two groups may be attributable to the intake of a large amount of omega-3 fatty acids from omega-3 eggs rather than to the small difference in cholesterol intake between the groups. The mechanisms to lower serum triglycerides in peoples with hypertriglyceridemia have been associated to suppressing the synthesis of triglycerides and very low-density lipoprotein (VLDL) cholesterol in the liver (Illingworth *et al*., 1984). The metabolism of omega-3 fatty acids results in the less

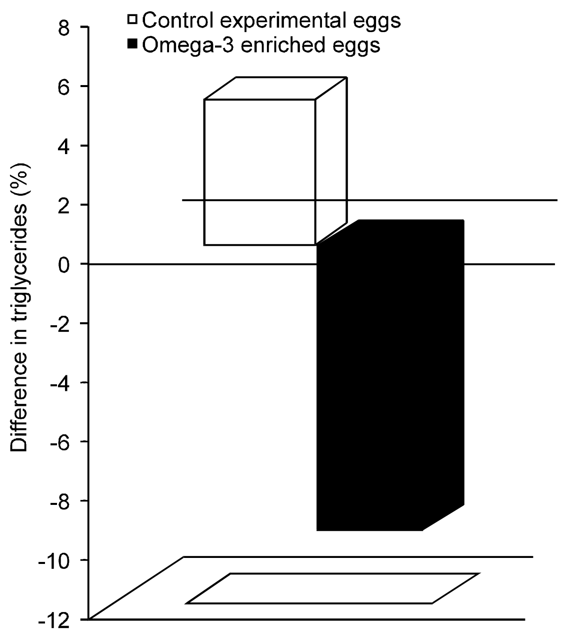

Fig. 1: Effect of experimental eggs consumption on percent serum triglycerides concentration as compared to reference (no consumption of eggs) in human subjects

production of pro-inflammmatory eicosanoids which encourage platelet aggregation. An increased intake of omega-3 fatty acids enriched diets has been recommended for reducing risk of coronary heart disease (Kromhout *et al.*, 1995).

Effect on serum total protein, albumin and glucose concentration: The human subject groups after consumption of control and omega-3 fatty acids-enriched experimental eggs showed non-significant changes in serum total protein, albumin and glucose concentration. The total protein, albumin and glucose concentration among human subjects fed on experimental eggs were found within the normal range. The consumption of eggs with increased contents of omega-3 fatty acids resulted in lower plasma glucose which can be associated to a reduced risk for cardiovascular mortality and diabetes (Ohman *et al.*, 2008).

Conclusion: The results of the present study demonstrated that eggs enriched with omega-3 fatty acids have a therapeutic use in managing serum HDL-cholesterol and triglycerides concentration without significantly altering the circulating total cholesterol levels in health conscious consumers. Novel omega-3 enriched designer egg is an affordable and acceptable food source of n-3 polyunsaturated fatty acids that must be include in diet pattern as nutritional food product and as a vector for the delivery of essential nutrients vital for human health. However, the serum cholesterol must be checked regularly to ensure that the consumers with hypertriglycemia or mild hypercholesterolemia symptoms are not responding to additional dietary cholesterol from omega-3 fatty acids-enriched eggs.

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