

## Fatty Acid Modified Eggs as a Delivery System for Functional Lipids

– Review –

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### Abstract

The chicken egg provides a perfectly packaged, portion controlled, highly nutritious food containing vital nutrients essential for maintaining human health. However, concern by health professionals over the possible association of diets high in fat and cholesterol to chronic diseases has led to a decrease in egg consumption. Several different strategies have been adopted by the poultry food industry to enhance the nutritional value of eggs. The major emphasis has been focused on the modification of polyunsaturated fatty acid composition and fat soluble vitamins. This review briefly describes the composition, lipid nutrient content and the positive health effects of egg lipid nutrients.

**Key words:** chicken egg, polyunsaturated fatty acids

### INTRODUCTION

The chicken egg has been a staple food for human beings since the dawn of civilization. The egg is both a biological and chemical entity. Eggs are unique in the sense that the nutrients in the egg are balanced in such a way as to support a new life upon incubation. The egg also provides a perfectly packaged, portion controlled, highly nutritious food containing vital nutrients essential for maintaining human health. However, the per capita consumption of egg has been declining over the past decades (1) (Fig. 1). Changing life styles and increased food choices have contributed to the decline, but the major cause of decline in egg consumption has been the concern expressed by health professionals that diets high in fat and cholesterol are associated with increased risk of chronic diseases such as coronary heart disease (CHD) (2). The poultry industry has been applying new technology for development of health-value-added eggs through manipulating lipid profiles of eggs to increase their nutritional value. This review briefly describes the composition, nutrient content, lipid nutrient modulation strategies, and studies in humans and animals that suggest a role or designer eggs as a delivery system for functional nutrients, with emphasis on lipids.

### EGG COMPOSITION

The egg is comprised of four main parts: yolk, egg white or albumen, shell membranes and shell. The composition of eggs was reported decades ago to consist of

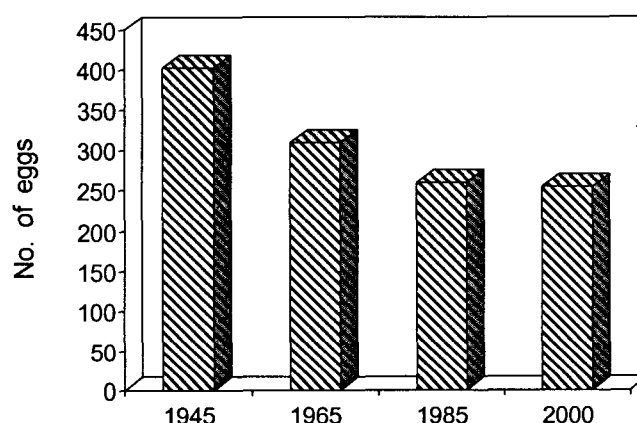


Fig. 1. Per capita consumption of eggs in US.

58% white, 31% yolk, and 11% shell (3). Manipulation of dietary nutrients, strain of the bird, and genetic selection for egg size and production may lead to changes in egg components. Recently, there has been a tremendous interest in specialty eggs. The composition of such specialty eggs reveals a wide variation in egg components such as yolk, white content, edible portion and yolk : white ratio. A comparison of the egg components of supermarket eggs to specialty eggs in US is shown in Table 1. In general, the shell contributes from 9% to 11%, yolk 25% to 33% and egg white contributes to 56% to 64% and the total edible portion constitutes 89% to 91% of the egg (4).

### EGG LIPIDS

Among the several nutrients in eggs, lipids have at-

**Table 1.** Comparison of egg components in regular and speciality eggs<sup>1)</sup>

Egg components	Control	Sp. eggs 1	Sp. eggs 2	Sp. eggs 3	Sp. eggs 4	Sp. eggs 5	SEM
Yolk	30.2 <sup>b</sup>	29.7 <sup>b</sup>	25.9 <sup>c</sup>	33.4 <sup>a</sup>	25.6 <sup>c</sup>	29.8 <sup>b</sup>	0.63
White	59.6 <sup>b</sup>	59.4 <sup>b</sup>	63.7 <sup>a</sup>	56.3 <sup>c</sup>	64.5 <sup>a</sup>	60.7 <sup>b</sup>	0.64
Shell	10.1 <sup>ab</sup>	10.8 <sup>a</sup>	10.3 <sup>ab</sup>	10.2 <sup>ab</sup>	9.9 <sup>b</sup>	9.5 <sup>b</sup>	0.35
Edible portion	89.8 <sup>ab</sup>	89.2 <sup>b</sup>	89.7 <sup>ab</sup>	89.7 <sup>ab</sup>	90.1 <sup>a</sup>	90.5 <sup>a</sup>	0.34
Yolk : White	50.6 <sup>b</sup>	50.0 <sup>b</sup>	40.6 <sup>d</sup>	59.0 <sup>a</sup>	39.6 <sup>d</sup>	49.0 <sup>c</sup>	0.59

Reported as percentages of egg weight.

<sup>a-d</sup>Within a row with no common superscripts indicates significantly different ( $p < 0.05$ ).

<sup>1)</sup>Sp. eggs 1, Sp. eggs 2 Sp. eggs 3, Sp. eggs 4, Sp. eggs 5, represents specialty eggs from hens fed vegetarian diet, certified organic free range brown eggs, uncaged unmedicated, brown eggs, cage free vegetarian diet brown eggs, or naturally nested uncaged hens fed diets with no steroids or no stimulants. Adapted from Cherian et al. 2002.

tracted more attention among scientists and consumers alike due to the link between high dietary fat consumption and CHD. This concern has led to a decrease in the consumption of chicken eggs (5). However, new evidence shows that increased egg consumption is unlikely to increase the risk of CHD in the general population (6). Chicken eggs are now being recognized and promoted as a highly nutritious and versatile functional food item.

The fat in eggs is exclusively in the yolk and comprises 5.5 to 6 g in an average 60 g egg. Almost all lipids are present as lipoprotein complexes associated with yolk. Trace levels of lipids have also been observed in egg whites. Egg lipids are composed mainly of triacylglycerol (TAG), phospholipids (PL) and cholesterol (Table 2). Fatty acids are the main components of TAG and PL. Fatty acids may be of different chain lengths and degrees of saturation as well as different configurations. Fatty acids are classified into three families: saturated fatty acids, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The predominant saturated fatty acids in eggs are palmitate (C16:0) and stearate (C18:0). The content of these two fatty acids in chicken eggs may range from 22~26% and 8~10%, respectively. The total saturated fatty acids in eggs may account for up to 30~35% of total fatty acids. The MUFA in eggs account for 42~46% of the fatty acid content, with oleic acid (C18:1) being the predominant MUFA.

**Table 2.** Major lipid classes and proportions in chicken eggs<sup>1)</sup>

Major lipid and fatty acid fractions	Percent proportion
Triglyceride	63~65
Cholesterol	4.9~5.0
Cholesterol ester	1.0
Phospholipids (PL)	30~31
Phosphatidyl choline	21.0
Phosphatidyl ethanolamine	7.3
Phosphatidyl serine	0.9
Sphingolipids	0.9

<sup>1)</sup>Adapted from Cherian, 2002 (18).

There are two families of PUFA in the eggs; n-6 and n-3 PUFA. The predominant n-6 PUFA in egg is C18:2 n-6 (linoleic acid). Other n-6 fatty acids in eggs may include C20:4 n-6, C22:4 n-6 and C22:5 n-6. The content of long chain n-6 PUFA (LCPUFA) (>20-carbon) may vary from 1~2% and is a reflection of the fatty acid composition of the laying hen diet (3). The most common n-3 fatty acids in eggs are  $\alpha$ -linoleic (LNA), eicosapentaenoic acid (EPA), docosapentaenoic (DPA) and docosahexaenoic acids (DHA). Among these, DHA is the major n-3 fatty acid in the egg (2). The LNA content in regular eggs is under 1% of total lipids. DHA may account for between 1~3% of total lipids. The content of n-3 PUFA is a reflection of dietary fat. Addition of flax oil, flax seeds, fish oil, or marine algae in laying hen diets leads to a significant increase in LNA and DHA in eggs (7).

The major sterol in yolk lipids is cholesterol and is found in the free form. The cholesterol content may vary from 11~14 mg/g yolk or 200~220 mg/ average egg. The cholesterol content of chicken eggs has received far more attention than any other component of egg from the media, consumers and health professionals due to the reported role of serum cholesterol in cardiovascular diseases. Although diet has very little effect on egg cholesterol (8) other factors like egg weight, yolk size, or strain of birds may affect the content of egg cholesterol (9). Several fat soluble pigments are also present in the yolk constituting to 0.02%. The major fat-soluble pigments include xanthophylls and carotenes. Leutin, zeaxanthin and cryptoxanthin belong to the xanthophyll group and  $\beta$ -carotene belongs to the carotene group. Yolk pigments are of aesthetic importance as deep yellow yolks are preferred over light-colored yolk in certain parts of the world. Feed containing yellow corn, canola, flax or alfalfa produces medium yellow yolks while feed containing wheat or barley produces lighter color yolks. Natural yellow-orange substances such as marigold petals are sometimes added to feeds to enhance the yolk

color.

## EGG LIPIDS AND HUMAN HEALTH STUDIES

### Cardiovascular Diseases

Although chicken eggs are a rich source of vital nutrients that are essential for maintaining human health, the per capita consumption of eggs has been declining steadily over the past four decades in the US (Fig. 1). Changing lifestyle, expanded food choices, and increasing consumer health awareness are some of the contributing factors. Due to its relatively high cholesterol content, the egg has been used as an exclusive source of dietary cholesterol in both animal and human experiments and has become the visual icon of high cholesterol. The results from some of studies have implicated cholesterol as a promoter of diet-related chronic diseases resulting in damage to the egg's reputation as a nutritious food item. However, recent research has shed more insight into the health effects of eggs, and egg consumption has been increasing since the late 1990's. Some of the recent research on human health effects of egg lipids are described.

In a large prospective cohort study that evaluated the food consumption patterns of more than 100,000 people, Hu et al., 1999 (6) reported no overall association between egg consumption (up to one egg per day) and the risk of CHD. The authors speculated that the positive health effects associated with egg consumption were attributable to health-promoting nutrients including PUFA, antioxidants, folates and other B vitamins. However, an adverse effect of egg consumption on diabetic subjects was observed by these authors. The increased risk of CHD may be associated with abnormal cholesterol metabolism in diabetics (6). The effect of dietary egg cholesterol on the ratio of total cholesterol to high density lipoprotein cholesterol was investigated by Weggemans et al., 2001 (10). After reviewing the data from 1974 through 1999, these authors concluded that egg cholesterol increases the ratio of total to high density lipoprotein cholesterol, thus adversely affecting the human cholesterol profile. Investigating the nutritional significance of eggs in American diets, Song and Kerver, 2000 (11) conducted a cross sectional and population-based study ( $n = 27,378$ ) during 1988 ~ 1994. Nutrient intake, egg intake and blood cholesterol concentrations of subjects were evaluated according to the frequency of egg consumption. These researchers reported no association between egg consumption and blood cholesterol concentration. In addition, consuming eggs contributed significantly to other nutrients such as vitamin B6, saturated and PUFA, vitamin A, E and B12 (11).

## CHICKEN EGG AS A FUNCTIONAL FOOD

Functional foods have been defined as foods that, by the virtue of the presence of physiologically active components, provide health benefits beyond basic nutrition. Eggs are a storehouse of several biologically active functional ingredients that can prevent or ameliorate the disease progression processes. These lipid-related functional ingredients include fat soluble vitamins, pigments, carotenoids, choline as phosphatidylcholine, sphingolipids (SL) and long chain n-3 and n-6 PUFAs.

### Eggs: Role in Sight

Age-related macular degeneration (ARMD) is a debilitating eye disorder of the elderly. Two carotenoids, leutin and zeaxanthin, have recently received attention for their potential role in delaying ARMD (12). Egg yolk contains 292 and 213  $\mu\text{g}$  of leutin and zeaxanthin, respectively; and they are more bio-available than plant sources of carotenoids. Eating eggs resulted in respective 50 and 114% increases in plasma leutin and zeaxanthin (12). In view of the potential role of these carotenoids in delaying ARMD, including eggs in the diets of the elderly should be reconsidered.

### Eggs: Source of Choline and Sphingolipids

Eggs are a rich source of choline (associated with phosphatidyl choline in phospholipids). Choline is responsible for the structural integrity and signaling function of phospholipid-rich cell membranes. Animal studies have found that choline plays an essential role in the development of brain function, memory and learning ability in mice (13). SL's are a complex class of compounds built upon a sphingoid base (sphingosine) and are rich in eggs. The SL content of egg has been reported to be the highest of any food at 2250  $\mu\text{mol/kg}$  (14). SL is critical for the maintenance of membrane structure and modulates the behavior of growth factor receptors and extra cellular matrix proteins. SL also functions at the binding site for microorganisms, microbial toxins, and viruses. Inhibition of early stage colon cancer and a decrease in the proportion of adenocarcinoma has been reported in mice fed SL (15). An inverse correlation between tissue and plasma concentrations of SL and cholesterol in normal and pathological conditions has also been reported. SL has been reported to alter cholesterol metabolism and, consequently, to affect cholesterol balance in the cell. A 30% reduction in plasma cholesterol in rats fed semipurified diets supplemented with a mixture of SL and glycosphingolipids at 1% of the diet was reported (16). However, very little information is available on the health effects of egg SL. Further research in this area is warranted.

## EGG LIPID NUTRIENT MODULATION

### n-3 Fatty Acids and Specialty Eggs

In a typical Western diet, 58% of dietary fat, 74% of saturated fat and 100% of cholesterol are supplied through animal products such as egg, meat and milk. The dietary recommendations set forth by health agencies include reduction in total fat intake to 30% or less, reduction in saturated fatty acids to 10% or less, increase in MUFA and PUFA to 15 and 10% of calories, respectively and to have a balance of PUFA to saturated fatty acids close to 1 (17). The egg industry has been very responsive to these recommendations and adopted feeding strategies for producing and marketing eggs with a more balanced ratio of n-6 to n-3 PUFA.

Several studies have demonstrated that increased consumption of n-3 PUFA may reduce the risk of CHD disease, atherosclerosis, and hypertension (18,19). Dietary sources of omega-3 fatty acids include LNA from plant sources (flax, canola, chia), and EPA and DHA from marine sources. The US consumption of marine foods is approximately one serving per week, thereby excluding fish as a primary source of dietary omega-3 fatty acids. American heart association recommends 1.8 to 3.5 g of omega-3 fatty acids for reducing CHD risk. An alternate means of providing a consistent dietary source of these essential nutrients is the enrichment of animal products such as eggs with omega-3 fatty acids. Addition of ground flax seed has been used for the production of n-3 PUFA-rich eggs. Eggs from hens fed diets containing 10–15% flax seed could provide up to 550–600 mg of n-3 PUFA compared to 60 mg of n-3 PUFA in regular eggs (7). The amount of n-3 PUFA supplied by one serving of n-3 fatty acid-enriched egg is equivalent to a 100 g serving of fish. Other oil seeds such as canola and chia also increase the n-3 fatty acid

content to a lesser extent. Other sources of n-3 PUFA for fatty acid enrichment include marine oils and algae. Eggs from hens fed marine oils contain higher levels of the long chain n-3 PUFAs, DHA and EPA. Using menhaden oil as a source of n-3 PUFA, Cherian et al. (20) and Van Elswyk (21) reported 314 and 268 mg/yolk of long chain n-3 PUFA, respectively. The positive health effects associated with consuming n-3 PUFA enriched eggs are shown in Table 3.

### Conjugated Linoleic Acids

Conjugated linoleic acid (CLA) is the generic name for a group of positional and geometric conjugated dienic isomers of linoleic acid (18:2 n-6) formed from linoleic in the rumen from rumen microorganisms. There are potentially sixteen different isomers of CLA. The most active form is believed to be c9, t11. CLA has received considerable attention for its anticarcinogenic, antiatherogenic, hypocholesterolemic, immunomodulatory, and body fat reduction properties (22). Current intake of CLA is estimated to be several hundred milligrams/day (23). Based on animal data, it is estimated that approximately 3 g /day of CLA would be required to produce beneficial effects in humans (24). Humans do not have the ability to synthesize CLA, so it can only be obtained from dietary sources. Dietary CLA is obtained from food products of ruminant origin such as dairy products and beef. As consumers are opting for low fat dairy products, it is possible that the dietary supply of CLA may be much lower than is reported. An alternate way to provide CLA is by enriching animal products with CLA through dietary manipulation. In this respect, development of CLA-enriched chicken eggs may be an alternative vehicle for delivering health-promoting fatty acids to consumers. Recent studies reported significant incorporation of CLA in eggs by dietary manipulation (25). Cherian

**Table 3.** n-3 Fatty acid-rich egg consumption and human health effects

Number of egg	Days fed	Reported responses
4 eggs/day	28	No change in plasma total cholesterol. Reduction in serum triglycerides, and systolic and diastolic blood pressure.
2 eggs/day	18	Increase in plasma HDL-cholesterol Reduction in plasma triglycerides. No change in total cholesterol, and LDL-cholesterol.
2 eggs/day	28	Reduction in plasma triglycerides, total cholesterol, systolic blood pressure
4 eggs/day	14	Reduction in serum triglycerides No change in total or HDL cholesterol. Increase in n-3 PUFA, DHA of platelet phospholipids
4 eggs/day	14	Increase in DHA, n-3 PUFA in platelet phospholipids. No change in plasma triglycerides, total cholesterol or HDL cholesterol
7 eggs/week	168	Increase in HDL cholesterol, EPA, DHA and total n-3 PUFA.
4 eggs/week	42	Decrease in platelet aggregation

<sup>1)</sup> Adapted from Cherian, 2002 (18).

et al. (26) used menhaden oil (as source of long chain n-3 PUFA) along with CLA in the diet of laying hens to produce n-3 PUFA-CLA rich eggs. An average CLA-n-3 PUFA rich chicken egg could provide upto 320 mg of CLA, along with other n-3 PUFA (26). Eggs high in CLA were rubbery when hard-boiled and developed a reddish tint upon storage (authors research). More work in the sensory quality and textural aspects of CLA-modified eggs has to be done before marketing.

## FAT-SOLUBLE ANTIOXIDANT VITAMINS

### Tocopherols, Retinols and Carotenenes

Both clinical and epidemiological studies have reported the protective effect of dietary antioxidants against the biggest health threats in the US such as heart disease, cancer and stroke (27,28). The Food and Nutrition Board of the National Academy of Sciences has increased the recommended daily amounts of certain antioxidant micronutrients. The consumption of health-enhancing antioxidant nutrients may benefit public health and reduce medical costs (28). Of the natural antioxidants, vitamin E has attracted much attention due to its powerful antioxidant activity both *in vivo* and *in vitro*. In biological systems it can function as an intracellular and intercellular antioxidant, thus neutralizing free radicals and preventing oxidation. In addition to tocopherols, other tocopherols such as tocotrienols and carotenoids also exhibit health benefits in the prevention of certain diseases such as cancer and CHD, and may improve immune functions.

Incorporation of tocopherols in hen diets has been reported to increase the vitamin E content of chicken eggs. Cherian et al. (20) reported a total of 7~8 mg vitamin E per average eggs from hens fed diets containing 400 µg/g of vitamin E. Feeding palm oil has been reported to increase the content of tocotrienols, carotenoids and retinols in eggs (29). As plant-derived natural antioxidants are gaining popularity, and poultry products represent a major segment of animal foods in the western diet, altering the tocopherols and carotenoids in eggs or further-processed egg products may provide an alternate source of such health-promoting nutrients to consumers. In addition to fat-soluble vitamins, eggs have been enriched with B-vitamins such as folates (30).

## BIOLOGICAL FUNCTIONS OF FATTY ACID MODIFIED EGGS IN HUMAN AND ANIMAL MODELS

### n-3 Fatty Acid Modified Eggs and Human Health Effects

Several authors have investigated the effects of n-3 fatty acid enriched eggs on human blood lipid parameters

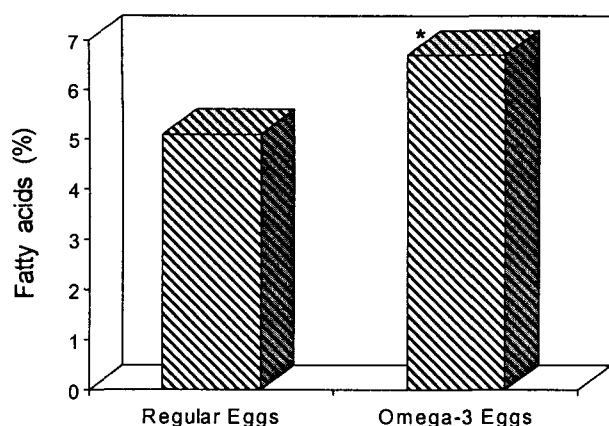
(Table 2). The n-3 PUFA-rich eggs were produced by feeding diets containing land or marine based n-3 fatty acids to hens. Therefore, the content of n-3 PUFA with respect to LNA and long chain n-3 fatty acids such as EPA and DHA varied among these eggs. In general, a significant increase in plasma n-3 fatty acids, platelet phospholipid n-3 fatty acids, and a reduction in serum triglycerides with a concomitant increase in HDL-cholesterol have been reported after the consumption of n-3 PUFA enriched eggs. A reduction in systolic blood pressure was also observed with the daily consumption of two n-3 PUFA modified eggs (7). These studies were conducted in general healthy populations consuming a normal diet along with n-3 PUFA modified eggs for 4 weeks to 6 months. However, the effect of n-3 PUFA egg consumption in people with hyperlipidemia, diabetics or people who are hyper responders to dietary cholesterol are still lacking.

### n-3 Fatty Acid Modified Eggs as a Source of Long Chain Polyunsaturated Fatty Acids for Infants

In the developing human brain, substantial quantities of LCPUFA such as arachidonic acid (20:4 n-6, AA) and DHA are deposited in the central nervous system during the brain growth spurt (31). Over 90% of brain growth occurs in the first 2 years of life, so the requirements for AA and DHA are very important during this period. Human breast milk contains DHA. However, commercially available infant formulas do not contain AA or DHA. The plasma levels of DHA are higher in breast milk-fed infants than formula-fed infants (32). The fat in the formulas are derived from one or more vegetable oils, usually coconut, soy, safflower or corn oil. All vegetable oils differ from human milk in their content of fatty acids, including AA and DHA. Cherian and Sim (33) used n-3 PUFA rich eggs as a vehicle for supplying n-3 PUFA to nursing infants. When nursing mother consumed two n-3 eggs as a part of their normal daily meal for six weeks, the total n-3 fatty acids in the mother's milk constituted to 3.6% compared to 1.9% for pre-test milk with a concomitant reduction in n-6 : n-3 ratio (6.7 vs. 3.0) ( $p < 0.05$ ). The long chain n-3 PUFA comprised 1.2% of the fatty acid content compared with 0.4% in the pre-test milk ( $p < 0.05$ ). After incorporating n-3 PUFA-rich eggs in weaning diets, Gibson et al. 1998 (34) reported a 27% increase in erythrocyte DHA of infants consuming 4 vs. 1 egg per week (Fig. 2).

### n-3 Fatty Acid Modified Eggs: Animal Studies

The effect of n-3 PUFA rich egg yolk powder consumption on tissue and plasma lipid composition of rats was examined by Jiang and Sim, 1992 (35). The plasma



**Fig. 2.** Erythrocyte docosahexaenoic acid content of infants consuming regular or omega-3 fatty acid enriched eggs at 12 months of age.

\*Indicates significantly different ( $p < 0.05$ ).

and liver cholesterol content was significantly reduced in rats fed high n-3 PUFA rich yolk powder. The n-3 LCPUFA content of rat tissues was also significantly increased by high n-3 PUFA rich yolk powder. In addition, prostaglandin  $E_2$  synthesis by skeletal muscle was decreased in rats fed yolk powder high in n-3 PUFA, suggesting immunomodulatory effects of n-3 PUFA rich yolk in animal models.

#### CLA-Rich Egg Yolk: Animal Studies

Although CLA modification of egg yolk has been documented, the health effects of CLA-rich yolk on animal models have not been reported. Cherian and Goeger (36) fed Sprague-Dawley rats yolk powder containing 0, 1.4 or 5.4% CLA for four weeks. Rat plasma was taken on day 28 and the activity of immunoglobulin G was measured. A significant reduction in plasma IgG of rats fed CLA-yolk was observed. Similarly, a significant incorporation of CLA was observed in the liver, plasma and splenocytes of rats consuming CLA-rich yolk powder. The concept of CLA-rich egg yolk could be appealing to health-conscious consumers interested in functional foods and a diet-based approach for disease prevention.

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