Antioxidative egg: high MUFA:PUFA and antioxidants reduce LDL oxidation

N. SHAPIRA

Stanley Steyer school of health professions, Tel-Aviv University, Ramat-Aviv, 69978, Israel
nivnet@inter.net.il

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Abstract

High egg consumption has been long suggested to affect plasma cholesterol levels but only recently suggested also to increase LDL oxidation. As oxidative modification of low-density lipoproteins (LDL) is considered a patho-physiologically relevant process in atherogenesis, a question raised regarding egg composition Vs. LDL oxidation. The effect of 2 regular eggs/day for 3 weeks on blood cholesterol, LDL and LDL oxidation was tested on two groups of 20 healthy adults each. Egg consumption significantly increased LDL oxidation, in average by 20% in the two groups, as shown by reduced lag time for oxidation, whereas plasma and LDL cholesterol were not increased significantly.

The possibilities of reducing the egg-induced increase in LDL oxidation by modifying egg composition, i.e. by increasing antioxidants, and MUFA:PUFA ratio was tested in different group of healthy adults that fed for periods of 3 weeks each, 2 regular eggs/ day, 2 high-antioxidants (HAOX) eggs enriched with vitamin E and carotenoids, and 2 HAOX eggs, that are also high in MUFA and MUFA:PUFA ratio (HAOX-HMUFA eggs). A significant reduction in LDL oxidation (p<0.01) was observed following 2 HAOX-HMUFA eggs/day, but not following HAOX eggs.

The significant physiological effect of eggs high in antioxidants, MUFA and MUFA:PUFA ratio HAOX-MUFA eggs, in spite of the minor impact on daily nutritional intake i.e. only 1-2 g extra MUFA from 2 eggs, suggests that the egg's fatty acid composition has a functional effect beyond its nutritional significance. Thus the composition of the egg should be considered. Especially with regard to LDL oxidation and its health implication. This strongly supports the notion that not all the eggs are the same and the advantage of applying functional health considerations, to the egg industry.

Introduction

Based on the notion that high cholesterol intake may increase plasma cholesterol (Grundy and Denke, 1990) and risk for coronary vascular disease, especially in cholesterol responders (Herron et al., 2004), the recommended daily intake was restricted to 300 mg/day and egg consumption to less than 1 egg/day. The egg restriction strategy was recently questioned generating a slight increase in consumption. This mostly due to the recognition of the small effect of dietary cholesterol on plasma levels, in average 2.2mg per 100mg/day (Mcnamara, 2000) as well as accumulated data which suggests that it is possible to maintain proper cholesterol levels with moderate egg consumption (Hu et al., 1999; Hu et al., 1997; Ascherio et al., 1996; Esrey et al., 1996; Clarke et al., 1997). Increasing egg consumption urges the question of the quality and functional effects of the egg on health risks, i.e. as related to the risk of LDL oxidation.

The possibility of modifying egg's fatty acid composition via changes in laying hen diet has long been known. Most of the egg modification efforts were directed to reduce blood cholesterol, i.e. by reducing saturated fatty acid content (Garwin, 1992) and to increase the heart friendly n-3 fatty acids, i.e. by fish oil and/or linseeds (Jiang and Sim, 1993; Weill et al., 2002; Smuts et al., 2003). But the recently suggested effect of eggs on increased LDL oxidation (Levy et al., 1996; Shapira, 1995 unpublished) was not yet approached and no effort was yet addressed to reduce PUFA and increase MUFA in the egg for the purpose of reducing LDL oxidation.

Oxidized LDL is toxic to endothelial cells, decreases vasodilatation and is accumulated by macrophages in an uncontrolled manner, thereby generating foam cells (Tsimikas et al., 1999) and fatty streaks in arterial walls. The susceptibility of the LDL to oxidation is influenced by the balance...
between the antioxidative capacity, i.e. vitamin E, Carotenoids, and proxidative substances like PUFA. The oxidation of LDL PUFA starts when most of the antioxidant defence has been lost. LDL PUFA’s are readily oxidized and increase the proinflammatory processes, which further facilitates LDL oxidation. Replacement of PUFA by MUFA reduces LDL oxidation, monocyte chemotaxis, adhesion to endothelial cells and platelet activation (Lee et al., 1998; Tsimikas et al., 1999).

This paper presents results showing the increased LDL oxidation following normal egg consumption, and describes an egg that was designed to reduce the egg induced LDL oxidation. The results are discussed with regard to the unexpected functional effect of the egg’s fatty acid composition on LDL’s oxidizability, which is far greater than their nutritional significance to the daily diet.

Subjects, methods and results

EXPERIMENT 1: EFFECTS OF REGULAR EGGS ON LDL OXIDATION

Free-living healthy young adults, in 2 groups (n=20 each), 30-50 y, BMI 23-26Kg/m², non-smokers, not using any medications, consuming a standard “Israeli” diet, were asked to add 2 eggs/day on a cross over design of 3 weeks periods, with eggs (2/day) and without eggs. Neither cholesterol nor LDL levels significantly increased (p= 0.54 and 0.66, for Total Blood Cholesterol and p=0.78 and 0.63 for total plasma and LDL cholesterol) in the two groups, but the lag time for LDL oxidation was significantly reduced (Figure 1), in average by 20-25% in both groups (Shapira, in press). This confirms previous findings regarding the major effect of normal egg consumption on increased LDL oxidation (by 37%, p<0.01) (Levy et al., 1996).

EXPERIMENT 2: ANTIOXIDATIVE MODIFICATION OF EGGS VS. LDL OXIDATION

Egg production and composition

Free living healthy adult subjects (n=17) 30-50 y, BMI 23-26Kg/m², non-smokers not using any medications consuming a standard “Israeli” diet (26% fat), added 2 regular or modified eggs/day, for 3 consecutive periods of 3 weeks each. Laying pullets (Yarkon, PUB) 4.5 weeks of age in 3 group were fed 1)standard feed mixture (regular eggs), 2) standard feed mixture enriched with vitamin E and biotene total premix (Eggland Best, Philadelphia,USA) (HAOX eggs) and 3) low-PUFA feed, enriched with biotene total premix, have reduced linoleic acid and increased oleic acid content (HAOX-HMUFA eggs).

Chemical analyses

Egg vitamin E , iodine and fatty acids were determined on pooled samples of three eggs per group. Egg iodine- according to the Food Chemical Codex method. Egg cholesterol - according to the Liebermann-Burchar Reaction. Fatty acid profile in feeds, yolks and plasma was determined by gas chromatography after lipid extraction, saponification and methylation (Folch et al., 1957; Aviram et al., 1986). Plasma vitamin E, A and carotenoids concentrations were determined using standard HPLC methods (Lowery, 1951). LDL was separated, incubated with CuSO₄ and oxidation analysed by conjugated diens absorbance at 234 nm (Esterbauer, 1989). Student's paired t tests were performed for all statistical analyses.
Egg composition vs. plasma levels

Vitamin E in the HAOX eggs increased by 5-8 compared to control eggs (from 17.4-22.9 to 82.5-112.7 mg/kg egg, p<0.01) and iodine by 2.1-2.5 (from 2.0-3.8 to 5.2-7.9 μg/g, p<0.01). Fatty acid analysis (Table 1) show that palmitic, oleic and linoleic acids are the main fatty acids in the egg. Due to reduction of linoleic acid in the chicken feed, the linoleic acid in the egg was reduced by 43% to 12.4% (p<0.01); the oleic acid increased by 33%, from 34.1-37.4% to 47.3% (p<0.01), corresponding to the decrease of linoleic acid. The oleic:linoleic acid (18:1/18:2) ratio increased by more than 2 times from 2.0 and 1.6 in control and HAOX, to 3.8 in the HAOX-HMUFA eggs, respectively (Shapira and Pinchasov, 1995 unpublished).

Vitamin E, A and carotenoids increased significantly (p<0.01) by 40% -50% following 3 weeks on 2 HAOX eggs. Following 3 weeks of 2 HAOX-HMUFA eggs/day also plasma oleic acid levels were increased significantly (p<0.01). Other fatty acids, such as linoleic acid gave inconsistent results.

Consumption of 2 regular eggs/day for 3 weeks, significantly increased LDL susceptibility to oxidation, as shown by the decrease in lag time (p<0.01) required for oxidation. Increased consumption of antioxidants, through HAOX eggs, did not reduce LDL oxidizability, despite the significant (p<0.01) increase of vitamin E, vitamin A and carotenoids in the plasma levels. However, consumption of 2 HAOX-HMUFA eggs/day did reduce LDL oxidation by 31% compared to regular eggs (p<0.01), back to the baseline levels of low eggs’ regime (Shapira, unpublished).
Table 1 Fatty acid composition of regular eggs, high antioxidant (HAOX), and HAOX, low
PUFA, high MUFA (HAOX-HMUFA) eggs (% of yolk fatty acids).

<table>
<thead>
<tr>
<th>Fatty acid %</th>
<th>Regular eggs</th>
<th>HAOX eggs</th>
<th>HAOX-HMUFA eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lauric (12:0)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Myristic (14:0)</td>
<td>0.3</td>
<td>0.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Palmitic (16:0)</td>
<td>30.1</td>
<td>26.1</td>
<td>27.2</td>
</tr>
<tr>
<td>Palmitoleic (16:1)</td>
<td>0.8</td>
<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>Stearic (18:0)</td>
<td>10.5</td>
<td>4.4</td>
<td>4.0</td>
</tr>
<tr>
<td>Oleic (18:1)</td>
<td>37.4</td>
<td>37.0</td>
<td>47.3*</td>
</tr>
<tr>
<td>Linoleic (18:2)</td>
<td>17.9</td>
<td>21.6</td>
<td>12.4*</td>
</tr>
<tr>
<td>Linolenic (18:3)</td>
<td>0.4</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Arachidic 20:0</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Lingoceric (24:0)</td>
<td>0.2</td>
<td>1.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

*p<0.01 vs. regular and HAOX eggs.

Discussion

A NEW PERSPECTIVE ON EGG RISKS

The new studies showing that high intake of regular eggs not only increases total plasma cholesterol and LDL levels, but also LDL oxidizability, and the unexpected effect of egg composition on LDL oxidation, may open new perspectives on egg risks and the potential of designing for functional protection. The research quoted here is the first to investigate the possibility of reducing egg induced LDL oxidation by modifying the chicken feed to enhance egg antioxidative capacity. This was attained by increased antioxidants, reducing PUFA and increasing MUFA and MUFA: PUFA ratio. Since the extent of increase of LDL oxidation was greater by 5-8 times than the increase of blood cholesterol, LDL oxidation may become a significant criterion for egg risks, i.e. increasing egg n-6 PUFA, for the purpose of reducing blood cholesterol, may contradicts the goal of reducing LDL oxidation. Increasing egg oleic acid can both decrease blood cholesterol and LDL oxidation.

It is long known that high antioxidants and high MUFA diet i.e. Mediterranean diet and oleic acid, reduces LDL oxidation. The unexpected finding that the same principles of the whole diet are 'condensed' to the small scale of the egg composition is a new understanding of the interrelationships between the eggs and in vivo cholesterol metabolism.

EGG MODIFICATION FOR REDUCING SFA AND PLASMA CHOLESTEROL

As the main limiting factor in eggs is cholesterol, the initial attempts were focused on reducing the egg induced increase in plasma cholesterol, mostly by reducing egg’s content of saturated fatty acids. This already proved to enable the inclusion of one daily egg as part of a cholesterol reducing diet (Garwin, 1992). However the potential of reducing egg-induced plasma cholesterol by reducing egg SFA may be rather limited because egg SFA assumed to be less affected by the hen feed (Grobas et al., 2001) and it seems more difficult to reduce blood levels with eggs.

EGG MODIFICATION FOR REDUCED LDL OXIDATION

Increasing egg antioxidants

In the present study increasing egg antioxidants did not reduce LDL oxidation despite the marked increase of plasma vitamin E, carotenoids and vitamin A, by about 40-47% (p<0.01). This finding was not surprising considering the relatively small amount of vitamin E intake (about 15 mg/2 eggs/day) whereas high amounts of vitamin E (400 mg/day) significantly reduced LDL oxidative susceptibility (Hodis et al., 2002). Also other antioxidants like beta-carotene or lycopene supplementation, failed to protect against LDL oxidation despite their marked increase in plasma levels (Carroll et al., 2000). In vivo data are inconclusive regarding antioxidants protective potential (Devaraj and Jialal, 2000) and epidemiological studies revealed that only one in five prospective studies showed beneficial effects of vitamin E supplementation on prevention of cardiac events or mortality (Bunout, 2000). Such
conflicting results may suggest that the impact of antioxidants is dependent on other nutritional factors, i.e. MUFA, which is expected to enhance the antioxidative capacity of vitamin E, in a synergistic way (Carr et al., 2000) i.e. in HMUFA egg, whereas HPUFA egg would be expected to reduce the egg vitamin E capacity to protect against LDL oxidation.

Reducing egg PUFA
In the present study egg linoleic acid in the HAOX-HMUFA eggs was reduced by 57% and 69% of yolk fatty acids, as compared to control and HAOX eggs respectively (Table 1). Ravean (1996) reported that LDL content of 18:2 (n-6) is strongly correlated with the rate and extent of its oxidation, whereas an increase of LDL-18:1 (n-9), closely correlates with reduced LDL-PUFA and delayed LDL oxidation. According to GC/MS analysis of in vitro oxidation of LDL samples (Spiteller and Spiteller, 1998), linoleic acid (LA 18:2) appears to be the main substance of LDL oxidation.

Increasing egg MUFA
Intervention studies have repeatedly confirmed that LDL enriched with MUFA is less susceptible to oxidation than with PUFA (Hargrove et al., 2001; Baroni et al., 1999; Castro et al., 2000; Ramirez-Tortosa et al., 1999). LDL resistance to in vitro oxidation was higher in rats fed a high MUFA than high PUFA diet (Fremont et al., 1998). In human subjects supplementation with olive oil (Baroni et al., 1999) and/or canola oil (Schwab et al., 1998) increased LDL oleic acid content (Gumbiner et al., 1998). In the present study egg MUFA (oleic and palmitoleic acids) in the yolk fat were increased by 36% and 25% in the HAOX-HMUFA compared with control and HAOX eggs, respectively (Table 1).

Increasing egg MUFA: PUFA ratio
In the present study, consumption of eggs with high MUFA and antioxidants (HMUFA-HAOX), lead to a significant reduction in LDL oxidation as shown by the increase in lag time for LDL oxidation as compared to regular eggs. As high antioxidants alone (HAOX eggs), did not reduce LDL oxidation effectively, the high MUFA and MUFA:PUFA ratio, in the HAOX-HMUFA eggs, appear to be the leading protective factors. High MUFA and/or MUFA:PUFA ratio probably act synergistically with the high antioxidant content for attaining the significant reduction of LDL oxidation.

The functional vs. the nutritional paradigm
The research presented here is the first to show that dietary modification of egg lipids towards reduced PUFA and increased MUFA can reduce egg-induced LDL susceptibility to oxidation. This effect was attained by the minute amount of 1-2g oleic acid in 2 eggs, whereas in former research it took 1-2 weeks of 50g (=38g oleic acid) olive oil/day to reduce LDL oxidation (Aviram and Elías, 1993). This can be partially explained by the effect on cholesterol esters i.e. increase in MUFA (by 45%) and decrease in PUFA-18:2 (by 21%) in plasma cholesterol esters following ingestion of Crete eggs (Aviram and Elías, 1993). These results emphasize the functional effect and the significance of close lipid environment of the dietary cholesterol, such as in the egg.

As cholesterol is extremely sensitive to oxidation, and oxidized LDL is known to be a major risk factor for atherosclerosis, the unknown factor of oxidative state could affect former research findings and conclusions, with regard to egg related heart risks. The high MUFA low oxidizable LDL would be especially important in subjects who are prone to oxidative stress such as subjects with impaired glucose tolerance, where diets high in PUFA tend to increase the susceptibility of LDL for oxidation (Schwab et al., 1998).

The results strongly support the application of healthy nutritional principles, i.e. high antioxidants and MUFA:PUFA ratio, which are compatible with the Mediterranean diet that was recently awarded as the Gold-standard for heart protection (Curtis and O’Keefe, 2002) to the egg industry. Further research regarding the interrelationships between egg composition and health risks and/or benefits may lead to optimization of the egg composition and more relevant recommendations regarding amounts of eggs/day for general population specific sections and for individual requirements.
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