Egg yolk as a source of long-chain polyunsaturated fatty acids in infant feeding[1,2]

Artemis P Simopoulos and Norman Salem, Jr

ABSTRACT In this paper we compare the fatty acid content of egg yolks from hens fed four different feeds as a source of docosahexaenoic acid to supplement infant formula. Greek eggs contain more docosahexaenoic acid (DHA, 22:6ω3) and less linoleic acid (LA, 18:2ω6) and α-linolenic acid (LNA, 18:3ω3) than do fish-meal or flax eggs. Two to three grams of Greek egg yolk may provide an adequate amount of DHA and arachidonic acid for a preterm neonate. Mean intake of breast milk at age 1 mo provides 250 mg long-chain ω3 fatty acids. This amount can be obtained from < 1 yolk of a Greek egg (0.94), > 1 yolk of flax eggs (1.6) and fish-meal eggs (1.4), or 8.3 yolks of supermarket eggs. With proper manipulation of the hens' diets, eggs could be produced with fatty acid composition similar to that of Greek eggs. *Am J Clin Nutr* 1992;55:411–4.

KEY WORDS Docosahexaenoic acid, arachidonic acid, ω3 fatty acids, infant formula, breast milk, preterm neonate, egg yolks, infant nutrition

Introduction

In previous studies we showed that the ω3 fatty acid content in eggs from range-fed Greek chickens was considerably higher than the ω3 fatty acid content reported in eggs by the US Department of Agriculture and our own analysis of US supermarket eggs (1). In fact, the ratio of ω6 to ω3 fatty acids (ω6:ω3) for Greek eggs was 1.3 and that of the supermarket egg was 19.9.

van Vliet and Katan (2) showed that there is a lower ratio of ω3 to ω6 fatty acids in cultured than in wild fish. Similarly, Crawford (3) showed that animals in the wild have more ω3 fatty acids in their carcass than do domesticated animals. In another study we compared the α-linolenic acid (LNA, 18:3ω3) content of wild purslane with that of spinach and other cultivated plants and found that purslane was much richer in LNA: 400 vs 89 mg/100 g for spinach (4). These studies indicate that industrialization and agricultural practices have systematically reduced the amount of ω3 fatty acids in the plants, eggs, fish, and meat that we eat. Most important, however, have been the studies indicating that infant formula is devoid of long-chain polyunsaturated fatty acids (LCPUFAs), including both the ω6 and ω3 families, whereas human milk contains both (5).

In newborn rhesus monkeys impaired visual development has been correlated with a diet that is high in linoleic acid (LA, 18:2ω6) and low in LNA and with decreased accretion rates of docosahexaenoic acid (DHA, 22:6ω3) in the developing brain (6–8). Because 18:2ω6 competes with 18:3ω3 for Δ-6 desaturation (7) the feeding of an artificial formula with high 18:2ω6 aggravates the effects of low-ω3 fatty acids. Nonhuman primates fed diets high in 18:2ω6 and low in 18:3ω3 during prenatal and early postnatal life had a low plasma phospholipid DHA content at birth and almost undetectable concentrations at age 12 wk (6, 8).

Studies by Liu et al (9) and Uauy et al (10) suggested that DHA is essential for the development of newborn infants and that preterm neonates who have deficient stores of DHA show deficits in visual function when not fed human milk or formula supplemented with marine oil containing eicosapentaenoic acid (EPA, 20:5ω3) and DHA. LNA supplementation failed to elicit normal rod electroretinographic responses, suggesting that LNA does not satisfy this requirement whereas marine-oil supplementation sustained rod function similar to that found in the infants fed human milk (10). These studies indicate that the desaturation steps necessary for the synthesis of DHA from LNA are inefficient in humans, suggesting that DHA is a required nutrient.

In the past, DHA from marine oil and egg-yolk oil was considered as a possible source of DHA for infant formulas (11). Jackson and Gibson (12) investigated the DHA content of weaning foods and concluded that weaning foods cannot replace breast milk as sources of LCPUFAs. These investigators examined egg yolk and fresh and canned baby foods and concluded that infants would have to eat unphysiologic amounts of foods and even then might not reach the amounts of LCPUFAs comparable with that found in human milk.

Attempts have been made to produce eggs that are rich in EPA or DHA because such eggs have been shown not to raise cholesterol and to lower blood pressure (13). We were therefore interested in investigating the composition of eggs enriched in ω3 fatty acids available in markets as a possible source of DHA to supplement infant formula, as a weaning food, or as a food for adults, particularly elderly people who may also have a limited capacity to elongate and desaturate LNA to EPA and DHA (14). We found two types of ω3-enriched eggs sold in markets. In one, the chickens were given fish meal to increase the amount of ω3

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[1] From The Center for Genetics, Nutrition and Health, Washington, DC, and the Laboratory of Membrane Biochemistry and Biophysics, National Institute of Alcohol Abuse and Alcoholism, Bethesda, MD.

[2] Address reprint requests to AP Simopoulos, The Center for Genetics, Nutrition and Health, 2001 S Street, NW, Suite 530, Washington, DC 20009.

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fatty acids (fish-meal egg) and in the other the major source of ω3 fatty acids in the chicken feed was provided by flax (flax egg). This paper presents data on the fatty acid composition of Greek eggs, supermarket eggs, fish-meal eggs, and flax eggs and compares them with the fatty acid composition and concentrations in human milk so that these eggs can be evaluated as a source of LCPUFAs as a supplemental or weaning food for infants.

Materials and methods

The eggs were hard boiled and their fatty acid composition and lipid content were assessed as previously reported (1, 4). Briefly, the total lipid extract was prepared by the method of Bligh and Dyer (15). A portion of this extract was transmethylated with BF_3 in methanol by using a modification of the method described by Morrison and Smith (16); the cosolvent used was hexane rather than benzene. An HP-5880 gas chromatograph (Hewlitt-Packard Co, Palo Alto, CA) was used with a 0.25 mm id by 30 m DB-FFAP (0.25 μm film thickness) capillary column (J and W Sci, Folsom, CA) with hydrogen as carrier gas (54 cm/s). The injector and detector temperatures were 240 °C and the column oven was programmed from 130 to 175 °C at 4 °C/min and then to 210 °C at 1 °C/min. The retention times of various fatty acid methyl esters were determined with standard mixtures such as Nu-Chek Prep 68A (Nu-Chek Prep, Elysian, MN).

Four different types of eggs were studied. The supermarket eggs were purchased from a Washington, DC, supermarket. The fish-meal eggs came from hens fed soybeans and fish meal as their principal fat source (The Country Hen, Hubbardston, MA). The feed contained ≈46% LA, 5% LNA, 1.4% EPA, and 2.1% DHA (22:6ω3). The flax eggs came from chickens fed high amounts of flax, some soy meal, alfalfa pellets, and corn (Essential Nutrient Research Corporation, Manitowoc, WI). The Greek eggs came from the Ampelistra farm in Greece. At the Ampelistra farm the chickens roam freely and feed on various types of fresh green grass leaves and wild plants including purslane, which is plentiful, and their diet is supplemented with fresh and dried figs, barley flour, and small amounts of corn. The chickens also eat insects of all kinds and sometimes eat worms when the weather is very dry.

Results

There are major differences in these four groups of eggs with respect to ω6:ω3 and fatty acid composition (**Table 1**). Supermarket eggs have the highest ω6:ω3 (19.9) and Greek eggs have the lowest (1.3). Although Greek eggs and the flax eggs have similar ω6:ω3s, flax eggs have a very high LNA content (21.3 vs 6.9 mg/g for Greek eggs) and smaller amounts of EPA and docosapentaenoic acid (DPA, 22:5ω3). The higher amount of total ω3 in flax eggs is therefore principally due to the high amount of LNA. Greek eggs have the lowest amount of LA, the supermarket and flax eggs are intermediate, and fish-meal eggs are the highest. Thus major differences are found in the composition of these four eggs in both the types of ω6 and ω3 polyunsaturates as well as in the ω6:ω3. Greek eggs have the highest amount of DHA, EPA, and DPA and trace amounts of 18:4ω3, 20:4ω3, and 21:5ω3, which were not detected in the other eggs.

Of interest is that the ratio of monounsaturates to saturates is similar for all four types of eggs whereas the ratio of polyunsaturates to saturates is twice as high for the fish-meal and flax eggs as for the Greek eggs. It is also of interest that 16:1ω7 is elevated in Greek eggs.

TABLE 1
Fatty acid concentrations in chicken egg yolks*

Fatty acid	Supermarket eggs	Fish-meal eggs	Flax eggs	Greek eggs
	mg fatty acid/g egg yolk			
Saturates				
14:0	0.7	1.0	0.6	1.1
15:0	0.1	0.3	0.2	—
16:0	56.7	67.8	58.9	77.6
17:0	0.3	0.8	0.5	0.7
18:0	22.9	23.0	26.7	21.3
Total	80.7	92.9	86.9	100.7
Monounsaturates				
16:1ω7	4.7	5.1	4.4	21.7
18:1	110.0	102.8	94.2	120.5
20:1ω9	0.7	0.9	0.5	0.6
24:1ω9	—	0.1	—	—
Total	115.4	108.9	99.1	142.8
Omega-6 polyunsaturates				
18:2ω6	26.1	67.8	42.4	16.0
18:3ω6	0.3	0.3	0.2	—
20:2ω6	0.4	0.6	0.4	0.2
20:3ω6	0.5	0.5	0.4	0.5
20:4ω6	5.0	4.4	2.6	5.4
22:4ω6	0.4	0.3	—	0.7
22:5ω6	1.2	0.2	—	0.3
Total	33.9	74.1	46.0	23.1
Omega-3 polyunsaturates				
18:3ω3	0.5	4.1	21.3	6.9
20:3ω3	—	0.1	0.4	0.2
20:5ω3	—	0.2	0.5	1.2
22:5ω3	0.1	0.4	0.7	2.8
22:6ω3	1.1	6.5	5.1	6.6
Total	1.7	11.3	28.0	17.7
Polyunsaturates:saturates	0.4	0.9	0.9	0.4
Monounsaturates:saturates	1.4	1.2	1.1	1.4
ω6:ω3	19.9	6.6	1.6	1.3

* Supermarket eggs, standard US Department of Agriculture eggs found in US supermarkets; fish-meal eggs, main source of fatty acids provided by fish meal and whole soybeans; flax eggs, main source of fatty acids provided by flax flour; and Greek eggs, free-ranging chickens.

Discussion

The studies of Crawford et al (5), estimates from paleolithic nutrition and from present day hunter-gatherer societies (17), the studies of van Vliet and Katan (2) on fish, our studies on purslane (4), and the present studies on the ω6 and ω3 fatty acid content of various chicken eggs point to the fact that major changes have taken place in the ω6-ω3 balance of our food supply. It is presumed that eggs eaten by early humans came from chickens fed under conditions similar to those of chickens producing Greek eggs. These conditions include free ranging and consuming green leafy vegetables, fresh and dried fruits, insects, and occasional worms. Eggs produced under these conditions

TABLE 2
Long-chain (LC) ω6 and ω3 fatty acid concentrations

Fatty acid	Supermarket eggs	Fish-meal eggs	Flax eggs	Greek eggs	Human milk*
		mg fatty acid/g egg yolk			*mg fatty acids/794 mL*
Sum LC ω6	7.5 (187.5)†	6.0 (150.0)	3.4 (85.0)	7.1 (177.5)	505.1
Sum LC ω3	1.2 (30.0)	7.1 (177.5)	6.3 (157.5)	10.6 (265.0)	250.0

* Mature human milk (infant aged 1 mo); 794 mL mean intake per day (from reference 23).

† Milligram fatty acid/egg yolk given in parentheses. For the purpose of this calculation, 25 g was taken as the weight of an egg yolk.

are rich in both ω6 and ω3 LCPUFAs. In general, human milk composition is influenced by the maternal diet (18). Harris et al (19) showed that adding DHA to a mother's diet increases the DHA content of her milk. Because mother's milk reflects the mother's diet and because earlier diets were richer in polyunsaturated fatty acids (PUFAs) than is the modern diet (3, 17), it may be surmised that infants at present are also receiving less PUFA from their mother's milk.

Fatty acid requirements of preterm neonates have been determined in various ways. One approach is to determine the amount necessary to match the intrauterine accretion rates (20–22). Another is to estimate the ω3 requirement by determining the amount necessary to maintain the red blood cell or plasma phospholipid concentration of DHA found at birth or in infants fed human milk. This amount of DHA has been estimated to be 11 $\text{mg} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (9). Uauy et al (10) found highly significant correlations for ω3 fatty acid status in plasma and red blood cell membranes with the indices of rod function. Our data show that 1 g of Greek egg yolk contains 10.6 mg of ω3 LCPUFAs (20:5ω3, 22:5ω3, and 22:6ω3) and 7.1 mg ω6 LCPUFAs (20:2ω6, 20:3ω6, 20:4ω6, 22:4ω6, and 22:5ω6) (**Table 2**). Therefore 2–3 g Greek egg yolk would provide an adequate amount of long-chain ω3 fatty acids for a 2-kg preterm neonate with respect to maintaining the blood concentrations of DHA.

Still another way to estimate the long-chain ω3 or ω6 PUFA requirement of infants is to estimate the intake of these nutrients from human milk. Recently Boersma et al (23) estimated the mean daily intake of fatty acids from mature milk samples from women in the Caribbean who completely breast-fed their infants aged 18–31 d. These investigators, in estimating the mean daily intake of human milk, used data from Neville et al (24). On the basis of these calculations, the infants consume an average of 794 mL human milk/d at age 1 mo. The total intake of long-chain ω3 PUFAs was 250 mg/d and the intake of the long-chain ω6 PUFAs was 505 mg/d. One Greek egg yolk weighs ≈25 g. From the data in Table 2, one Greek egg yolk provides 265 mg of ω3 LCPUFAs acids and 177.5 mg ω6 LCPUFAs; < 1 egg yolk (0.94 egg yolk, or 23.6 g) would provide 250 mg ω3 fatty acids. It therefore appears that a diet supplemented with one such egg yolk would supply the necessary amount of ω3 LCPUFAs and of DHA, in particular, for a 1-mo-old infant. Similarly 250 mg ω3 LCPUFAs would be provided by 1.6 flax egg yolks (39.7 g egg yolk), 1.4 fish-meal egg yolks (35.2 g egg yolk), and 8.3 supermarket egg yolks (208.3 g egg yolk). The latter is an enormous amount and therefore is inappropriate for consideration as a weaning or supplemental food. However, with appropriate manipulation of chicken feed, fish-meal and flax eggs could be made to resemble the Greek eggs.

Infant formula does not contain LCPUFAs. One Greek egg yolk, in addition to providing the essential fatty acids, vitamins, and minerals found in human milk, also provides ≈4 g protein (11). Full-term neonates are given formulas containing different amounts of protein ranging from 11 g/L (the amount found in human milk) to 32 g/L and have thrived on it (25). The additional 4 g protein provided by one Greek egg yolk should not be a problem, provided that the formulas selected are in the lower range with respect to protein content. For preterm neonates 2–3 g Greek egg yolk would provide the requirements for ω3 fatty acids and an additional 0.34–0.51 g protein/d. This amount can be easily adjusted by controlling the amount of protein in the formula. Therefore adjustments in the formulas could be made if necessary. The average protein content of human milk is 11 g/L but in the first few months it is 13 g/L, so for 1-mo-old infants 750 mL of human milk (average intake) provides 9.75 g protein/d. Fomon (26) recommends a minimum protein concentration in infant formulas of 2.2 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for infants between 1 and 2 mo of age and 1.8–1.5 $\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ for infants > 3 mo of age. Because in the Greek and other cultures, babies have been fed egg yolks without adverse effects, it appears that infants at age 1 mo can tolerate the additional amount of protein provided by the egg yolk. In addition to providing DHA, Greek and fish-meal eggs are also good sources of arachidonic acid (AA), which may be necessary to prevent the reduction of AA in red blood cells and plasma phospholipids brought on by the administration of marine oils in the formulas fed to very-low-birth-weight infants (27).

We conclude that a Greek egg yolk would be useful as a supplemental or weaning food just as it has been used for thousands of years. Greek eggs have almost equal amounts of AA and DHA and therefore are particularly suited to infant feeding. It is interesting to note that in many old cultures such as in Greece, China, and the Middle East, the first supplemental food for breast-fed infants is one egg yolk mixed with sugar or honey given at age 1 mo.

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