Lipid profile after alpha-linolenic acid (ALA) enriched eggs diet: a study on healthy volunteers

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ABSTRACT

The correction of a subtle nutritional deficiency that may reduce the risk of a future chronic disease is indeed a challenge. One of the most intriguing current and future impacts on public health may come from a greater intake of omega-3 fatty acids such as alpha-linolenic acid (ALA). We investigated the effect of an increased amount of dietary a-linolenic acid (ALA) from enriched eggs on the lipid profile and inflammatory markers in healthy volunteers. 62 subjects were voluntary enrolled after they gave their informed consent. They were randomly assigned in either control or omega group. Control group consumed normal eggs while omega group consumed eggs enriched in omega 3 fatty acids. The content of ALA in omega 3 eggs was 5 times greater than that of control eggs. During the study, all subjects maintained their habitual diets except that egg consumption. Each subject had to consume 6 eggs a week during a 6-week period. Blood samples were collected at day 0 (baseline) and at the end of the study. Triglycerides, cholesterol, HDL, LDL cholesterol, ApoA, ApoB, CRP and fibrinogen were measured in serum samples. We compared the measured values of the biochemical parameters at baseline and after egg consumption both in control and omega group. Triglycerides were significantly reduced in omega group ($p=0.002$) after ALA enriched eggs consumption but not in control group. Fibrinogen level was significantly decreased ($p<0.001$) by ALA enriched eggs consumption whereas in control group there were no significant changes. No significant changes were found in the other parameters of the lipid profile or CRP. In conclusion, the alpha-linolenic acid dietary supplementation decreases tryglicerides and fibrinogen level. Alpha-linolenic acid enriched eggs can be considered as functional food with beneficial effects on human health.

Keywords: alpha-linolenic acid, eggs, diet, human health.

INTRODUCTION

An increasing number of physiological functions have been attributed to omega-3 fatty acids, including calcium movement inside and outside the cell,

muscle contraction and relaxation, clotting, regulation of digestive enzymes and hormones, fertility, cell division and growth, brain development. Omega 3 fats may protect against cardiovascular disease by lowering blood pressure and heart rate, reducing serum triglycerides, inflammatory markers (Bhatnagar D, 2003) and arrhythmias (Geelen A, 2004) and improving endothelial function, insulin sensitivity and plaque stability (Thies F, 2003).

The major dietary sources of omega-3 fatty acids in are fish, fish oil, vegetable oils, walnuts, wheat germ, and some dietary supplements (Das UN, 2006). The class of unsaturated fatty acids can be divided into monounsaturated and polyunsaturated fatty acids. Monounsaturated fatty acids (the primary constituents of olive and canola oils) contain only one double bond. Polyunsaturated fatty acids (PUFAs) (the primary constituents of corn, sunflower, flax seed and many other vegetable oils) contain more than one double bond.

Mammalian cells can introduce double bonds into all positions on the fatty acid chain except the n-3 and n-6 position. Thus, the short-chain alpha-linolenic acid (ALA, chemical abbreviation: 18:3n-3) and linoleic acid (LA, chemical abbreviation: 18:2n-6) are essential fatty acids. No other fatty acids found in food are considered 'essential' for humans, because they can all be synthesized from the short chain fatty acids.

Omega-3 and omega-6 fatty acids are not interconvertible in the human body and are important components of practically all cell membranes. Whereas cellular proteins are genetically determined, the polyunsaturated fatty acids (PUFA) composition of all cell membranes is to a great extent dependent on the dietary intake. Therefore appropriate amounts of dietary omega-6 and omega-3 fatty acids need to be considered in making dietary recommendations, and these two classes of PUFAs should be distinguished because they are metabolically and functionally distinct and have opposing physiological functions. Their balance is important for homeostasis and normal development. The metabolisms of fatty acids of the n-3 family and of the n-6 family (arachidonic acid [20:4(n-6)]) are of particular interest because of the biological actions of their metabolites (eicosanoids) in vivo. For example, eicosanoids derived from arachidonic acid are pro-inflammatory and pro-aggregator agonists, whereas those derived from n-3 PUFAs tend to inhibit platelet aggregation and be anti-inflammatory (Simopoulos AP, 1991).

The correction of a subtle nutritional deficiency that may reduce the risk of a future chronic disease is indeed a challenge. One of the most intriguing current and future impacts on public health may come from a greater intake of omega-3 fatty acids such as alpha-linolenic acid (ALA).

Objective: We investigated the effect of an increased amount of dietary a-linolenic acid (ALA) from enriched eggs on the lipid profile and inflammatory markers in healthy volunteers.

MATERIAL AND METHODS

Subjects and methods

62 subjects were voluntarily enrolled after they gave their informed consent. They were randomly assigned in either control (C), 31 subjects or omega group (O), 31 subjects. Control group consumed normal eggs while omega group consumed eggs enriched in omega 3 fatty acids. The content of ALA in omega 3 eggs was 5 times greater than that of control eggs.

During the study, all subjects maintained their habitual diets except that egg consumption. Each subject had to consume 6 eggs a week during a 6-week period.

Blood samples were collected in the morning after a 12-h fast at day 0 (baseline) and at the end of the study. Aliquots of serum were obtained and stored at -80 C.

Triglycerides, cholesterol, HDL, LDL cholesterol, ApoA, ApoB, CRP and fibrinogen were measured in serum samples.

Total cholesterol, high-density lipoprotein (HDL) cholesterol, Apo A, Apo B100, triglycerides and high-sensitive C-reactive protein were assessed using commercial enzymatic tests (Roche Diagnostics, Mannheim, Germany).

Fibrinogen was assayed by a quantitative method. Briefly, the blood sample taken on Na citrate 3.81% is centrifuged at 2000 rpm, 10 min. 2 ml of resulted plasma is mixed with 2 ml $CaCl_2$ 0.025 M and incubated at 37°C, in a water bath for 30 min. The resulted gel is washed with distilled water, ethanol, ether/acetone and is air dried for 24 h. The product is weighted and represents the fibrinogen quantity.

Intervention

The **ALA enriched egg** was obtained by the National Research - Development Institute for Animal Biology and Nutrition by feeding the lying hens with diets enriched in vegetal oils and flax seeds. To produce linolenic acid (omega-3 polyunsaturated fatty acid)-rich eggs the layers received a compound feed based on corn, wheat, soybean meal, corn gluten, full fat soy and flax seeds. Table 1 shows the quality indices of the compound feed.

Table 1

Metabolisable energy MJ / kg	11.85 ± 2.00
Crude protein, %	18.33 ± 2.00
Ether extractives, %	7.03 ± 2.00
Fiber, %	4.66 ± 2.00
Methionine, %	0.41 ± 2.00
Methionine + cystine, %	0.75 ± 2.00
Lysine, %	0.94 ± 2.00
Calcium, g%	3.6 ± 0.60
Available phosphorus, g%	0.34 ± 0.50
Linoleic acid, %	3.85 ± 0.50
Linolenic acid, %	0.46 ± 0.50

The subjects were randomized to control or ALA enriched eggs consumption. During the study, all subjects maintained their habitual diets except that egg consumption.

RESULTS AND DISCUSSION

Omega-3 enriched egg

The fat composition of the eggs used in this study is presented in figures 1 and 2.

The omega-3 egg has a higher content of saturated fatty acids (33.74% vs. 26.17%) and a reduced content of monounsaturated fatty acids (45.04% vs. 38.63%). The polyunsaturated fatty acids content is reduced by 1.18%. The alfa linolenic acid content was 4.74 times greater in omega-3 egg as compared to control egg (2.65 g/100 g fat vs. 0.56 g/100 g fat) as is shown in figure 3.

Fig. 1 Fat composition of control egg

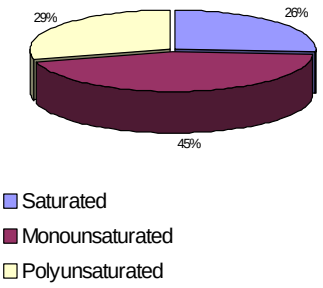

Fig. 2 Fat composition of omega-3 egg

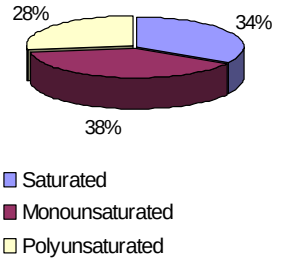

Fig. 3 Alfa linolenic acid(C18: 3n-3) content of egg yolk

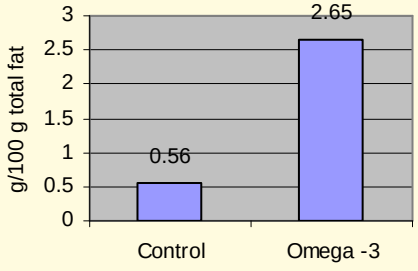

Fig. 4 Age distribution of the subjects

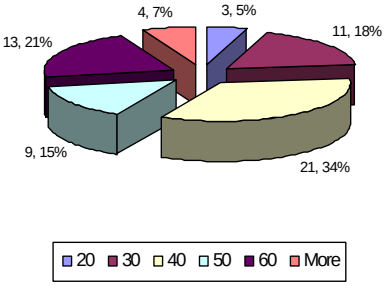

Table 2 presents the age and BMI of the two groups expressed as mean ±ES. There were not significant differences between study groups. Figure 4 shows the age distribution of the subjects.

Table 2 Subjects characteristics

	Control (n=31)	Omega 3 (n=31)
Age, yrs	37.9±5.55	38.83±4.07
BMI, kg/m2	22.70±1.21	23.67±1.06

Tables 3 and 4 show the values of the biochemical and inflammatory markers at the initial and final visit in control and omega-3 group, respectively.

We compared the measured values of the biochemical parameters at baseline and after egg consumption both in control and omega group. Triglycerides were significantly reduced in omega group ($p=0.002$) after omega-3 enriched eggs consumption but not in control group. Fibrinogen level was significantly decreased ($p<0.001$) by omega-3 enriched eggs consumption whereas in control group there were no significant changes. No significantly changes were found in the other parameters of the lipid profile or CRP.

Table 3. The effect of control egg on biochemical profile and inflammatory markers

Analyt	UM	Initial, M	±ES	Final, M	±ES	P
Creatinine	mg/dl	0.993	0.095	0.945	0.115	ns
Alkaline phosphatase	U/l	93.250	13.223	66.875	6.607	0.010
Calcium	mg/dl	9.300	0.182	9.119	0.219	ns
Cholesterol	mg/dl	200.250	8.976	187.750	13.667	ns
Triglycerides	mg/dl	96.125	19.224	95.125	20.390	ns
Uric acid	mg/dl	3.569	0.428	4.999	0.633	0.002
Magnezium	mg/dl	1.969	0.068	2.099	0.096	ns
AST	U/l	13.950	1.100	20.200	2.045	0.001
ALT	U/l	16.663	3.370	23.286	6.070	ns
Glucose	mg/dl	88.125	4.015	87.000	3.352	ns
UREA	mg/dl	34.550	7.190	32.671	10.629	ns
Phosphorus	mg/dl	3.611	0.218	3.863	0.220	ns
Total protein	g/dl	7.178	0.104	7.306	0.139	ns
HDL	mg/dl	55.463	2.529	57.400	3.804	ns
LDL	mg/dl	118.500	9.816	114.750	11.498	ns
apoB	mg/dl	86.100	6.756	82.813	6.314	ns
apoA1	mg/dl	166.438	4.295	150.600	4.800	0.008
CRP(C-reactive protein)	mg/dl	0.185	0.066	0.328	0.139	ns
Fibrinogen	mg/dl	393.62	46.24	456.12	53.65	ns

Table 4. The effect of omega-3 egg on biochemical profile and inflammatory markers

Analyt	UM	Initial, M	±ES	Final, M	±ES	P
Creatinine	mg/dl	0.820	0.027	0.832	0.023	ns
Alkaline phosphatase	U/l	60.300	4.182	65.300	3.004	0.040
Calcium	mg/dl	9.395	0.087	9.238	0.061	ns
Cholesterol	mg/dl	199.433	7.823	207.433	10.870	ns
Triglycerides	mg/dl	112.400	13.215	90.967	10.437	0.002
Uric acid	mg/dl	3.810	0.192	5.311	0.300	<0.001
Magnezium	mg/dl	1.936	0.037	1.979	0.043	ns
AST	U/l	16.300	1.199	22.433	1.552	<0.001
ALT	U/l	20.900	2.570	14.853	1.855	<0.001
Glucose	mg/dl	91.467	1.789	93.300	1.627	ns
UREA	mg/dl	25.690	1.800	33.493	1.424	<0.001
Phosphorus	mg/dl	3.559	0.081	3.754	0.104	0.019
Total protein	g/dl	7.350	0.062	7.526	0.074	0.005
HDL	mg/dl	51.013	2.263	51.907	2.244	ns
LDL	mg/dl	121.467	8.274	128.900	8.840	ns
apoB	mg/dl	89.837	5.756	89.013	6.170	ns
apoA1	mg/dl	155.443	4.112	154.980	5.000	ns
CRP(C-reactive protein)	mg/dl	0.378	0.13	0.378	0.147	ns
Fibrinogen	mg/dl	436	23.68	308.2	22.64	<0.001

DISCUSSIONS

Egg nutritional value can be easily influenced by the structure and composition of hen's food. Polyunsaturated fatty acids are essential nutrients for human health. The animal nutritionists modified the fat composition of the egg yolk and obtain an omega-3 (ω 3) enriched egg that has 2,65g/100g total fat acid α linolenic (ω 3) compared to control egg.

Correcting low levels of PUFAs and the balance between pro-inflammatory and anti-inflammatory molecules may aid in the prevention of cardiovascular and other inflammatory diseases (Das UN, 2008).

Previous studies showed that biochemical and behavioral abnormalities due to low-levels of omega-3 fatty acids are partially reversed by a dietary supplement as omega-3-rich egg yolk extracts (Bourre JM, 2004).

We tested the effect of consumption of this egg on healthy volunteers in a blind randomized study.

Our outcomes were the trygliceride levels, apo B and inflammatory markers, fibrinogen and C-reactive protein.

The results showed that omega-3 egg consumption on a dose of 6 eggs a week for 6 weeks significantly decreased the tryglicerides level of the intervention group. No significant changes were found in Apo B. We also found

a significant reduction of fibrinogen level in the omega-3 group, but no effect on CRP.

CONCLUSIONS

The omega-3 enriched egg (2.65 g/100 g fat) consumed in a dose of 6 eggs a week for 6 weeks significantly reduced the tryglicerides and fibrinogen level in healthy volunteers. These results confirm our hypothesis and make the ω 3 egg a functional food with benefic effects on human health.

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REFERENCES

Bhatnagar D, Durrington PN. Omega-3 fatty acids: their role in the prevention and treatment of atherosclerosis related risk factors and complications. Int J Clin Pract, 2003;57:305-14.

Geelen A, Brouwer IA, Zock PL, Katan MB. Antiarrhythmic effects of n-3 fatty acids: evidence from human studies. Curr Opin Lipidol 2004;15:25-30.

Thies F, Garry JM, Yaqoob P, Rerkasm K, Williams J, Shearman CP, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet 2003;361:477-85.

Das UN, Essential fatty acids: Biochemistry, physiology and pathology, Biotech J., 2006, I(4):420-439

Simopoulos AP. Omega-3 fatty acids in health and disease and in growth and development. Am J Clin Nutr 1991; 54:438–63.

Das UN, Can endogenous lipid molecules serve as predictors and prognostic markers of coronary heart disease?, Lipids in Health and Disease, 2008, 7:19.

Bourre JM, Roles of unsaturated fatty acids (especially omega-3 fatty acids) in the brain at various ages and during ageing, 2004, J. Nutr. Health Aging, 8(3):163-174.