# Biochemical Effects of Consumption of Eggs Containing Omega-3 Polyunsaturated Fatty Acids

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## Abstract

Today, eggs with an increased content of ω-3 fatty acids are available but there are few publications on the effects of consumption of such eggs on the lipoproteins and acute phase markers in humans. The aim of the present study was to evaluate the effects of consumption of standard eggs and ω-3 enriched eggs on lipoproteins, glucose and inflammation markers. Nineteen healthy volunteers consumed one extra egg per day of either standard eggs or ω-3 enriched eggs in a double-blind, cross-over study. The duration of each period was 1 month. The effects of the different egg diets on apolipoprotein A1 and B (Apo A1 and B), lipoprotein (a), creatinine, cystatin C, C-reactive protein, serum amyloid protein A, interleukin 6, triglycerides, glucose, total-, high-density lipoprotein and low-density lipoprotein cholesterol concentrations were analyzed. Addition of one regular egg per day to the normal diet had no negative impact on blood lipids or inflammation markers. Consumption of ω-3 enriched eggs resulted in higher levels of ApoA1, lower ApoB/ApoA1 ratio and lower plasma glucose. These effects have been associated in previous studies with a reduced risk for cardiovascular mortality and diabetes.

## Introduction

Cardiovascular disease (CVD) is one of the major causes of death in the Western world (1). The CVD risk is influenced by several factors including our diet (2). During the past decades, reduction in fat intake has been the main target of national dietary recommendations to decrease risk of CVD. Despite a reduction in dietary fat intake in the U.S., the prevalence of obesity and type 2 diabetes has grown dramatically (3,4). However, several studies indicate that the types of fat consumed have a more important role for CVD risk than the total amount of fat in the diet and that replacing saturated fat with unsaturated fat is more effective than simply reducing total fat consumption and that an increased intake of ω-3 fatty acids substantially lowers the risk of cardiovascular mortality (5).

There is also a strong link between lipids, lipoproteins and inflammation (6).

This has become one of the main themes in the pathogenesis of CVD over the past decades. It is proven that fat-tissue synthesizes tumour necrosis factor α (TNF-α) and interleukin-6 (IL-6) (7), cytokines that are involved in the inflammatory process and that stimulate C-reactive protein (CRP) and serum amyloid protein A (SAA) synthesis, but also play a major role in plaque formation in the vascular tree (8). Also, the widely used statins reduce low-density lipoprotein (LDL) and increase high-density lipoprotein (HDL) cholesterol (9, 10) therewith reducing inflammation and thus the acute phase response (11).

In 1972, the American Heart Association published dietary recommendations for the public, limiting the egg consumption to no more than three eggs per week. To this date, this is the only food-specific restriction listed in the dietary guidelines (12). Such a recommendation assumes that all eggs are the same, which is not correct. It has been shown that it is possible to strongly modulate the lipid content of consumer eggs by modifying the feed given to the hen (13).

Today, eggs with an increased content of ω-3 fatty acids are available in several markets but there are few publications on the effects of consumption of such eggs on the lipoproteins and acute phase markers in humans. Eggs are a relatively inexpensive protein source with a very good amino acid composition for humans (14). The very high nutritional density makes the egg well suited for elderly individuals who often have a low nutritional status. A poor nutritional status is associated with an increased rate of complications and mortality during hospital care (15, 16).

The aim of this study was to evaluate the effects of consumption of standard eggs and ω-3 enriched eggs on lipoproteins, glucose and inflammation markers. The study population consisted of healthy individuals over the age of 45 years.

## Materials and methods

### Study population

The study was performed in 2005 and included 19 healthy Swedish Caucasian volunteers (8 males and 11 females). The study design was a double-blind, cross-over study with half of the volunteers starting with ω-3 eggs and the other half with standard eggs. The daily study intake was one egg and the duration of each period was 1 month. After the end of the first study period the study subjects changed to the other egg type for another month. Apart from the addition of one egg per day, the volunteers had no other diet restrictions and continued with their normal diet. Inclusion criteria for the study were: healthy individuals with an age over 45 years and without medication that could influence inflammatory parameters or blood lipids. Exclusion criteria were known malignancy. The volunteers were informed not to use estrogens, steroids (except inhalation steroids), NSAID, statins or fibrates. The study was approved by the Ethics Committee at Uppsala University and all participants gave their informed consent.

## Eggs

The eggs were analyzed prior to the study by AnalyCen, Lidköping, Sweden. The ω-3-eggs were from hens that had been on a feed containing rapeseed oil. The test results showed a higher content of ω-3 fatty acids in the ω-3-eggs (9.3 % alpha-linolenic acid (ALA); 0.2 % eicosapentaenoic acid (EPA); 1.5 % docosahexaenoic acid (DHA)) than in the regular eggs (0.7 % ALA; 0 % EPA; 1.3 % DHA). The non ω-3-eggs had higher contents of ω-6 fatty acids (1.8 % arachidonic acid (AA); 17.4 % linoleic acid (LA)) than the ω-3 eggs (0.2 % AA; 13.7 % LA).

## Sample collection

Blood samples were drawn from an antecubital vein in the morning after 12-hour (overnight) fasting and collected in vacutainer tubes without additive (for fatty acid analysis) and lithium-heparin (LH PST™ II, BD Vacutainer Systems, Plymouth, UK). Blood samples were collected immediately prior to the start of the study (baseline) and at the end of each period. The samples were immediately centrifuged and frozen at -20°C until analysis.

## Laboratory assays

All plasma samples were analyzed for the following parameters: Apolipoprotein A1 (total CV 0.9% at 2.25 g/L), Apolipoprotein B (total CV 1.2% at 1.73 g/L), lipo-protein (a) (total CV 5.6% at 265 mg/L), creatinine (total CV 4.8% at 94 µmol/L), cystatin C (total CV 4.2% at 0.96 mg/L), C-reactive protein (total CV 0.9% at 22 mg/L), glucose (total CV 1.0% at 4.4 mmol/L), total cholesterol (total CV 0.5% at 5.7 mmol/L), HDL-cholesterol (total CV 0.8% at 2.2 mmol/L), LDL-cholesterol (total CV 1.2% at 2.3 mmol/L) and triglycerides (total CV 1.4% at 2.1 mmol/L). These assays were performed using an Architect Ci8200® analyzer (Abbott, Abbot Park, IL, USA). SAA was measured by nephelometry (Dade Behring, Deerfield, IL, USA) using a Behring BN ProSpec® analyzer (Dade Behring). The total analytical imprecision of the method was 5.9% at 12.8 mg/L. IL-6 was measured using an enzyme-linked immunosorbent assay (ELISA) method (R&D Systems, Minneapolis, MN, USA). The total analytical imprecision of the IL-6 method was less than 7 %.

All assays were performed independently without prior knowledge of patient data.

## Determination of the composition of fatty acids in serum phospholipids

Fatty acids were analyzed as methyl esthers on a HP 5890 series II gas chromatograph equipped with a FID and a HP-FFAP capillary column (30 m x 0.25 mm x 0.25 µm) (17).

## Statistical calculations

All calculations were performed with the statistical software package (StatView, SAS Institute Inc., Cary, NC, USA). Differences between groups were tested with Kruskal-Wallis using relative values (%) at the end of each study period in relation to the sample collected just before the initiation of each study period. P-values < 0.05 were regarded as statistically significant throughout the study.

# Results

## Consumption of regular eggs

No significant changes were observed for any of the studied parameters when adding a regular egg to the normal diet (Table 1).

## Consumption of ω-3 eggs

Consumption of ω-3 eggs resulted in significant increases in ApoA1 (p = .017) and significant decreases in ApoB/ApoA1 (p = .019) and plasma glucose (p = .018) (Figures 1–3). Consumption of ω-3-eggs resulted in an almost significant increase in SAA.

*Table 1.* Mean values and SEM for the analytes at the beginning and end of each consumtion period

| Analyte | Before omega-3 eggs | SEM | After omega-3 eggs | SEM | Before ordinary eggs | SEM | After ordinary eggs | SEM |
|---|---|---|---|---|---|---|---|---|
| SAA, mg/L | 5.52 | 1.10 | 6.70 | 1.10 | 6.03 | 1.40 | 5.60 | 1.10 |
| Cholesterol, mmol/L | 6.25 | 0.30 | 6.22 | 0.30 | 6.25 | 0.30 | 6.22 | 0.30 |
| HDL-C, mmol/L | 1.43 | 0.10 | 1.49 | 0.10 | 1.45 | 0.10 | 1.48 | 0.10 |
| LDL-C, mmol/L | 3.92 | 0.24 | 3.85 | 0.26 | 3.89 | 0.24 | 3.88 | 0.24 |
| ApoA1, g/L | 1.62 | 0.05 | 1.68 | 0.06 | 1.67 | 0.06 | 1.66 | 0.05 |
| ApoB, g/L | 1.15 | 0.06 | 1.12 | 0.06 | 1.13 | 0.06 | 1.14 | 0.06 |
| Triglycerides, mmol/L | 1.44 | 0.16 | 1.42 | 0.12 | 1.48 | 0.14 | 1.45 | 0.16 |
| Cystatin C, mg/L | 0.69 | 0.05 | 0.72 | 0.04 | 0.76 | 0.04 | 0.75 | 0.05 |
| CRP, mg/L | 3.00 | 1.02 | 2.84 | 0.73 | 2.68 | 0.98 | 2.96 | 1.04 |
| Glucose, mmol/L | 5.40 | 0.22 | 5.21 | 0.17 | 5.19 | 0.19 | 5.43 | 0.21 |
| IL-6, ng/L | 17.80 | 8.34 | 17.40 | 8.50 | 17.10 | 8.28 | 18.26 | 8.41 |
| LDL/HDL | 2.92 | 0.26 | 2.71 | 0.22 | 2.84 | 0.23 | 2.80 | 0.25 |
| ApoB/ApoA1 | 0.72 | 0.05 | 0.68 | 0.04 | 0.69 | 0.04 | 0.70 | 0.05 |
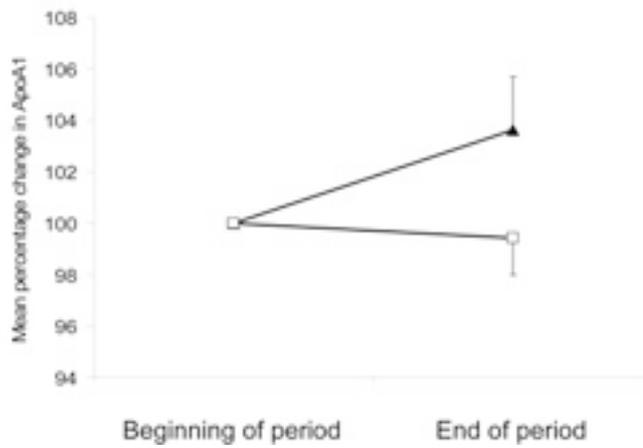
*Figure 1.* Change in percentage in ApoA1 during consumption of regular eggs (□) and ω-3-enriched eggs (▲) in relation to values at the beginning of the period. The results are presented as mean and standard deviation for each group.
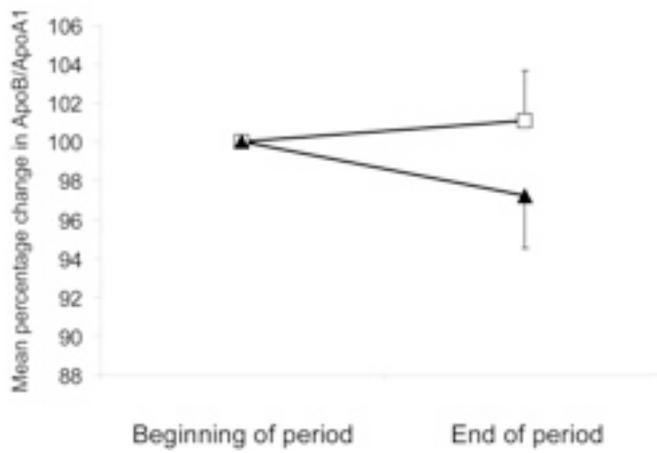


*Figure 2.* Change in percentage in ApoB/ApoA1 during consumption of regular eggs (□) and ω-3-enriched eggs (▲) in relation to values at the beginning of the period. The results are presented as mean and standard deviation for each group.
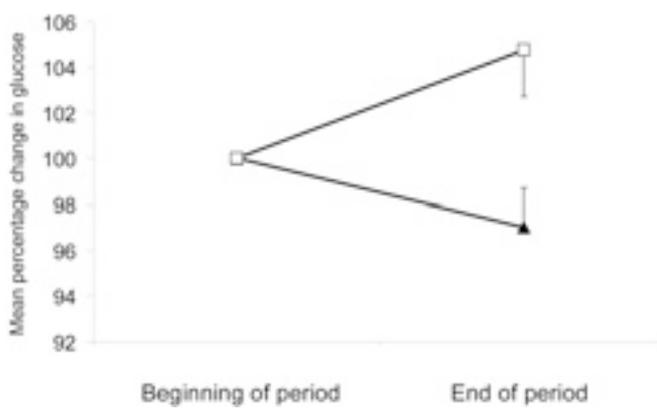


*Figure 3.* Change in percentage in glucose during consumption of regular eggs (□) and ω-3-enriched eggs (▲) in relation to values at the beginning of the period. The results are presented as mean and standard deviation for each group.

## Fatty acid composition in serum phospholipids

Consumption of ω-3 eggs resulted in significant increase in C 18:3 ω-3 (alpha-linolenic acid) (Figure 4), while consumption of regular eggs resulted in significant increase in C 20:4 ω-6 (arachidonic acid).

## Discussion

Despite the long-standing interest in the diet-heart hypothesis, the number of cohort studies that have directly addressed associations between dietary fat intake and risk of CHD is surprisingly small and the results are not consistent (5). It is often difficult to change the diet of large populations. Despite the recommendations of the American Heart Association to limit the egg consumption to no more than three eggs per week, the mean egg consumption in the US has remained significantly higher. It is much easier to change the composition of daily food products e.g. fortification of food products with vitamins or minerals. Similarly, one could probably exchange one type of egg for another with a better lipid composition.

In this study we used ω-3 enriched eggs from hens fed rapeseed oil. ω-3 Enriched eggs are obtained by giving the layer hens an ω-3 enriched food. Such a feed usually contains fish, rapeseed or flax products. The egg fatty acid composition varies between hens fed fish, rapeseed or flax (18). In metabolic studies, different classes of saturated fatty acids have different effects on serum lipid and lipoprotein levels (19).

We studied the effect of addition of one egg a day of either standard eggs or eggs enriched with ω-3 fatty acids to the normal diet in a double-blinded cross-over study. The volunteers were informed not to change their normal diet apart from adding an extra egg to the diet. The average egg consumption in Sweden is approximately 0.7
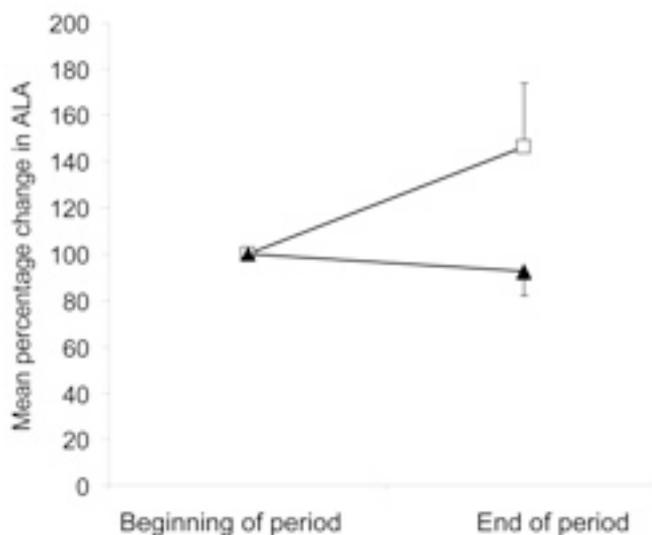


Figure 4. Change in percentage in alpha-linolenic acid (ALA) during consumption of regular eggs (□) and ω-3-enriched eggs (▲) in relation to values at the beginning of the period. The results are presented as mean and standard deviation for each group.

eggs per day. The larger part of this egg consumption is not as whole eggs. It is thus very difficult to accurately define the daily egg consumption in a study population. We thus chose a cross-over setup to compare the effect of consuming the two egg types. Analysis of C 18:3 ω-3 (alpha-linolenic acid) was included in the study as an indicator of compliance. Consumption of ω-3 eggs resulted in a significant increase in alpha-linolenic acid. We found no significant effect on total cholesterol or triglycerides either in comparison with baseline values or between the two groups of eggs. This is in agreement with previous reports that egg consumption has a very limited effect on total cholesterol in healthy individuals. We found a significant increase in ApoA1 and a significantly improved ApoB/ApoA1 ratio in the group that consumed ω-3 enriched eggs. Apolipoproteins and the ApoB/ApoA1 ratio are considered to be superior as CVD risk markers to the traditional blood lipid markers (total cholesterol, LDL- and HDL-cholesterol) (20–22). They have also been shown to be better markers for treatment effects with statins (23, 24). There was a significant increase in SAA but not in IL-6 or CRP in the group that consumed ω-3 enriched eggs. The same group also showed a significant increase in Apolipoprotein A1, which is a marker for HDL-cholesterol. SAA, but not CRP, is bound to the HDL particles. Theoretically, the SAA increase could thus be secondary to the ApoA1 increase and not due to an inflammatory response. We measured the fatty acid composition of serum phospholipids, which reflect weeks-to months qualitative dietary intake of fatty acids (17). We found increased levels of C18:3 ω-3 in the subjects that consumed ω-3 enriched eggs and significantly lower glucose levels. This is in agreement with previous reports on increased insulin sensitivity in rats fed alpha-linolenic acid (25, 26).

In conclusion, addition of one regular egg per day to the normal diet had no negative impact on blood lipids or inflammation markers. Consumption of ω-3 enriched eggs resulted in higher levels of ApoA1, lower ApoB/ApoA1 ratio and lower plasma glucose concentrations. All these changes have in previous studies been associated with a reduced risk for cardiovascular mortality and diabetes.

## Acknowledgements

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