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## Abstract

This study was designed to compare the effects of the consumption of one omega-3 (n-3) polyunsaturated fatty acid (PUFA)-enriched egg or one conventional egg on serum lipids in physically active adults. A total of 12 adults (mean age  $33 \pm 7$  years, mean body mass index [BMI]  $24 \pm 3$ ) were recruited, and dietary treatments were randomly assigned. After a 2-week lead-in period (baseline), participants received each 4-week treatment in a crossover arrangement with a 4-week washout period between treatments. Participants completed a 3-day food record at baseline and during each treatment period. Food records were analyzed for carbohydrates, protein, total fat, saturated fat, monounsaturated fatty acids, PUFA, n-3 PUFA, and cholesterol using the Food Processor Nutrition and Fitness (ESHA) software. Blood samples were collected at the end of each treatment period and analyzed for total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, and n-3 PUFA. Dietary intake of  $\alpha$ -linolenic acid ( $1.196 \pm 0.116 \text{ g} \cdot \text{day}^{-1}$ ) and docosahexaenoic acid ( $0.087 \pm 0.013 \text{ g} \cdot \text{day}^{-1}$ ) and serum  $\alpha$ -linolenic acid ( $10.52 \pm 0.581 \text{ ng} \cdot \text{mL}^{-1}$ ) were higher during the n-3 PUFA-enriched egg treatment than during the conventional egg treatment ( $P < 0.05$ ). Serum triglycerides were higher ( $P < 0.05$ ) with n-3 PUFA-enriched eggs ( $86.54 \pm 5.84 \text{ mg} \cdot \text{dL}^{-1}$ ) than with conventional eggs ( $67.56 \pm 5.48 \text{ mg} \cdot \text{dL}^{-1}$ ). Daily consumption of one n-3 PUFA-enriched egg resulted in higher serum  $\alpha$ -linolenic acid and triglycerides in physically active adults than did daily consumption of one conventional egg.

**Keywords:** Polyunsaturated fatty acids,  $\alpha$ -Linolenic acid, Docosahexaenoic acid, Cholesterol, Triglycerides, Runners

## 1. Introduction

Runners are at risk for sudden death because of the stress on their hearts during bouts of vigorous exercise [1]. Dietary intakes of omega-3 polyunsaturated fatty acids (n-3 PUFA) are associated with a reduced risk of primary cardiac arrest and both sudden and non-sudden death [2, 3]. Furthermore, dietary n-3 PUFA demonstrate serum lipid lowering, antithrombotic, antiarrhythmic, and anti-inflammatory effects [4-7]. The precise mechanisms for the positive cardiovascular disease (CVD) affects are under investigation. However, it is known that the n-3 PUFA can prevent cardiac arrhythmias. Daviglus et al. reported that heart attack risk is decreased by 50% in populations that consume six or more servings of fish each month [3]. A higher dietary intake of  $\alpha$ -linolenic acid (LNA), an n-3 PUFA, is associated with reduced risk of fatal ischemic heart disease (IHD) in women [5]. The n-3 PUFA may also affect serum lipid levels. Stone [8] reported in a review that when fish oil is consumed and saturated fat intake is constant, serum LDL cholesterol (LDL-C) levels either do not change or may increase. Recently, it has been reported that supplementation of n-3 PUFA (approximately  $4 \text{ g} \cdot \text{day}^{-1}$ ) from fish oil could increase LDL-C by 5-10% and increase HDL cholesterol (HDL-C) by 1-3% [9]. Fish oil supplementation ( $3$  to  $5 \text{ g} \cdot \text{day}^{-1}$ ) can also have hypotriglyceridemic effects in individuals with marked hypertriglyceridemia ( $>750 \text{ mg} \cdot \text{dL}^{-1}$ ) [9].

Exercise has health benefits including a favorable modification in plasma lipids and lipoprotein concentrations [10, 11]. Observations from numerous investigators have shown that physically active individuals experience a higher cardiorespiratory fitness and improved lipid profile [12, 13]. However, there appears to be a marked inconsistency in the responsiveness of blood lipids to exercise training [14]. The most common observed change is an increase in HDL-C [13], however reductions in total cholesterol, LDL-C, and triglycerides are less frequently observed [14]. Leon et al. [14] indicated that exercise training alone or combined with dietary intervention provided inconsistent results in improvement of the blood lipid profile. Therefore, further investigations involving physically active adults and the effects of dietary intervention on blood lipids are needed.

New scientific studies have strengthened the conclusion that there is little relationship between conventional egg consumption and heart disease. One analysis indicated that a change of 100 mg in dietary cholesterol intake would cause blood cholesterol to change by an average of approximately  $2.5 \text{ mg} \cdot \text{dL}^{-1}$  [15], and another analysis indicated that the change would be  $2.2 \text{ mg} \cdot \text{dL}^{-1}$  [16]. Thus, a large change in cholesterol intake induces a small response in blood cholesterol. The main dietary determinant of blood levels of LDL-C is saturated fat intake [9], and a single conventional "large" egg provides only 2.2 g of saturated fat. In two studies, one involving more than 37,000 men in the United States and 8 years of follow-up, and the other involving more than 80,000 women in the United States and 14 years of follow-up, no association was found between egg consumption (up to one egg per day) and the risk of coronary heart disease [17].

The new Dietary Reference Intakes (DRI) for LNA, established by the National Academy of Sciences [18] are  $1.6$  and  $1.1 \text{ g} \cdot \text{day}^{-1}$  for male and female individuals, respectively,  $\geq 14$  years of age. Consumption of long-chain n-3 PUFA is low among certain popu-

lation subgroups in the United States, especially in people living in the Midwestern states and those who dislike fish [19]. Omega-3 PUFA-enriched eggs provide a dietary source of n-3 PUFA, particularly for persons who do not include fish in their diets.

The purpose of our study was to compare the effects of n-3 PUFA-enriched eggs or conventional eggs on dietary n-3 PUFA intake and on serum lipids in physically active adults. We hypothesized that the serum fatty acids would be improved with the consumption of the n-3 PUFA-enriched eggs.

## 2. Methods and materials

### 2.1. Subjects

A total of 14 adult volunteers were recruited through flyers placed at the local Young Men's Christian Association (YMCA), instructional talks during marathon and triathlon training group meetings, and word of mouth. This study was approved by the Institutional Review Board of the University, and all participants gave their written informed consent to participate. All of the participants were exercising regularly as members of a running training group sponsored by the local YMCA, and all were non-Hispanic white.

Participants completed an initial screening interview during which the requirements of the study were explained and the participants' weight, eating, and exercise habits were assessed. Persons being treated with eating disorders or depression, or those unable to eat eggs were excluded. Participants underwent a medical evaluation (performed by their personal physician) designed to identify contraindicators to participation in regular exercise or egg consumption. Subjects using medications known to affect serum lipids were excluded.

### 2.2. Study design

This study was designed as a 4-week randomized, two-treatment, two-period crossover, with a 4-week washout period between treatments and a 2-week baseline period before treatment. Dietary treatments were randomly assigned and applied after the 2-week baseline period. Each participant received each treatment and served as his/her own control [20]. The washout period before the second treatment was designed to minimize possible carryover effects. The two treatments were 1) six conventional eggs per week, and 2) six n-3 PUFA-enriched eggs per week added to volunteers' self-selected diet. Participants were instructed to consume one egg per day for 6 days and no eggs on day 7. Eggs enriched with n-3 PUFA can be produced by incorporating sources of these fatty acids into poultry diets [21, 22]. The n-3 PUFA-enriched eggs used in this study were produced by the addition of flaxseed to the hens' diet. Each egg provided 350 mg of n-3 PUFA. The conventional egg provided 60 mg of n-3 PUFA (Table 1). Volunteers were given 12 (1 dozen) of the appropriate eggs at the beginning and midpoint of each treatment period.

**Table 1.** Nutrient composition of omega-3-enriched egg and conventional egg (60-g large eggs)

	Omega-3-Enriched Egg	Conventional Egg
Calories	75	75
Protein (g)	6.0	6.0
Carbohydrate (g)	0.6	0.6
Total fat (g)	6.0	6.0
Saturated fat (g)	1.5	2.2
MUFA (g)	2.8	2.4
PUFA (g)	1.35	0.90
n-6 Fatty acids (g)	0.75	0.80
n-3 Fatty acids (g)	0.35	0.06
LNA (g)	0.25	0.04
DHA (g)	0.10	0.02
Cholesterol (mg)	180	210

Abbreviations as in text.

### 2.3. Diet

Volunteers completed a 3-day food record at baseline and during the last week of each treatment period (weeks 4 and 12). Participants received instructions from a dietitian on how to keep an accurate food record. Food records were analyzed using the Food Processor Nutrition and Fitness Software (ESHA Research, Inc., version 7.4, Salem, OR). Food items not in the database were substituted with a similar food. Food records were analyzed for energy (kilocalories), carbohydrate, protein, total fat, saturated fat, monounsaturated fat (MUFA), LNA, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and cholesterol.

### 2.4. Blood analysis

Fasting blood samples were drawn from participants at baseline and at the end of week 4 of each treatment period (weeks 4 and 12). Blood was drawn into 10-mL separator tubes; serum was analyzed at the University Health Center within 24 hours for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides. Serum fatty acids were analyzed using gas chromatography [23].

### 2.5. Exercise

Participants were required to complete 7-day exercise logs during the same week that they completed food records. Data were analyzed based on a percentage of total responses of type of primary exercise reported (running, biking, cross-training, or other exercise) as well as the duration of the primary exercise reported ( $\leq 30$ , 30–45, and  $\geq 45$  min  $\cdot$  day $^{-1}$ ).

### 2.6. Statistical analyses

Dietary intakes and serum lipids were analyzed with the SAS-GLM procedure using analysis of variance (SAS version 6.12, Cary, NC). Level of significance was set at  $\alpha = 0.05$ .

Exercise logs were analyzed over time with a simple frequency test. The  $\chi^2$  test was used to compare frequency of types of exercise during treatment periods. Data are reported as a percentage of total responses for the type and duration of the primary exercise reported by participants.

### 3. Results

#### 3.1. Subjects

A total of 14 volunteers began the study. One participant withdrew after learning that she was pregnant, and another participant withdrew from the study for personal reasons. The volunteers (eight men and four women) were  $33 \pm 7$  years (mean  $\pm$  SD), with a body mass index (BMI) of  $24 \pm 3$ .

#### 3.2. Dietary intake

Dietary intakes are presented in Table 2. The mean energy intake at baseline was approximately  $3000 \text{ kcal} \cdot \text{day}^{-1}$ , and there was no difference in mean energy intakes during the two treatments. Participants' diets provided 61% of total kilocalories from carbohydrate, 16% from protein, and 22% from fat.

Total fat intake and fat as a percentage of kilocalories was somewhat higher during the egg treatments than at baseline, but the difference was not significant. Total MUFA and PUFA intakes were highest during the omega egg treatment, but the difference from baseline or conventional egg treatment was not significant. Intakes of LNA and DHA were significantly higher ( $P < 0.05$ ) during the omega egg treatment than at baseline. Dietary cholesterol intake was significantly higher ( $P < 0.05$ ) during the egg treatments than at baseline.

#### 3.3. Serum lipid responses

Figure 1 shows responses of serum lipids to the egg treatments. Serum total cholesterol, LDL-C, and HDL-C did not change significantly with the dietary treatments. Serum triglycerides were higher ( $P < 0.05$ ) with n-3 PUFA-enriched eggs ( $86.54 \pm 5.84 \text{ mg} \cdot \text{dL}^{-1}$ ) than with conventional eggs ( $67.56 \pm 5.48 \text{ mg} \cdot \text{dL}^{-1}$ ).

Serum LNA was higher ( $P < 0.01$ ) with the n-3 PUFA egg treatment ( $10.52 \pm 0.58 \text{ ng} \cdot \text{mL}^{-1}$ ) than with conventional egg treatment ( $7.99 \pm 0.53 \text{ ng} \cdot \text{mL}^{-1}$ ). There was a numerical increase in serum DHA with n-3 PUFA-enriched eggs ( $24.14 \pm 0.99 \text{ ng} \cdot \text{mL}^{-1}$ ) compared to the conventional eggs ( $22.51 \pm 0.91 \text{ ng} \cdot \text{mL}^{-1}$ ), but this difference was not statistically significant.

#### 3.4. Exercise

Running was the most common type of activity (64% of the time spent exercising), followed by biking (20%) and cross-training (9%). There were no statistical differences for

**Table 2.** Dietary intakes in physically active adults ( $N = 12$ )\*

	Baseline†	Omega-3-Enriched Egg‡	Conventional Egg‡
Energy (kcal)	3015 ± 161§	2973 ± 161	2992 ± 153
Carbohydrate (g)	459 ± 33	422 ± 33	455 ± 31
(% of kcal)	61 ± 4.3	57 ± 4.4	61 ± 4.1
Protein (g)	119 ± 9	127 ± 9	109 ± 9
(% of kcal)	16 ± 1.1	17 ± 1.2	14.5 ± 1.2
Fat (g)	75 ± 8	90 ± 8	83 ± 8
(% kcal)	22 ± 2	27 ± 2	26 ± 2
Saturated fat (g)	25.4 ± 3.8	29.9 ± 3.8	27.5 ± 3.6
(% kcal)	7.5 ± 1.1	9.0 ± 1.1	8.2 ± 1.0
MUFA (g)	25.7 ± 3.4	30.1 ± 3.8	24.5 ± 3.2
(% kcal)	7.6 ± 1.0	9.1 ± 1.1	7.3 ± 1.0
PUFA (g)	12.6 ± 8.2	15.6 ± 8.2	13.4 ± 7.8
(% kcal)	3.7 ± 2.4	4.7 ± 2.4	4 ± 2.3
LNA (g)	0.851 ± 0.116 <sup>a</sup>	1.196 ± 0.116 <sup>b</sup>	0.993 ± 0.111 <sup>a</sup>
(% kcal)	0.25 ± 0.034	0.36 ± 0.035	0.29 ± 0.033
EPA (g)	0.007 ± 0.005	0.004 ± 0.005	0.005 ± 0.005
(% kcal)	0.002 ± 0.001	0.001 ± 0.001	0.001 ± .001
DHA (g)	0.026 ± 0.013 <sup>a</sup>	0.087 ± 0.013 <sup>b</sup>	0.048 ± 0.012 <sup>a</sup>
(% of kcal)	0.007 ± 0.003	0.026 ± 0.003	0.014 ± 0.003
Total n-3 (g)	0.887 ± 0.121 <sup>a</sup>	1.288 ± 0.121 <sup>b</sup>	1.057 ± 0.115 <sup>a</sup>
(% kcal)	0.26 ± 0.036	0.38 ± 0.036	0.31 ± 0.034
Cholesterol (mg)	209 ± 37 <sup>a</sup>	311 ± 37 <sup>b</sup>	331 ± 36 <sup>b</sup>

Abbreviations as in text.

\* Mean daily intake calculated from 3-day food records.

† Self-selected diet.

‡ One omega-3-enriched egg or one conventional egg added to self-selected diet per day.

§ Mean ± SD.

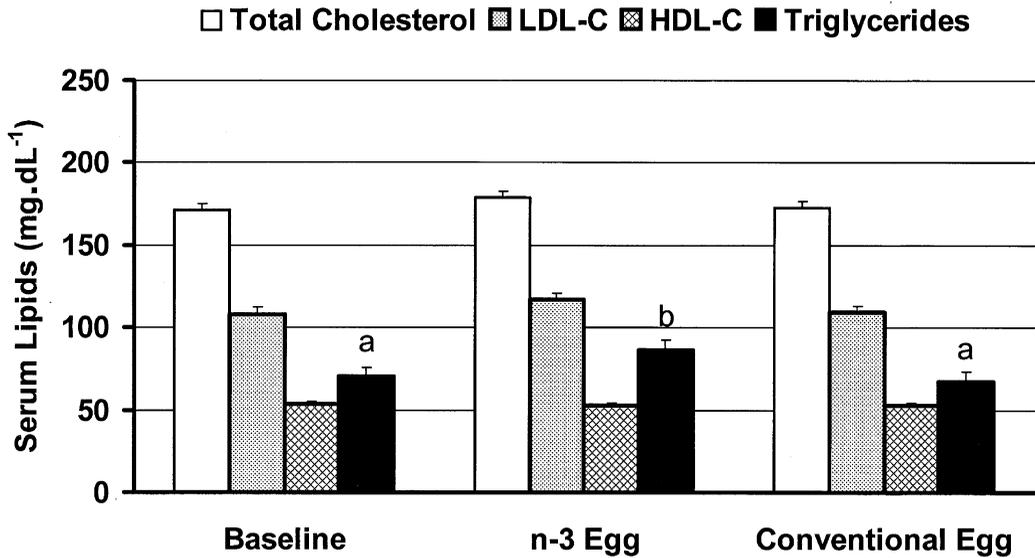
a,b,c Different superscript letters within the same row indicate significant difference ( $P < 0.05$ ).

the primary types of exercise (running, biking, cross-training, or other activity) that volunteers participated in during the treatments.

#### 4. Discussion

This research was conducted to examine the effects of n-3 PUFA-enriched eggs or conventional eggs on serum lipids in physically active adults. The addition of one n-3 PUFA-enriched egg per day significantly increased dietary intake of the n-3 PUFA, LNA and DHA, and increased serum LNA and triglycerides. Other serum lipids were not affected.

Dietary intake of n-3 PUFA is important in runners. Consumption of LNA, DHA, and total n-3 PUFA were significantly higher during the n-3 PUFA egg treatment compared to baseline intake. Total n-3 PUFA consumption during the n-3 PUFA egg treatment was within the DRI established by the National Academy of Sciences (1.1–1.6 g · day<sup>-1</sup>). Albert et al. [24] reported that higher serum levels of long-chain n-3 fatty acids are strongly associated with a reduced risk of sudden death among men without evidence of prior car-



**Figure 1.** Serum lipids of physically active adults ( $N = 12$ ) consuming self-selected diets (baseline) and with one omega-3 fatty acid-enriched egg or one conventional egg added. <sup>b</sup>Significantly different from baseline and conventional egg treatment (mean  $\pm$  SE,  $P < 0.05$ ).

divascular disease. Hu et al. [5] reported that higher dietary intakes of LNA are related to a lower risk of fatal ischemic heart disease in women. Increasing the intake of n-3 fatty acids by eating foods enriched with n-3 PUFA, such as the omega egg, is an intervention that could be applied at low cost and with little risk [24].

In our study, serum triglycerides increased when participants consumed n-3 PUFA-enriched eggs. This finding is in contrast to previous studies, which have shown a reduction or no effect of dietary n-3 PUFA on serum triglycerides [25]. In a previous study in our laboratory, serum triglyceride levels were reduced by 14% with the addition of two n-3 PUFA-enriched eggs in adults with hypercholesterolemia. Despite the increase, serum triglyceride levels in our subjects remained  $<200 \text{ mg} \cdot \text{dL}^{-1}$  and therefore were within the recommended desirable serum triglyceride level [9].

Energy, fat, saturated fat, and MUFA intakes of runners in this study were within guidelines established for Americans [9, 18, 26]. Dietary cholesterol consumption was higher during the egg treatment periods ( $311 \pm 37.3 \text{ mg} \cdot \text{day}^{-1}$  during the n-3 PUFA-enriched egg treatment and  $331 \pm 35.5 \text{ mg} \cdot \text{day}^{-1}$  during the conventional egg treatment) than during the baseline period ( $209 \pm 37 \text{ mg} \cdot \text{day}^{-1}$ ;  $P > 0.05$ ). The American Heart Association currently has no limitation on the number of eggs that a healthy person can eat; however, the recommendation for cholesterol intake is  $300 \text{ mg} \cdot \text{day}^{-1}$  [9]. The addition of one egg per day resulted in an increase in cholesterol intakes above baseline levels, although this did not affect serum cholesterol levels.

Serum lipids were analyzed to detect the risk for developing coronary heart disease (CHD) in a population that is physically active. Serum total cholesterol, LDL-C, and HDL-C did not change significantly in response to the egg treatments. This finding is consis-

tent with previously reported research [16]. Research suggests that n-3 PUFA have only a minimal effect on serum lipid levels [25]. Addition of two n-3 PUFA-enriched eggs per day to diets of individuals with hypercholesterolemia had no significant effect on serum lipid levels [21]. In contrast, Farrell [27] reported an increase in LDL-C with the addition of one n-3 PUFA-enriched egg per day compared to one standard egg per day.

Regular physical activity results in an increase in serum HDL-C concentration and is considered a protective factor against the development of CHD [14, 28, 29]. In our subjects, HDL-C levels were  $> 50 \text{ mg} \cdot \text{dL}^{-1}$  at baseline.

In conclusion, runners can increase their n-3 PUFA intakes using omega-3 fatty acid-enriched eggs. The addition of one n-3 PUFA-enriched egg per day significantly increased LNA and DHA intakes and serum LNA. This is important for runners who are at risk for sudden death.

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