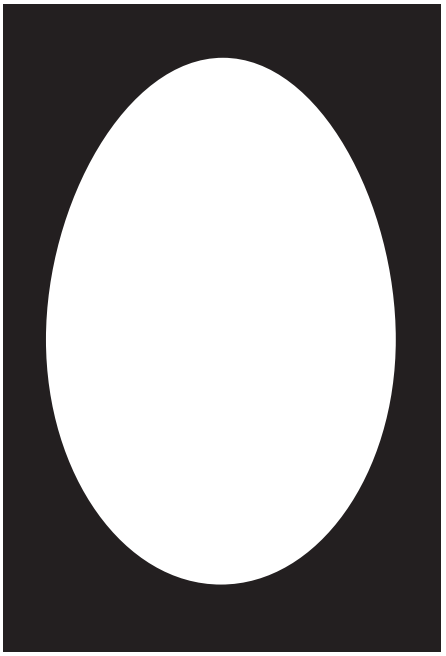
Eggs and Health Promotion

Edited by Ronald Ross Watson



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lar wall. Hansson and Frostegard et al.[38,39] found a substantial proportion of activated T lymphocytes in carotid plaque specimens, the activation pattern of which was similar to that occurring under chronic inflammatory conditions. Moss[39] confirmed that inflammation was the strongest predictor of stenosis, while cholesterol was not significantly related to the degree of stenosis. If egg intake–increased serum cholesterol level contributes to a high incidence of CHD, the presence of other possible risk factors is also required for deleterious effects to become evident.

Eggs are readily available and inexpensive. Eggs are rich sources of protein, all the essential amino acids, thiamine, riboflavin, pantothenic and folic acid, vitamin B_{12}, biotin, vitamin D_3, vitamin E, and phosphorous. The relationships among egg intake, serum cholesterol, and CHD are inconsistent. These conflicting results could be attributed to other extraneous factors. Refined theories and research designed to resolve these inconsistencies are required. Before making a decision to restrict egg intake especially in a healthy person, we should individually evaluate the advantages and disadvantages of eating eggs. Recommendations should be based on overall nutritive content, not on cholesterol content alone.

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Eggs and Saturated Fats: Role in Atherosclerosis as Shown by Animal Models

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Introduction

Many different animal models have been used to study the effects of dietary saturated fat on lipoprotein concentrations and the development of atherosclerosis. Although, in general, the animal data support observations in humans, they should be interpreted cautiously because (1) certain animal species have lipoprotein profiles during the normal and hypercholesterolemic states that are dissimilar to those of humans, (2) many studies used either no dietary cholesterol or pharmacologic doses, which can significantly influence the size of the fatty acid effect, (3) some diet treatments used variable energy densities, (4) more than one dietary component was varied in a study, and (5) the saturated vegetable oil or fat was substituted for an unsaturated vegetable oil or fat instead of a neutral control.

Animal data for the effects of individual fatty acids on plasma LDL-C concentrations and metabolism and, thus, the development of atherosclerosis are sparse. The evidence suggests that caproic acid (6:0), caprylic acid (8:0), and capric acid (10:0) are neutral with respect to their LDL-C–raising properties and their ability to modulate LDL metabolism. Lauric acid (12:0), myristic acid (14:0), and palmitic acid (16:0) are approximately equivalent in their LDL-C–raising potential by reducing hepatic LDL receptor activity and increasing the LDL-C production rate, apparently via modulation of sterol O-acyltransferase activity. Stearic acid (18:0) appears to be neutral in its LDL-C–raising potential and how it affects LDL metabolism.

For many years, diet-induced hypercholesterolemia as a result of consumption of saturated fat and/or cholesterol feeding was considered an equivalent risk factor for cardiovascular disease. Although in the last several years more emphasis has been given to saturated fat consumption as a result of the greater incremental increase in plasma cholesterol, there are many who continue to argue that dietary cholesterol has an independent effect on atherosclerosis. The epidemiological studies of Stamler and Shekelle[1] and Shekelle and Stamler[2] have often been used to suggest that dietary cholesterol increases the risk for atherosclerosis beyond its plasma LDL-C–raising effects, though to

this day the mechanism remains unknown. Although these studies are epidemiological in nature, they are often overinterpreted.

Numerous recent studies have described the effects of saturated fat and individual saturated fatty acids on plasma total and lipoprotein cholesterol concentrations in animal models (for review, see Spady et al.[3]). In contrast, the few recent experimental animal studies that examined the effects of saturated fat on atherosclerosis are not readily available, and virtually no data exists on the influences of individual fatty acids on the atherogenic process. However, one review article summarized several studies conducted nearly four decades ago to test whether different fatty acids added to a cholesterol-enriched diet influenced the incidence and extent of atherosclerosis in hypercholesterolemic rabbits.[4]

While animal studies have shown a clear relationship between saturated fat consumption and atherosclerosis, the association of cholesterol consumption alone with atherosclerosis is less clear because many studies included saturated fat along with the dietary cholesterol and/or fed pharmacological doses of dietary cholesterol.

Steps in the Development of Atherosclerosis

The role of hypercholesterolemia in the development of atherosclerosis as described in the following section represents a synthesis of information derived from the reviews by Ross,[5] Steinberg et al.,[6] and Nicolosi and Stucchi.[7] Elevated serum cholesterol levels associated with the uptake by arteries of atherogenic lipoproteins such as LDL, very low-density lipoprotein (VLDL) remnants, and /or β-VLDL appear to be a necessary prerequisite for initiating atherogenesis (Fig. 11.1). The enhanced uptake of these atherogenic lipoproteins may be associated with nonspecific endothelial dysfunction or with endothelial injury resulting from risk factors such as hypertension, smoking, or diabetes. If endothelial dysfunction exists, once these lipoproteins move into the subintimal space, their residence time may be extended by their interaction with proteoglycan substances that make up the connective tissue matrix of the blood vessel wall and bind avidly to apo B–containing lipoproteins such as LDL and VLDL remnants. This increase in the residence time of these lipoproteins provides a greater opportunity for oxidative modification of LDL. Oxidative LDL acts as a chemoattractant, causing more monocytes to adhere to and penetrate the endothelium. In the presence of endothelial dysfunction, there is increased adhesion of monocytes to the endothelial and movement into the subendothelial space, where they are converted to macrophages. Macrophages, having receptors for modified LDL, can take up more of the modified LDL, forming many intracellular cholesterol ester–laden lipid droplets, ultimately resulting in conversion of the macrophage into a foam cell. The foam cells, engorged with oxidized lipid and apoproteins, may lyse, releasing their content into the extracellular space. This oxidized material is cytotoxic and causes further endothelial injury.

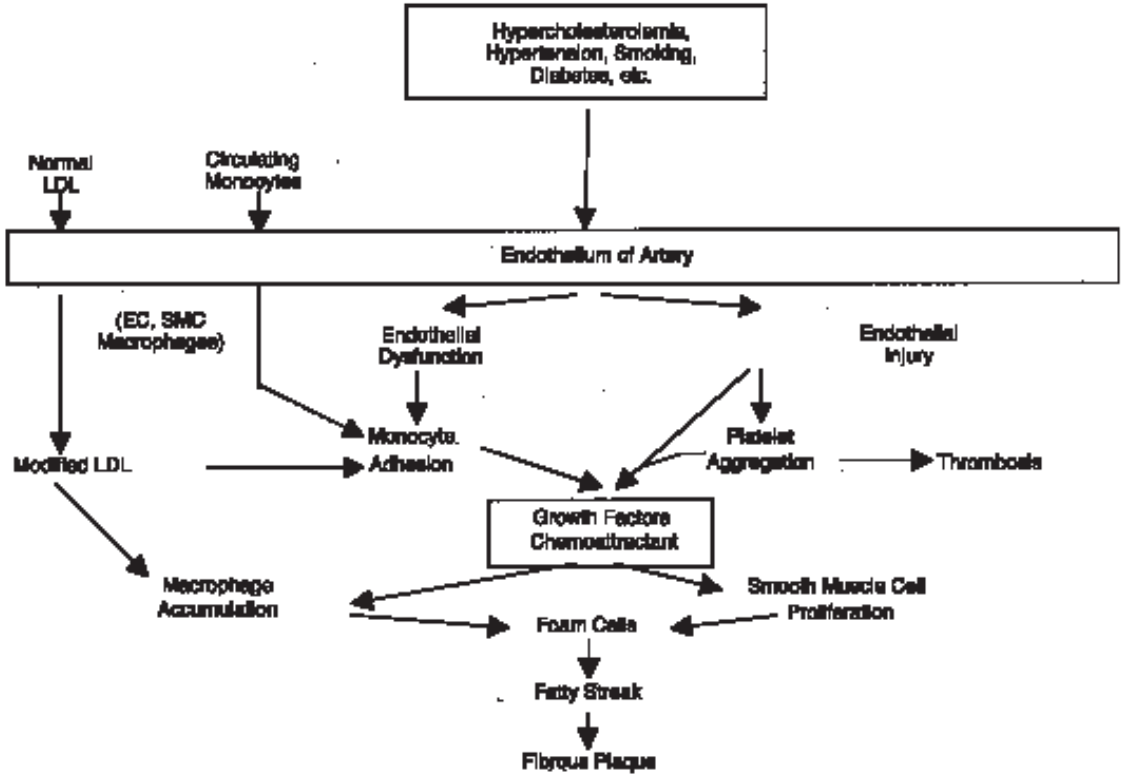

Figure 11.1. Steps in atherosclerosis. LDL = low-density lipoprotein, EC = endothelial cells, and SMC = smooth muscle cells.

Studies of Atherosclerosis in Nonhuman Primates

Nonhuman primates represent an ideal animal model for human atherosclerosis. They develop arterial lesions, which resemble those seen in human beings, and often respond to lipidemic stimulus with the production of lipoproteins resembling those seen in humans. The disadvantages are that nonhuman primates are expensive to purchase and maintain and they are often difficult to handle. Nevertheless, there has been considerable activity in this area of research. The extent of the research effort is a function of consistent availability, and there are now some locally based breeding colonies that assure a reliable, steady supply and that allow for testing of genetic characteristics through selective breeding.

Cebus Monkeys (Cebus albifrons)

Wissler et al.[8] fed cebus monkeys diets containing 0.5 percent cholesterol and 25 percent butterfat, coconut oil, or corn oil. Aortas from all the coconut oil–fed monkeys contained lesions compared with a 75 percent and 0 percent incidence for the butterfat– and corn oil–fed monkeys, respectively.

Rhesus Monkeys (Macaca mulatta)

Almost a half-century ago, Mann and Andrus[9] found that when adult rhesus monkeys were fed a diet high in fat and cholesterol for almost 4 years, they exhibited extensive atherosclerosis of the aorta and all its major branches. The diet was based on dried egg yolk (45 percent) plus 5 percent added cholesterol (providing 6.5 g cholesterol per 100 g diet) and 10 percent corn oil as fat. After 10 months of feeding, serum cholesterol had risen to 1200 mg/dl (31 mM/l) and remained elevated for the duration of the study. The serum ß-lipoproteins (S_f 12-20) were also grossly elevated. The vascular lesions were similar to those seen in human atherosclerosis. In a study with monkeys fed high concentrations of dietary cholesterol (2 percent) and butter, peanut oil, or corn oil (25 percent), less atherosclerosis was found in the monkeys fed corn oil.[10]

Cynomolgus Monkeys (Macaca fascicularis)

The cynomolgus monkey exhibits naturally occurring atherosclerotic lesions.[11] These lesions are present in the aortic arch as well as in the thoracic and abdominal aorta. Diet-induced atherosclerosis in this species was observed by Armstrong,[12] Kramsch and Hollander,[13] and Malinow et al.[14] among others. Wagner et al.[15] found that compared with rhesus monkeys, aortic lesions in cynomolgus monkeys show greater intimal thickening, more extracellular lipid, a greater fibrogenic response, and higher mineral content. Bond et al.[16] also observed myocardial infarctions in cynomolgus monkeys fed a diet containing 25 percent lard and 0.5 percent cholesterol.

African Green (Vervet) Monkeys (Circopithecus aethrops)

The aortic lesions and lipoprotein changes elicited by diet in the African green monkey model resemble those seen in man.[17–19] There is an expanding literature regarding diet and atherosclerosis in the vervet. Much of the data regarding effects of dietary fat on cholesterolemia and atherosclerosis have been accumulated by Rudel and his coworkers.[20–23] They fed vervet monkeys an atherogenic diet (0.8 mg/kcal cholesterol and 40 cal percent as saturated fat) for 11 weeks and then divided them into groups of roughly equal plasma cholesterol and saturated, monounsaturated, or polyunsaturated fat (35 cal percent). There were two phases to the study, one in which monkeys were fed fat mixtures and the other in which they were fed individual fats. Total plasma cholesterol levels were maintained on the saturated fat and fell when the monkeys were fed either mono- or polyunsaturated fat. The relative percentages of LDL- and HDL-cholesterol (high-density lipoprotein–cholesterol) were generally the same on either the mono- or polyunsaturated fat diets with LDL-/HDL-cholesterol ratios between 2.4 and 3.96. In monkeys fed the mixture providing polyunsaturated fat, the LDL/HDL ratio rose to 3.23 (suggesting a drop in HDL-cholesterol levels), whereas in monkeys fed the oleic acid–rich safflower oil it fell to 1.32 (suggesting a fall in the percentage of LDL and a substantial rise in percentage of HDL).[20] Another experiment[21] showed that fish oil, fed for 2.5–3 years, led to lower plasma cholesterol levels than did lard, 231 ± 37 mg/dl (5.97 ± 0.96 mM/l) compared with 360 ± 44 mg/dl (9.31 ± 1.14 mM/l). Aortic atherosclerosis was significantly less severe in the monkeys given fish oil. Subsequent studies showed that feeding polyunsaturated fat from an early age reduced risk for coronary artery atherosclerosis.[22,23]

A study using African green monkeys also supports the atherogenicity of saturated fat.[24] From birth to the age of 5 years, monkeys were fed diets containing 40 percent of energy as fat as either lard (saturated fat) or safflower oil (polyunsaturated fat) with 191 ng cholesterol/J (0.8 mg/kcal). The animals fed the polyunsaturated fat had coronary intimal lesions one-fourth the size of the lesions in animals fed the saturated fat. In addition, the abdominal aorta of the group fed saturated fat contained more sterol clefts and free cholesterol, suggesting more complicated lesions. A similar study in African green monkeys fed 40 percent of energy as fat with 22 percent of energy from the n-3 polyunsaturated fat menhaden oil or from the saturated fat lard with 191 ng cholesterol/J (0.8 mg/kcal) confirmed the atherogenicity of saturated fat.[25] Monkeys fed the diet containing saturated fat for up to 36 months had significantly more coronary artery disease (CAD) and more atherosclerosis in the thoracic aorta and common carotid arteries than did monkeys fed the polyunsaturated fat.

Baboons (Papio ursinus)

There is considerable literature about the effects of diet on blood cholesterol and lipoprotein metabolism in baboons; however, there is relatively little information concerning diet and atherosclerosis development. Gillman and Gilbert[26] published a study of 59 female

and 26 male baboons (*Papio ursinus*) aged between 3 and 15 years subsisting on a diet low in cholesterol. From their study they concluded (1) a one to one relationship does not exist between level of dietary fat and serum lipids or between serum lipids and atherosclerosis, (2) accumulation of aortic fat is not necessarily dependent on blood lipids but appears to be secondary to alterations in the aortic tissue that favor binding fat and calcium, and (3) the functional and structural integrity of the intima and media are determined not only by their intrinsic properties but also by factors arising beyond the vascular tree.

McGill, Jr., et al.[27] examined the arteries of 163 free-ranging Kenyan baboons and found that about 75 percent of the adults exhibited some degree of fatty streaking in the aorta. The amount of streaking increased with age. Strong and McGill, Jr.,[28] fed baboons high-cholesterol diets for 2 years. Serum cholesterol increased moderately, and there were some fatty streaks but no advanced lesions. A 4-year experiment in which a diet high in fat and cholesterol was fed[29] led to moderate cholesterolemia, but on necropsy, the principal lesion was still only a fatty streak. Cholesterol-fed baboons exhibited a positive relationship between aortic fatty streaks and elevated serum levels of LDL- and VLDL-cholesterol. There was also a negative relationship between HDL-cholesterol levels and aortic fatty streaks.[30]

In many of these studies, fat was fed along with high concentrations of cholesterol, making it difficult to identify relative contributions of the different dietary fats to atherogenesis. However, in one study in which squirrel and cebus monkeys were fed the saturated fat coconut oil but no dietary cholesterol, elevated plasma cholesterol concentrations were associated in squirrel monkeys with significant atherosclerosis compared with monkeys fed corn oil.[31] Similarly, cynomolgus monkeys fed 41 percent of energy as fat (butter, olive and corn oil) with 0.1 percent cholesterol had significantly more atherosclerosis than did monkeys fed a diet in which butter was replaced with peanut oil.[32]

The foregoing studies suggest that primate models of atherosclerosis are variable and can be influenced by diet and stress. A major aim of human studies is to effect regression of pre-established lesions. Regression of atherosclerosis in primates has been reviewed by Stary[33] and Malinow.[34] Reversion of primates from an atherogenic diet to one low in fat and cholesterol will lead to diminution of lesion severity in rhesus and cynomolgus monkeys. Wagner et al.[35] fed rhesus monkeys an atherogenic diet for 19 months. Twelve animals were necropsied at 19 months to provide baseline data. The remaining 36 monkeys were fed diets designed to maintain their plasma cholesterol levels at about 200 or 300 mg/dl for an additional 48 months. More cholesterol was lost from arteries of monkeys maintained at 200 mg/dl than those kept at 300 mg/dl. Eggen et al.[36] have shown that pre-established lesions in rhesus monkeys can regress even when fed a diet free of cholesterol but high in saturated fat for 30 or 52 weeks. Vesselinovitch et al.[37] have demonstrated that rhesus monkeys fed a prudent diet exhibit about 70 percent fewer aortic lesions than those fed an atherogenic diet for 14 months and 77 percent fewer lesions than monkeys fed the same diet for 48 months. The primate model is useful for the study of both progression and regression of atherosclerosis, and it is closer to humans than the other available models.

Studies of Atherosclerosis in Rodents

In rodent studies, the effect of feeding saturated fat is often evaluated in animals fed high concentrations of cholesterol, which makes identifying the specific fat effects impossible.

Hamsters

In one study, hamsters were fed coconut oil along with high concentrations of dietary cholesterol (3 percent), which prevented assessing different dietary fat effects on atherosclerosis.[38] However, in other studies, this same saturated fat was shown to produce early atherosclerosis at more typical concentrations of dietary cholesterol.[39,40] Recently in a study conducted in our laboratory,[41] golden Syrian hamsters were fed diets containing various levels of dietary coconut oil (saturated fat) or cholesterol and 10 percent cocoa butter (neutral fat) for 10 weeks. When the data were compared based on similar plasma LDL-C, the hamsters fed the cholesterol-containing diets had significantly less early atherosclerosis compared with the animals fed the coconut oil-containing diets, at lower plasma LDL-C levels (<1.3 mmol/l). However, at higher plasma LDL-C (>1.9 mmol/l), the hamsters fed the cholesterol-containing diets had early atherosclerosis significantly more often than hamsters fed the coconut oil–containing diets.

In a recent study,[42] hamsters were fed a diet containing 20 percent total fat and 0.12 percent cholesterol where the different test diets contained either 8:0, 14:0, *cis*-18:1, or *trans*-18:1 at approximately 50 percent of the total fat. While plasma LDL-C concentrations were significantly higher in the 14:0 diet only, formation of early atherosclerotic lesions was greater in both the 14:0 and 8:0 diets compared with the other diets.

Rabbits

For rabbits with comparable serum cholesterol concentrations, a diet containing cholesterol and methyl stearate was more atherogenic than diets containing methyl oleate or methyl linoleate.[43] A study on the effects of naturally occurring fats on pre-established atheroma in rabbits fed 2 percent cholesterol and either 5 percent corn oil or coconut oil found that corn oil retarded the progression of lesions relative to coconut oil.[44] In another series of investigations examining the effects of fat saturation on atherosclerosis in rabbits with comparable serum cholesterol concentrations, palm oil feeding resulted in more atherogenesis than did cocoa butter feeding, but both produced more severe lesions than did corn oil treatment.[45] In a similar experiment with rabbits, palm oil was found to be as atherogenic as coconut oil, and cocoa butter was more atherogenic than corn oil but less than coconut and palm oils.[46] This raised the question of whether 12:0, 14:0, 16:0, and 18:0 affect atherogenicity differently.

To address this question, for several weeks rabbits were fed corn oil or corn oil in which the triacylglycerol structure was interesterified with 12:0, 14:0, 16:0, and 18:0, and athero-

genesis was then evaluated. A measure of the atherogenic index revealed that 12:0-, 14:0-, and 16:0-enriched diets produced more atherosclerosis than did the 18:0-enriched diet, but that all were higher than the index for the control corn oil diet.[47] In swine fed butter or two different concentrations of corn oil for 12 weeks, the reductions in serum cholesterol concentrations for both corn oil treatment groups relative to butter were accompanied by rather striking decreases in atherogenesis.[48]

Apo E–Knockout Mice

Apolipoprotein E3-Leiden (APO) transgenic mice develop hyperlipidemia and are highly susceptible to diet-induced atherosclerosis. The progression and histopathology of lesions in this animal model show similar features to those observed in humans and other species, including fatty streaks, necrotic cores, and fibrous caps.[49–51]

Calleja et al.[52] investigated the effect of palm, coconut, olive, and sunflower oils (10 percent wt/wt) without the addition of cholesterol for 10 weeks on the development of atherosclerosis in apo E–knockout mice. None of the diets induced changes in plasma cholesterol concentrations, whereas plasma triglycerides were uniformly reduced in all diet groups. Some diets caused significant reductions in the size of atherosclerotic lesions in males and others in females; males responded most to sunflower oil and females to palm and olive oils.

In another study,[53] apo E-knockout mice were fed a diet containing 15 percent cocoa butter, 0.5 percent cholate, and 1 percent cholesterol for 12 months. After 12 months on the diet, lesions in the aortic arch showed calcification and lesions with a fibrous cap were observed at both the right and left carotid artery bifurcations. Moreover, 5 of 12 mice showed calcifications in their coronary arteries. Groot et al.[54] investigated APOE*3 Leiden mice fed a high–saturated fat/high-cholesterol/0.5 percent cholate diet for 6 months. They observed 5 to 10 times greater lesion areas in transgenic mice compared with nontransgenic mice.

Eggs and Cholesterol Consumption and Atherosclerosis in Animal Models

In the majority of animal studies that examined the influence of eggs/egg yolk consumption on plasma cholesterol levels and the development of atherosclerosis, eggs were used for the single purpose of being a dietary source of cholesterol to produce a hypercholesterolemic state in the animal model. However, a few studies did examine the direct effect of feeding eggs/egg yolk on plasma cholesterol levels.

In a study conducted by Srilatha et al.,[55] healthy, nonobese, young rabbits developed hypercholesterolemia following daily intake of fresh egg yolk for 8 weeks. On a quantitative basis, this diet had a profound effect on serum cholesterol level, which rose to 15 or 30 times the baseline value depending on whether the test group consumed the yolk content of one or two eggs during the study. In another study,[56] the feeding of powdered ver-

sus fresh egg yolks to young male chickens up to 30 and 43 weeks of age, respectively, produced approximately the same increase in plasma cholesterol, liver fat, and liver cholesterol when compared with the feeding of a soybean-based cholesterol-free diet. Also, aortic atherosclerosis, however, was found to be more severe in a group of birds fed fresh egg yolks than in those fed powdered egg or the cholesterol-free diet.[56]

However, when consuming eggs or egg yolks, dietary cholesterol is not the only nutrient that is consumed. Other possible cholesterol-lowering nutrients, such as lecithin, are found in sufficient quantities in eggs. O'Brien and Corrigan[57] compared the influence on plasma and tissue lipids of dietary soybean and egg lecithins, which have contrasting fatty acid compositions, in hypercholesterolemic guinea pigs. Among the most noteworthy observations were the 49 percent decrease in total plasma cholesterol of animals fed 7.5 percent soybean lecithin without decreasing HDL-C and the 177 percent increase in plasma HDL-C of the animals fed 7.5 percent egg lecithin without a significant increase in total cholesterol compared with control-fed animals.[57]

Conclusion

There are substantial data related to the hypercholesterolemic properties of animal fat and vegetable oils that contain high amounts of saturated fatty acids. Early studies conducted mostly in rabbits but also pigs and monkeys indicate that diet-induced hypercholesterolemia is associated with atherosclerosis. These studies suggest that, in general, the atherogenicity is comparable for animal fat and vegetable oils enriched in 12:0, 14:0, and 16:0, and the atherogenicity of these is greater than that of fats enriched in 18:0. The data relating specific fatty acids to atherosclerosis are sparse, and although the relative effects of 12:0, 14:0, and 16:0 appear to be similar collectively (and probably individually), they are more atherogenic than 18:0. Additional studies are needed to clarify the cholesterolemic and atherogenic effects of the individual saturated fatty acids.

While consumption of eggs, particularly egg yolks, has become a concern for most people because of their dietary cholesterol, there are many other nutrients that may have other beneficial health effects for humans. Most recent is the development of chicken eggs that have been naturally incorporated with omega-3 polyunsaturated fatty acids through the chickens' diet.

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12

Health Effects of Docosahexanoic Acid (DHA)–Enriched Eggs

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Introduction

Docosahexanoic acid (DHA, 22:5 n-3), one of the major n-3 long-chain polyunsaturated fatty acids (LCPUFAs), is essential for the growth and functional development of the brain in infants as well as for maintenance of normal brain function in adults. The inclusion of plentiful DHA in the diet improves learning ability, whereas deficiencies of DHA are associated with deficits in learning. DHA is good for the eyes and helpful in recovery from certain visual dysfunctions. The visual acuity of healthy, full-term, formula-fed infants is increased when their formula includes DHA. DHA also has a positive effect on diseases such as heart disease, thrombosis, and atherosclerosis.

Humans obtain DHA primarily from their diets because they are capable of synthesizing only small amounts of DHA.[1] Therefore, researchers have explored a variety of food supplements. This chapter discusses how DHA-enriched eggs promote health. The nutritional manipulation of the diets of laying hens to include sources of n-3 fatty acids (FAs) promotes the deposition of these nutrients into egg yolk.[2] Therefore, DHA-rich eggs may provide an exciting alternative food source for enhancing consumer intake of these healthful FAs.

Interactions between Polyunsaturated Fatty Acids (PUFAs)

Fat is an essential component of the human diet. The essential fatty acids are the critical components of fat. Essential fatty acids include linoleic acid (LA) and its n-6 derivative, arachidonic acid (AA). More recently, the vital role of α-linolenic acid (α-LNA) and its n-3 derivative, DHA, has been recognized.[1] The active components in both series are the longer-chain acids such as AA and DHA. These are produced by desaturation and elongation or obtained from the diet. A high ratio of LA to α-LNA causes a depletion of the longer-chain n-3 fatty acids (FAs), including DHA, by competing for the enzymes necessary for desaturation and elongation.[3]

Humans originally consumed a diet rich in the n-3 FAs and low in saturated FAs because wild and free-range food animals have much higher contents of n-3 FAs than do present-day commercial livestock. The dietary supply of FAs previously contained a 1:1 ratio of n-6 to n-3 PUFAs.[1] The present ratio in the United States is greater than 10:1, causing a deficiency of the n-3 FAs. An increased intake of LA and an elevated ratio of n-6 to n-3 FA is a major risk factor for Western-type cancers, thrombosis diseases, apoplexy, and allergic hypersensitivity.[1] The World Health Organization is now recommending a ratio of between 3:1 and 4:1 for n-6 to n-3 FAs, an increase of the n-3 FAs portion in the human diet. As we know, dietary α-LNA can be metabolically converted to DHA; however, the enzymes involved in this conversion are common to the pathway for the elongation and desaturation of LA, and competition with n-6 FAs will reduce the amount of α-LNA converted (Fig. 12.1).[3,4]

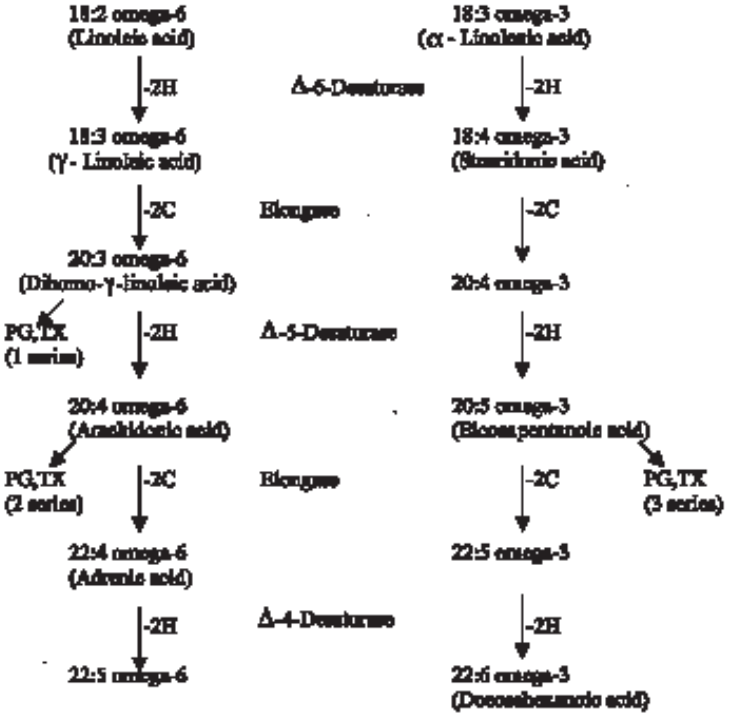

Figure 12.1. Interactions among polyunsaturated fatty acids. Modified from Reference 3.

Food Sources of DHA

DHA is more prevalent in fatty fish (salmon, tuna, and mackerel) than in meat and eggs. DHA is usually present in human milk instead of in infant formula.[1] Eicosapentanoic acid (EPA), another long-chain n-3 fatty acid, also exists in fatty fish. However, fish is not consumed regularly or in large quantities in many Western countries.[5] In the United Kingdom and the United States, daily average intakes of DHA and EPA are extremely small, and a dietary insufficiency of n-3 PUFAs is likely to occur.

Fish oil is currently the primary dietary source of EPA and DHA, which is associated with reduced cardiovascular diseases. However, fish oil consumption is still very limited due to availability and cost.[6,18] Various sources of DHA have been developed for human DHA intake. Poultry meat is often an important source of these n-3 PUFAs in the United States because fishmeal may be added to poultry diets.[5]

Other forms of DHA are now being developed. It is well-known that the composition of the fatty acids in egg yolk can be altered.[5] Eggs enriched with DHA are on the market in several countries. Woobang Science Company, Ltd., is now producing Edison 300TM eggs, containing at least 300 mg DHA per 100 g of egg, in Korea. This company provided a feed supplement for laying hens containing fish oil, flaxseed, and a mixture that stimulates the conversion of n-3 PUFAs to DHA.[6] Experiments were also conducted to investigate the usefulness of a natural golden marine algae (MA) as a poultry ration supplement for the production of shell eggs rich in n-3 FAs.[7] A fermentation production technology for *Schizochytrium* was developed that utilized inexpensive nutrients and achieved high culture densities, short production cycles, and very high concentrations of DHA in the algae. DHA-enriched eggs were produced by feeding dried *Schizochytrium* fermentation products to laying hens (Table 12.1).[8,9] Because eggs can be made into so many products, eggs enriched with DHA by feeding hens a microalgal feed source provide consumers with a wider variety of food sources containing DHA than does fish oil or flaxseed.[10] Research on the DHA enrichment of eggs and their effects on humans as they consumed these eggs and egg products has been conducted in several areas.

Health Benefits of DHA

Nervous System in Infants

The brain is 60 percent structural lipid, which universally uses AA (20:4n-6) and DHA (22:6n-3) for growth, function, and integrity.[11] Experimental evidence in animals has demonstrated that the effect of essential fatty acid deficiency during early brain development is deleterious and permanent. The risk of neurodevelopment disorder is highest in the very low birth weight babies. Babies born with low birth weight or prematurely are most likely to have been born to mothers who were inadequately nourished, and the babies tend to be born with AA and DHA deficits. Because brain development disorders can be permanent, proper provision should be made to protect the AA and DHA status of both term and preterm infants to ensure optimum conditions for the development of membrane-

Table 12.1. Lipid components in DHA-enriched eggs laying hens

Lipids	Market egg	DHA egg
Saturated fatty acids		
C14:0	34	50
C16:0	2,226	2,310
C18:0	784	790
C20:0	10	<10
C22:0	12	<10
Monounsaturated fatty acids		
C14:1	8	<10
C16:1	298	300
C18:1	3,473	3,530
C20:1	28	20
C22:1	3	<10
Polyunsaturated fatty acids		
C18:2 n-6	1,148	1,100
C18:3 n-3	33	150
C20:4 n-6	142	100
C22:6 n-3	37	[a]270
Cholesterol	425	455

Source. Modified from Reference 9.
[a]Significantly different content between market and DHA eggs.

rich systems such as the brain and the nervous and vascular systems. Another evidence of DHA physiological function in nervous tissue membrane is that the concentration of DHA in the membranes of the retina and brain is very high and the deficiency of DHA results in loss of visual acuity in monkeys and human infants.[4]

DHA plays an important role in the maintenance of normal neural functions, a role that n-6 FAs cannot fill. The outgrowth of neurites induced by nerve growth factor is promoted by DHA. Part of the unique function of DHA in the nervous system seems to relate to the synthesis of phospholipids (PLs) for the membranes needed for neurite elongation.[1] During fetal development, DHA can be preferentially transported across the placenta into the fetal circulation to match the requirement of fetal growth, especially in the brain and vascular systems.[12] DHA deficits may lead to deficits in learning ability because DHA is involved in cell signaling. DHA is the predominant structural FA in the brain's gray matter and retinal tissues in humans and other mammals.[1] Infant brain growth requires the formation of large amounts of neural membrane. Synaptic membranes are especially rich in two LCPUFAs: DHA and AA. Potential sources of LCPUFA for supplementation include fish oils and egg lipids. In animal studies dietary shortages of both DHA and its precursor α-LNA have resulted in reduced levels of DHA in the brain and adverse effects on early brain development (e.g., visual deficits in a primate model and altered learning behaviors in a rat model).[13]

Many independent studies indicate that the mental development and visual acuity of infants are positively affected by breast-feeding and that breast-fed infants have higher levels of DHA in the brain tissues and enhanced mental ability later in life when compared with those fed infant formula not containing DHA.[1] A study was done to determine whether DHA supplementation of breast-feeding mothers increases the DHA contents of breast milk and infant plasma PLs. Breast-feeding women were randomly assigned to three DHA-supplementation groups (170–260 mg/d) and a control group.[14] Group 1 (n = 6) consumed an algae-produced high-DHA triacylglycerol; group 2 (n = 6) consumed high-DHA eggs; group 3 (n = 6) consumed a high-DHA, low-EPA marine oil; and group 4 (n = 6) received no supplementation. Correlations between the DHA contents of maternal plasma and breast milk and of milk and infant PLs were significant. Breast milk and maternal and infant PL 22:5n-6 concentrations were lowest in group 2. DHA supplementation increases the plasma and breast milk DHA concentrations of lactating women, resulting in higher PL DHA concentrations in infants. There were significant correlations between the contents of all long-chain (containing >18 carbons) n-3 and n-6 polyunsaturated fatty acids in maternal plasma phospholipids and the contents of these fatty acids in milk. The correlation between maternal plasma phospholipid DHA and milk DHA was particularly strong. Similar, although weaker, correlations were observed between the contents of these fatty acids in milk and those in infant plasma phospholipids.

Since DHA is not available in most infant formulas, supplementing could be an alternative source of DHA. For example, eggs laid from Greek chickens (called Greek eggs) contain more DHA (22:6 n-3) and less LA (18:2 n-6) and LNA (18:3 n-3) than do eggs from hens fed fish meal or flaxseed. Two to 3 g of Greek egg yolk may provide an adequate amount of DHA and AA for a preterm neonate.[15] With proper manipulation of the hens' diets, eggs could be produced with FA composition similar to that of Greek eggs as a source of DHA to supplement infant formula.

Cardio-Protective Effects

The leading cause of death in Western countries is cardiovascular disease.[16] The increase in deaths due to coronary heart disease in these countries has been blamed on the increased consumption of saturated fats. The American Heart Association estimates that 57 million Americans have cardiovascular disease, causing 954,000 deaths annually and costing $259 billion per year. Many billions of dollars are spent annually on developing drugs. Although drugs such as cholestyramine and clolibrate are effective in reducing triglycerides and LDL-cholesterol and increasing HDL-cholesterol, they have significant side effects. Better nutrition with plenty of long-chain n-3 FAs, especially DHA, can produce the same lipid changes and positive effects with no side effects and much less expense.

Epidemiological studies have shown a strong correlation between fish consumption and reduction in sudden death from myocardial infarction. The reduction is approximately 50 percent with 200 mg per day of DHA from fish. DHA is the principal active component in fish for cardiovascular protection.[17] DHA supplements increase the HDL-/LDL-choles-

terol ratio and decrease the total cholesterol/HDL ratio, suggesting a decreased risk for coronary artery disease. Even an intake of only 2.9 g of EPA and DHA per month was associated with a 30 percent reduced risk. Blood samples taken after 16 and 22 weeks from fasting subjects showed significant increases in EPA, DHA, and total n-3 PUFAs in subjects consuming enriched eggs compared with controls.[5] Serum triglyceride concentrations in human subjects consuming the n-3 fatty acids EPA plus DHA are consistently reduced, and platelet n-3 fatty acids consistently increased.[4] In addition, the ratio of n-6 to n-3 PUFAs in plasma was significantly reduced from 12.2:1 to 6.5–7.7:1 in subjects consuming enriched eggs compared with controls (Table 12.2). Consumption of only one enriched egg daily can contribute substantially to the recommended daily intake of n-3 PUFAs. Moderate lowering of plasma triglyceride concentrations was observed in human subjects consuming eggs enriched in EPA and DHA from hens fed fish oil. Feeding hens ground flaxseed has produced eggs high in α-LNA. Although less pronounced than for α-LNA, the DHA concentration is also significantly increased in these eggs.[4]

Platelet function and blood coagulation play important roles in coronary artery disease. Flaxseed oil decreased collagen-induced platelet aggregation and thromboxane production.[4] Both platelets from human subjects consuming DHA and human platelets incubated in vitro with DHA exhibited depressed platelet reactivity. Excessive platelet aggregation can lead to arterial thrombosis, while n-3 FAs are associated with reduced platelet aggregations as well as the risk of coronary heart disease. The accumulation of DHA PLs, as observed for modified-egg consumers, provided some added health benefits in addition to its role in neuronal functioning. Additionally, EPA can inhibit the aggregation of blood platelets, an important factor in reducing the incidence of cardiovascular disease. DHA can also fulfill this function through retroconversion to EPA. The intermediate compound in this process is docosapentanoic acid (DPA).[5]

On the other hand, in the pathogenesis of atherosclerosis, vascular smooth muscle cell growth is an important component. These cells have the potential to proliferate and accumulate lipids. Cyclins and cyclin-dependent kinases control progression through the eukaryotic cell cycle and thus the proliferation of cells. DHA and EPA inhibit DNA synthesis through G1 cyclins, cyclin-dependent kinase inhibitor (p27), and stop the progression from the G1 to the S phase. By this mechanism, DHA and EPA inhibit the proliferation of vascular smooth muscle cells.[1]

Conclusion

Eggs are not only widely accepted as cheap and complete nutritional packages but also can now be used to meet a substantial part of the recommended dietary intake of n-3 PUFAs because the ratio of n-6 to n-3 FAs can be properly balanced by ingesting specialized hen eggs. Attempts have been made to produce eggs that are rich in DHA; such eggs have been shown to not raise cholesterol and lower the ratio of n-6 to n-3 (Table 12.1). Upon consumption of DHA-enriched eggs, serum and platelet lipid alterations have been associated with a reduced risk of myocardial infarction and thrombosis. People on a Western diet may also improve their cardiovascular risk factors by consuming DHA-

Table 12.2. Fatty acid composition of lipid in control and modified eggs

Fatty acid	Amount of flax in diet of hen		
	0%	10%	20%
	(% by wt. of total fatty acids)		
16:0	23.3 ± 0.5	22.5 ± 0.3	21.0 ± 0.4
16:1	2.1 ± 0.2	2.4 ± 0.3	3.0 ± 0.3
18:0	7.8 ± 0.1	8.1 ± 0.1	6.3 ± 0.8
18:1	40.8 ± 0.9	39.4 ± 0.6	36.5 ± 1.2
18:2n-6	16.8 ± 1.1	15.2 ± 0.4	16.3 ± 0.5
18:3n-6 (α-LNA)	0.5 ± 0.04	5.5 ± 0.4	10.7 ± 0.4
20:4n-6	2.2 ± 0.16	1.1 ± 0.1	0.9 ± 0.06
20:5n-3 (EPA)	0.1 ± 0.05	0.2 ± 0.05	0.2 ± 0.03
22:4n-6	0.4 ± 0.07	0.3 ± 0.02	0.2 ± 0.04
22:5n-3 (DPA)	0.2 ± 0.05	0.3 ± 0.03	0.4 ± 0.1
22:6n-3 (DHA)	1.0 ± 0.05	[a]1.7 ± 0.08	[a]1.8 ± 0.08
Saturates	32.3 ± 0.5	31.7 ± 0.3	28.2 ± 0.7
ΣMonounsaturates	43.8 ± 0.7	42.4 ± 0.5	4.0 ± 1.2
ΣPolyunsaturates	23.9 ± 1.2	25.9 ± 0.6	31.8 ± 0.9
Σn-6 PUFAs	21.4 ± 1.1	17.7 ± 0.4	18.2 ± 0.6
Σn-3 PUFAs	2.4 ± 0.2	8.2 ± 0.4	13.5 ± 0.5
Ratio of n-6 to n-3	9.3 ± 0.7	[a]2.2 ± 0.1	[a]1.4 ± 0.05

Source: Modified from Reference 4.

Note. α-LNA = α–linolenic acid, EPA = eicosapentanoic acid, DPA = docosapentanoic acid, DHA = docosahexanoic acid, and PUFA = polyunsaturated fatty acid.

[a]Significantly different from 0% treatment group, $p < 0.05$.

enriched foods. DHA from egg yolk and from mother's milk is very helpful to the infant brain and vascular system development as well as visual acuity.

Although the FA composition of hen eggs can be changed by dietary means, eggs with significant amounts of n-3 PUFAs frequently have an unusual flavor.[18] This can be overcome by using a mixture of commercially available chemical antioxidants and vitamin E.[4] Making the egg flavor as normal as possible is very important if people are going to accept these eggs as food rather than as "medicine."

However, due to the complexity of fatty acids' interactions, sufficient availability of both n-3 and n-6 LCPUFAs, rather than n-3 alone, is required for optimal structural and functional development in infants.[13] Inclusion of 0.2–0.3 percent DHA ensures maximal DHA accretion in the retina of newborn piglets, but cosupplementation with AA is necessary (1) to achieve the same status of blood lipids as that which results from maternal feeding and (2) to prevent any possible imbalance between n-6 and n-3 FAs.[19] One issue that has not been resolved is the origin of DHA and AA for infant neural development.[20] Hence, additional study should be undertaken on whether infants should be dependent on dietary sources before adopting routine use of LCPUFA. Another question concerns the quantity of supplement. In a previous study,[4] we found that eggs are obtained with DHA

of 81 and 87 mg/egg by feeding hens diets containing 10 and 20 percent ground flaxseed, which resulted in no significantly different DHA composition between the 10 and 20 percent flax groups (Table 12.2). This result leads to the question of why the concentration change of DHA eggs is not linear to the amount of flax added. It is possible that eggs may have a saturation curve for the maximum absorption of DHA from diet, which might be related to the metabolism of DHA in chickens. Further study needs to be done in order to determine the most efficient quantity of supplementation and to obtain an optimum n-6 to n-3 ratio as well.

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13

The Correlation between Cholesterol Oxidation Products and Eggs

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Introduction

Cholesterol has remained the nemesis of coronary heart disease (CHD) and atheroschlerosis for many years. Research has shown, however, that dietary cholesterol is not as potent to serum cholesterol as once thought. While the debate over dietary cholesterol ebbs and flows, oxysterols have become the center of a new debate. Oxysterols include lipid peroxidation products as well as cholesterol oxidation products. Because the cholesterol content of eggs is higher than the lipid content, the cholesterol oxidation products are of more importance. Cholesterol oxidation products include the following:

7α-hydrocholesterol
7β-hydrocholesterol
7-ketocholesterol
cholestanetriol
epoxycholesterol
26-hydroxycholesterol
25-hydroxycholesterol

Cholesterol oxidation products (COPs) result from the auto-oxidation of cholesterol in vitro or the metabolic products of cholesterol in vivo. Although foods that provide abundant amounts of cholesterol usually also contain COPs, there is no direct link between cholesterol disappearance and COP creation.[9] The creation of COPs in high amounts is proportional to determining factors such as light exposure, heat application, and storage time. It is now believed that COPs may elevate the risk of heart disease by contributing to atherogenicity and cytotoxicity (cell death). While it is primarily accepted that dietary cholesterol does not directly influence atherosclerosis, COPs are believed to be involved. However, the exact concentration of ingested COPs that could lead to health complications remains unknown.[4]

Health Risks Associated with COPs

COPs are now being suspected of causing a number of cellular and cardiovascular health problems. Factors that support this claim include the ability of COPs to inhibit sterol biosynthesis, cause membrane dysfunction, and increase cellular calcium leading to cell death.[4] The COP known as 25-hydroxycholesterol has been shown to cause damage to the wall of the aorta, thus beginning the atherosclerotic process.[9] Other COPs have the ability to inhibit the activity of HMG-CoA reductase. A decrease in the activity of this enzyme has the effect of disallowing cholesterol biosynthesis by the body. Cholesterol is needed for the structure and rigidity to be maintained within the plasma membrane. With a decrease in cholesterol, COPs can take the place of the cholesterol in the membrane but cannot provide the membranous support offered by cholesterol. This substitution can lead to cytotoxic problems[9] within the membranes of cells and eventually lead to cell death. Cytosolic and free calcium, although not affected by cholesterol, are influenced by COPs. Both cytosolic and free calcium are increased after COP exposure. Increases in intracellular calcium are known to cause cells to autodestruct. Therefore, by a similar mechanism, high levels of COPs may also lead to autodestruction.[6]

Factors Involved in COP Formation

Powdered eggs have been found to be particularly high in COPs.[1] Egg powder as a food ingredient has become increasingly popular for use in pastas and baked goods. Because of this, eggs (fresh and powdered) and foods that contain powdered eggs have been the focus of much COP research. The parameters of packaging, heating, irradiation, and storage provide a wide range of COP concentrations in egg yolk powder.[2] Spray-dried egg yolk powders were found to contain a significantly higher COP concentration at room temperature than at refrigeration temperature.[2] Pasta made with dried eggs has been shown to have significant levels of COPs. Pasta drying methods include heating at 70–90°C for 10 or more hours. These conditions allow for COP formation, and it has been shown that manufacturing does significantly influence COP formation. COP formation may be controlled by monitoring time and temperature during drying.[1] The actual drying process can also have a substantial effect on the amount of COPs formed. Drying via direct heating with gas produces more COPs than drying via indirect heating with electric heat.[3] This difference was also noted after several months of storage but began to decline after the third month. Free radicals formed during the drying process known as NOx (nitric oxide and nitrite) were present in the air of the gas drying environment but not in that of the electric. When NOx was added to the electric heating environment, COP formation increased.[3] It can be deduced that the higher concentration of COPs in the gas-heated drying method can be attributed to NOx formation during that heating process. While storage has the effect of raising COP concentration within certain foods, irradiation has been shown to have a greater effect of COP formation.[9] This difference may be due to the fact that most storage occurs without light exposure.

Cholesterol Oxidation Products in the Serum

Because of their high cholesterol content, eggs have been avoided in an effort to ward off CHD. Both COPs and lipid peroxidation products are present in eggs and other dehydrated foods, but whether or not these compounds can be absorbed by the body is the focus of some recent research. Foods shown to be high in COP concentration were fed in large amounts to a group of people. The relative amounts of COPs in the blood of these individuals were tested prior to administration and postprandial. A definite rise in blood concentration of COPs was observed, but some discrepancy exists in determining how long serum levels remain elevated.[7] The time interval of serum COP elevation may be important in determining the risk of atherogenecity associated with COPs. The highest COP concentration in the blood occured 24 hours after the meal and remained stable for another 24 hours.[7] Also of some interest is the COP route of travel after being absorbed. When a particular COP was labeled and traced throughout the human body, it was found that COPs can travel in serum bound to albumin or lipoproteins as opposed to cholesterol, which is transferred primarily by lipoproteins.[8] Thus, COP transport was shown to thrive in both regular serum as well as serum that is deficient in lipoproteins. COPs are more polar in nature than cholesterol, which may explain their affinity for albumin when lipoproteins are either unavailable or not present in usable quantities. Moreover, COP concentration was markedly greater in lipoprotein deficient serum with or without albumin present.[8] This leads to the conclusion that COPs are more mobile in the body than is cholesterol and thus have more opportunity to cause damage.

Polyunsaturated Fatty Acids

The intermingling of free oxygen and the double bond in cholesterol leave cholesterol vulnerable to oxidation. It has been suggested that the byproduct of polyunsaturated fatty acid (PUFA) oxidation is needed for the initiation of oxidation of cholesterol.[9] This is supported by the notion that PUFAs are likely to be present in large amounts in foods that contain cholesterol. For example, eggs contain a large amount of oleic and linoleic fatty acids. Furthermore, there is an increased risk of cholesterol oxidation via lipid peroxidation either by natural occurring PUFAs or by eggs coming from hens that ate a PUFA-supplemented diet. PUFA damage appears to worsen as degrees of "unsaturation" and chain length increase.[5] Saturated triglycerides as well as long-chain monounsaturated fatty acid (MUFA) yield little or no cholesterol oxides. The peroxy radicals stemming from these PUFAs may be responsible for the attack on cholesterol that yields the COPs. COP formation is enhanced both by the amount of PUFAs in a food as well as the presence of transition metals, such as iron, that can promote oxysterol formation.[9]

Cholesterol Oxidation Products and Food

Because COP concentration in the blood can be influenced by dietary factors, it is important to analyze the COP concentration within the diet. Dehydrated foods such as heat-

dried egg yolk powder contain more COPs than fresh foods in general, and fresh eggs do not contain COPs in any more than a trace amount. Zunin et al. reported that when fresh eggs were frozen and stored, no COPs were formed. Baked biscuits and snacks made with egg powder yielded more COP formation than the same foods made with fresh eggs.[4] Water as an ingredient or byproduct of heating in foods seems to inhibit cholesterol oxidation. Foods that exhibit the most surface area tend to have higher levels of COPs. This partially explains the large concentrations of COPs found in powdered eggs.[4] COPs do not usually all show up in one product. Most likely different foods contain different COPs.

The Effects of Antioxidant Administration

Zunin et al. proposed that naturally occurring caretenoids in eggs inhibit COP formation by competing for free radicals.[1] When antioxidants were added to the eggs being dried in a gas-heated environment, there was no significant effect on COP formation. Egg powders from α-tocopherol–supplemented hens had a lower concentration of COPs after heating and during storage. As storage time increased, available α-tocopherol decreased. Though vitamin E appears to be able to decrease the formation of COPs, it has not been shown to prevent cytotoxicity in cells from existing COPs.[6] Studies have shown that vitamin E failed to protect cells from the induced cytotoxic effects of some of the more potent COPs. Vitamin E has been shown to protect against atherosclerotic damage by acting as a preventative antioxidant. However, in the event that COPs have already initiated damage, such as taking over a cell membrane, the time period for preventative maintenance may have expired, leaving vitamin E without the ability to decrease COP concentrations.

Conclusion

Although cholesterol oxidation products have been shown to be absorbed and cause damage within the body, the COP concentration necessary to initiate the damage remains unknown. While fresh foods containing cholesterol have only trace amounts of COPs, foods that have been dehydrated, exposed to light, or stored contain a much higher percentage of COPs. Eggs have been the focus of many studies due to their extensive use in the food industry and high initial amounts of intact cholesterol. Though eggs have numerous health benefits, the presence of COPs could be a disadvantage. This cannot be determined until further research has demonstrated exactly what range of COPs causes harmful effects within the body. Further research is also needed to illustrate the COP concentration differences among foods. Results of research in this nature could provide evidence of the degree of potential damage by eggs as compared with other cholesterol-rich foods. Research has shown no significant evidence of any substances having the ability to halt or hinder COP formation. If COPs are actually found to cause illness, more studies will be needed to search for a substance, antioxidant or otherwise, that has the ability to decrease COP formation. Cholesterol oxidation products have the potential to perform harm within the body, and although it has been shown that COP concentrations are higher in eggs than in most foods, the concentration necessary to threaten health remains unknown.

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Section 3

Eggs and Disease: Health Promotion

14

Whole Eggs: The Magic Bullet?

H.L. "Sam" Queen

mág•ic búl•let, "something that cures or remedies without causing harmful side effects."

Whole Eggs: The Magic Bullet?

Consider for a moment the following hypothetical event. Someone hands you the magic bullet for heart disease, which is then injected into a dear family member who has high cholesterol and grossly narrowed arteries. This magic bullet would enable your loved one to overcome the heart- and life-threatening consequences of such a scenario, with relatively little effort, low cost, and no unpleasant side effects. What if that magic bullet was actually (gasp!) an egg?

Whole eggs have long been maligned as a cause of high serum cholesterol and heart disease, mostly because they contain substantial amounts of fat and cholesterol. Strangely, this attitude has been taken by health authorities without ever studying whole eggs directly. Recommendations to limit daily consumption were initially based on cholesterol content and epidemiological evidence, such as Ancel Keys' Seven Country Study.[1] Over the next 30 or so years this impression was supported by a continuous deluge of animal and human studies, which heart authorities contended was indisputable evidence of cause and effect.[2] So certain were authorities that the cause of heart disease had been found that they formed the National Cholesterol Education Program (NCEP), fully expecting (through education) to eradicate heart disease by the year 2000. Well, 2000 has come and gone, fewer eggs are being consumed as planned, but the problem remains just about as it was.

Looking back, nearly all of the early egg studies had serious flaws. They were primarily performed in metabolic wards and cages—settings that were far removed from the free living conditions experienced by most people and animals. Adding to the unnatural design, the test diets generally consisted of dried egg products rich in oxidized cholesterol rather than whole eggs that were free of oxidized products. So the outcome, while convincing to many scientists, fell far short of typifying the conditions under which eggs are normally consumed by people.

Recent studies that you'll read about throughout this book have lightened the charge against eggs. Nevertheless, the strength and weight of past evidence continues to discourage researchers from proposing a direct clinical trial of whole eggs. Adding to the resist-

ance, institutional review boards generally take the attitude that feeding whole eggs to people would be cruel and inhumane, given the "facts." So scientists who wish to examine the relationship of eggs and heart disease have taken to evaluating long-term heart and diet patterns of free-living individuals. They compare the cardiovascular endpoints of people who eat eggs against endpoints of those who eat few to no eggs per day.

Two such examinations in which this has been done are the Nurses Health Study[3] (involving more than 80,000 female subjects) and the Health Professionals Study[4] (involving more than 40,000 male subjects). To the general dismay of those who have advocated fewer eggs per day, no definitive link could be made between whole egg consumption patterns, higher serum cholesterol, and heart attack risk. Other major studies have resulted in similar conclusions (see chap. 8). This lack of support for past recommendations has prompted the new conclusion that eggs are neither a risk factor nor a cause of heart attack.

With the recent overturning of the past indictment of whole eggs, the lever needed to lobby for a direct, clinical trial is now in place, just waiting for someone to apply the pressure. For those who are still concerned about safety, it is suggested they review the data of cohorts within the previously mentioned studies and compare the people who ate the most eggs with those who ate the least. Since the bottom line shows no difference in heart disease risk among egg eaters and non–egg eaters, it is logical that cohorts will be found within each group that fared far better and far worse. The differences are likely to be accounted for not just by the number or frequency of eggs eaten but by the differences in genetics and the accompanying diet and lifestyle choices. Understanding the differences may make all the difference in determining whether you think of eggs as the problem or the magic bullet.

Searching for That Magic Bullet: Would We Recognize It If We Saw It?

Let's consider our hypothetical event in which you've received a magic bullet for your loved one. If the potion lived up to its claim, what immediate change would you expect in serum cholesterol level and appearance and makeup of arterial lesions? Indeed, an immediate physical change would be seen in the lesions. Whether the change was immediately for the better would be debatable.

The change seen in serum cholesterol would be less debatable. You would, of course, see a rise in serum cholesterol, since it would be exiting the lesion and entering the bloodstream. Although this initial rise would logically be a sign of success, researchers and clinicians have never regarded any rise in cholesterol as a good thing. A rise in cholesterol from any challenge—even the magic bullet—would be regarded by them as a bad thing. Given this flawed perception, it is possible (and likely) that science has stumbled onto the magic bullet many times without realizing it.

Supposing again that eggs were the magic bullet, what changes in serum cholesterol would you expect over an extended period when eggs were consumed by free-living people who began with a slight baseline elevation? Logic tells us that serum cholesterol would rise even more, especially at first, as the bloodstream is at the confluence of cholesterol entering via the diet and cholesterol that's being released from the material that makes up

arterial lesions. However, as the lesions regressed in size and severity due to this magic bullet, and as the excess cholesterol got redistributed, the serum level would later return to baseline or below.

To evaluate the possibility that eggs may bring about a cholesterol curve that mimics the magic bullet effect, a stair-step study of free-living people was carried out in the late 1970s by Elliott,[5] who looked first at the combined effect of egg feeding on serum cholesterol and LCAT (the enzyme responsible for esterification, which packs more cholesterol into high-density lipoprotein [HDL] than might otherwise occur). The study, involving an isocaloric substitution of four eggs per day, was carried out for 12 weeks in 15 test subjects who served as their own controls, 12 of whom completed the study. Beginning with the 5th week, the test subjects added a daily supplement of 2 g of vitamin C to the egg regimen. On the 9th week the test subjects added regular exercise to the vitamin C and egg regimen. Too, since cholesterol can only exit the body through the intestinal tract, only those people who reported having two or more bowel movements per day were chosen for the study. To assist in keeping cholesterol and bile from being reabsorbed, a high-fiber diet was also required, and all participants agreed to adjust calories and exercise so as to maintain a constant body weight throughout the study period.

Total serum cholesterol rose at first in all participants, as anticipated, in response to the increase in egg consumption. By week 12 the cholesterol level had subsided in all but one person to a level that was either equal to or less than the baseline reading. In the lone exception, serum cholesterol went up and stayed up and was accompanied by failure of the LCAT enzyme to respond (rise). This scenario was indeed consistent with what you might expect from administering any hypothetical magic bullet under similar diet and lifestyle circumstances. Yet, because the study involved whole eggs, because it produced a rise in serum cholesterol early in the study as experts anticipated, because it involved so few people, and perhaps because it failed to get reported in a key heart journal, the findings were not considered remarkable. Nevertheless, the study offers a model of the changes you might expect in serum cholesterol in response to any magic bullet that uses cholesterol to fight cholesterol.

A second common flaw in searching for the magic bullet comes from the fact that science tends to look at data from the disease perspective while ignoring the health perspective. An example is the discovery of a specialized protein that brought about the need for this discussion and that is largely responsible for the magic bullet effect of eggs, *apoprotein E* (apo E).

Apoprotein E

Let's begin with a little background. Apoproteins comprise the major protein components of all lipoproteins. A search in 1963 for the cause of abetalipoproteinemia (an unusual condition characterized by a very low total serum cholesterol and neurological symptoms) revealed that there are five major apoproteins, known today as apo A, B, C, D, and E.[6] All five are in short supply in that condition, which suggested that a deficiency of these proteins is not a good thing. Further work soon revealed that apoproteins perform the following basic functions:

1. They assist in the maturation process of lipoproteins.
2. They serve as cofactors for the enzymes that promote lipid metabolism (i.e., lipoprotein lipase and LCAT) by providing a suitable lipid interface upon which the enzymes can work.
3. They participate in the cell-to-cell distribution and redistribution of cholesterol, thereby serving as precursors for steroid production.
4. They play a significant role in escorting cholesterol not just through, but out of, the body.

Apo E is fundamental to the mechanism by which eggs, given the right circumstances, may impart a magic bullet effect. Apo E is associated with very low-density lipoprotein (VLDL) and two unique HDL particles (HDL1 and HDLc), which are not normally included in routine HDL testing. The two particles contain far more cholesterol than is contained in the HDL that's normally measured. The usual testing procedure, performed by the heparin/manganese precipitin method, measures only the cholesterol attributed to HDL2 + HDL3. The cholesterol measured in HDL1 and HDLc (derived mostly from eating eggs and butter) shows up in the total cholesterol reading.

This difference is *quite* significant, as HDL1 and HDLc are perhaps the most important particles for redistributing cholesterol collected from areas of high concentration for delivery to where it is either needed or excreted. This *reverse cholesterol transport* system begins with apo A, which is responsible for picking up cholesterol from areas of high concentration and loading it aboard HDL3 and HDL2. Once aboard, the LCAT enzyme esterifies the cholesterol so that it can be tightly packed and stored in the triglyceride-rich core. HDL's tightly packed cholesterol load can then be delivered either to the liver for excretion or redistributed to areas where cholesterol is needed. This latter benefit, which is sometimes seen as a means by which the arterial intima accumulates too much cholesterol, can largely be attributed to apo E, the key apoprotein of egg-induced HDL.

Early studies of apo E by the research team of Dr. Robert Mahley at the Gladstone Foundation in San Francisco revealed that diets high in eggs and other cholesterol sources brought about a rise in serum cholesterol in the majority of his subjects.[7] While the cholesterol response was initially looked upon with suspicion, it was nevertheless found to be due to a rise in a cholesterol-rich HDL particle that differed from the usual HDL. Apo E accounted for the altered HDL particle and served a potentially helpful role in disposing of the additional cholesterol load.[8] As a ligand, or binding agent, it tended to target receptors prevalent in the brain, the nervous system, steroid-producing glands, and the liver's catabolic pool.[9]

Upon seeing this reality, it became clear how apo E was designed to work together with apo A to our benefit. The process has been extensively explained,[10] but it largely occurs in this way: Apo A picks up cholesterol from areas of high cholesterol concentration, after which apo E docks with certain tissues so that the cholesterol load can be delivered to where it is needed or to where the excess can be excreted (where cholesterol is delivered to the liver's catabolic pool).[11]

When apo E was first discovered and understood, the health benefit credited to it seemed

sufficiently clear, enough that by the mid-1980s many credible scientists saw it as playing a positive role in the reversal of atherosclerosis.[12] It was at this point that the health perspective got replaced by a disease perspective, due mostly to the fact that the receptors for the so-called bad cholesterol of low-density lipoprotein (LDL) tended to also show affinity for cholesterol delivered by apo E. Since arterial disease was being associated with oxidized LDL, then apo E was lumped under the same "bad" label. Being disease oriented, authorities then turned their focus totally away from the health-promoting function of apo E and toward the bad. Rather than acknowledging that 80 percent of people benefited from apo E, doctors warned that it raised the risk for developing type III hyperlipoproteinemia (a rare condition) and risked making type II and type IV hyperlipoproteinemia worse (even more rare). From that point forward, without being shown due respect for their benefits, eggs and apo E were considered either bad or suspect.

While Mahley's research team could not control how authorities used its findings, it nevertheless continued to look at the potential benefit from apo E in areas other than heart health. For starters, the researchers were impressed with the fact that the sciatic nerve of a rat had the propensity to mend itself when severed and that the mending process was accompanied by a massive accumulation of cholesterol at the site of injury. Quite naturally, they and other apo E enthusiasts began wondering what role cholesterol played in the mending process of nerves and if apo E might be responsible for directing cholesterol to the injured site. Mahley's team had, after all, just revealed that the macrophages that emerged following injury were a primary endogenous source of apo E.

Research into the process of nerve repair and remyelination revealed that apo E from macrophages that arises following injury serves to sequester the axon debris along with membrane cholesterol, fatty acids, and other lipids.[13] The sequestered cholesterol is then metabolized to pregnenolone and progesterone, just as it is in the gonads and the adrenal glands, which provide the steroidal effect needed to regenerate and remyelinate the nerve. Among neurologists, this finding was big stuff, as it suggested that remyelination and repair of human nerves might be possible by learning how to express apo E to its maximum. To answer this question, Mahley's research team pointed to earlier work where the team had shown that the addition of four to six eggs per day to the diet of six healthy men and women volunteers had brought about a six-fold increase in the expression of apo E. This finding caused apo E researchers to conclude two important points:

1. Apo E is highly active in the redistribution of cholesterol.
2. Adding four to six eggs per day to the diet increases the activity of apo E up to six-fold and thereby improves the mechanism by which nerves are repaired and myelin is regenerated. (Fig. 14.1 lists some factors other than eggs involved in apo E biosynthesis.)

The health benefits that can be credited to the redistribution process extend far beyond rebuilding and remyelinating nerves. Here is one example of which I have intimate knowledge.

In the early 1970s, I worked in a large clinical laboratory, performing bicarbonate determinations with the historically important Van Slyke Apparatus. The Van Slyke Apparatus

Apo E Biosynthesis

Apo E biosynthesis is stimulated by thyroid hormone.[1] By the same token, receptors for apo E serve to facilitate thyroid hormone uptake into a variety of tissues.[2] Thus, thyroid activity may serve as a marker of apo E activity. A low level may negatively influence the production of apo E. Based on this observation, researchers confirmed that the level of insulin is not only related inversely to thyroid function but also to apo E biosynthesis.[3] Not surprising, then, a diet based on sugar and white flour will stunt apo E output, while (as Mahley's group had demonstrated) a diet based on eggs and butter tends to stimulate production of apo E the most.

Apo E is also synthesized by the liver and macrophages following ingestion of a fatty, sterol-rich meal and during the healthy acute inflammatory response. Conversely, either a fat-free diet or chronic inflammation (or both) tends to depress the total output of apo E.[4]

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Figure 14.1. Biosynthesis.

held about 600 ml liquid mercury in a "closed" system. In the normal process of using the apparatus, laboratory workers were exposed to mercury vapors and liquid mercury that spilled from leaky stopcocks. After far too many technologists had either died or become seriously ill, OSHA came to the aid of the laboratory staff by requiring that the Van Slyke be used thereafter only under a vented hood. Today, it is seldom used at all.

As for me, I suffered from brain stem deterioration due to significant chronic exposure to mercury and was not expected to live. My recovery was credited largely to the practice of eating six eggs per day, also including a regimen of daily aerobic exercise, good bowel function, a high fiber diet, and supplementation with an ultramegadose vitamin C, based on the knowledge I'd gained from what others have researched. In retrospect, I believe my recovery can most accurately be credited to apo E stimulated by the regular consumption of eggs. In contrast, people with this condition who do not eat eggs generally fail to survive.

Some other established health benefits are the building of steroid hormones and the making of bile. In the process, the shuttlelike mechanism credited to apoproteins allows heavy metals to be picked up and removed from metal-sensitive brain and nerve cells. For those brave souls who can bring themselves to accept the reality that a rise in cholesterol can be useful, the benefit comes from the redistribution of cholesterol from areas of excess to areas of need. Just such a scenario is what you would expect in anyone who had been given a magic bullet not only for cholesterol-laden arteries but also for nerves affected by toxic pesticides and metals.

The desired outcome, however, would not likely be seen in someone who failed to follow the rules for good cholesterol turnover:

1. Consume sufficient vitamin C each day to complete the synthesis of bile.
2. Engage in regular exercise.
3. Eat a high-fiber diet.
4. Have 2 or more formed bowel movements per day.

We consider this "earning the right to eat eggs."

All People Do Not Receive Equal Benefit from Apo E or Eggs

Although we come from the same conceptual design, we are all different. This difference, which is clearly reflected in genetics, also results from factors that are mostly under our control, such as diet, lifestyle, stress response, choice of profession, and environmental exposures. Collectively, these and differences that may not be so obvious help explain the mechanism by which eggs can be beneficial to some people but not to others. Of greatest consideration, however, especially where apo E is involved, is genetics.

Genetics plays a major role as to whether you can expect to receive the magic bullet effect from eating eggs without additional special effort. It also goes a long way toward determining if you will be more or less susceptible to coronary artery disease (CAD) or Alzheimer's disease, which tend to occur together (although arterial disease often kills a

person before the Alzheimer aspect can manifest).[14] Apo E can play a protective role, and a deficiency leads to the most severe cases of arterial disease. Yet, as others have pointed out, an increase may lead to both arterial disease and Alzheimer's disease.[15] To help unravel the mystery for how the latter might happen, and how you might respond in a positive way to offset unfavorable genetics and outcomes, the following discussion is offered.

Understanding the Apo E/Egg Connection

Eggs stimulate apo E activity in accordance with your inherited phenotype, and it is the genes that make up the phenotype that determine the good or bad outcome from eating eggs. Genetically, the genes for apo E appear as either apo E-2, E-3, or E-4. Each person inherits two copies of the gene, one from each parent, resulting in six possible phenotypes:

- apo E-2/2
  apo E-2/3
  apo E-2/4
- apo E-3/3
  apo E-3/4
- apo E-4/4

People who are most likely to derive the magic bullet effect are those with either the apo E-2/3 or 3/3 phenotypes. The following discussion provides background information needed to develop a plan so that every person can experience the magic bullet effect if he or she is willing to work at it.

Apo E-2

Some combination of genes involving apo E-2 is found in about 10–13 percent of the population and tends to offer the most protection against Alzheimer's but less protection against arterial disease. Yet even that risk is small. About 1 in 1,000 people who carry the E-2/2 phenotype develops a risk to arterial disease from type III hyperlipoproteinemia (due to reduced affinity for binding to receptors in the liver). A second cohort of people with apo E-2/2 tends to have a great risk for infection, angina, and high blood pressure and thus may have a higher risk for arterial disease.

Apo E-3

The apo E-3 gene, positioned in three of the six phenotypes, apo E-3/2, E-3/3, and E-3/4, is the ancestral allele isoform that most people have, which helps protect against arterial disease as well as Alzheimer's disease. Nearly 80 percent of people have inherited at least one gene of apo E-3 from their two parents, and it is this 80 percent of the population that can benefit most from eating eggs. Again, however, the tendency by authorities has been to impose restrictions on all egg eaters rather than to look at how to improve the health of that 20 percent of the population who carry the health-sensitive apo E-2 and E-4 isoforms.

Apo E-4

The apo E-4 gene, existing in one of three combinations (either E-4/4, E-2/4, or E-3/4), predisposes people to Alzheimer's disease and CAD. Although not everyone who has these phenotypes gets Alzheimer's or CAD, 95 percent of Alzheimer's people carry at least 1 allele for E-4.[16] With regard to treatment, the standard drug used to treat Alzheimer's disease works best on people who are free of the apo E-4 allele.

Apo E-4 also predisposes people to atherosclerosis and to a variety of hyperlipidemias, including the potentially fatal type II hyperlipoproteinemia, which is generally a more serious condition in those people who carry an apo E-4 gene from each parent and who express the apo E-4/4 phenotype. To assess the total heart disease contribution of apo E-4, regardless of the lipid phenotype, a group of Italian researchers looked at 106 young European adults who had a history of myocardial infarction.[17] True to form, the presence of one or more apo E-4 alleles demonstrated (with a 95 percent confidence interval) a strong independent predictor of adverse events, with each allele raising the risk for heart attack (in people over age 65) by a factor of 2.84. A similar relationship could be found in a study of the people of Saudi Arabia.[18]

Looking further at the general contribution of apo E isoforms to arterial disease, researchers have recently demonstrated—using CT scans—that in people bearing apo E-4 there is a far greater extent of coronary artery calcification than in people who carry other apo E alleles.[19]

Apo E-4 may further contribute to a rise in coronary events and CAD severity through producing more free radicals than its apo E counterparts.[20] Oxidized LDL, for instance, is more prevalent in people who carry one or more apo E-4 alleles than in other people. The excess free radicals generated by apo E-4 can be accounted for by the excesses of nitric oxide produced by the arginine substrate and from failure to bind with and extract heavy metals due to lack of cysteine. (See Table 14.1 for a description of the amino acid differences in apo E.)

The amino acid differences in the various apo E isoforms give some hints as to why some phenotypes are more and less protective than others. Knowing these differences may also help in developing a strategy for improving the outcome of eating eggs no matter what the person's apo E phenotype.

Table 14.1. Amino acid differences among the three Apo E isoforms and their relationship to Alzheimer's disease and coronary artery disease (CAD) risk

Apoprotein	Cysteine	Arginine	Alzheimer's risk	CAD risk
apo E-2	2	0	Lowest Risk	Average Risk
apo E-3	1	1	Average Risk	Lowest Risk
apo E-4	0	2	Highest Risk	Highest Risk

Problems with E-4

Degeneration of cells of the nerves, brain, and arteries tend to occur most readily with an apo E-4/4 phenotype. This can largely be explained by the substitution of arginine for cysteine on codon 112 and 158 that characterize the various apo E phenotypes.[21] (Codons are bases located on a strand of DNA that direct the incorporation of a specific amino acid into a polypeptide chain.) Cysteine, directed by codon 112 in the apo E-2 and apo E-3 alleles, is characterized by the conspicuous presence of a terminal $^{-}$SH group that allows it to latch onto a heavy metal or pesticide during its interchange with the target tissue.[23] (Thiols bearing the $^{-}$SH group bind to portions of any compound that is deficient in electrons—making them good conjugates of heavy metals and pesticides.) In contrast, apo E-4 is absent of cysteine and thereby lacks the necessary $^{-}$SH group to latch onto heavy metals and select pesticides. So wherever heavy metals or pesticides are causing damage—such as in the brain, the nerves, the adrenal glands, or the arteries—the cysteine portion of apo E present in egg-induced HDL serves to remove the metal and protect the tissue site. The substitution of arginine for cysteine in apo E-4 allows heavy metals to do damage where they might otherwise be neutralized and extracted from affected tissues.

Arginine, directed by codon 158 on apo E-4 and E-3, provides its own beneficial role by serving as substrate for nitric oxide (NO), an acidic free radical that serves both as an antibacterial and as a smooth muscle relaxant needed to control blood pressure and prevent spasms of the arteries and other NO-sensitive tissues. However, when arginine is present in excess, such as would most likely occur in someone who was homozygous for apo E-4, and accompanied by an excess of the enzyme that induces its synthesis (iNOS), NO becomes a liability rather than a benefit due to excessive acidity and free radical output. So the differences in amino acid content of the various apo E isoforms help to account for why the cysteine-rich apo E-2 and E-3 isoforms are more protective against brain metals and why arginine-rich apo E-4 might raise the risk for Alzheimer's, lower the risk for metal toxicity, cause early-onset arterial disease, and result in a higher risk for brain damage following a stroke (where acidity becomes the determinant of damage). Reactive oxygen species (ROS), for instance, are known to be produced in greater quantity in an acidic environment than in a more alkaline environment.[24]

From this relationship it is reasonable that people with E-4, lacking the metal-binding cysteine, are the people who may want to limit egg consumption until a health program can be tailored to fit their needs. Otherwise, these are the people who react first to low-level exposure to toxic metals and who tend to develop Alzheimer's disease where heavy metals become a precipitating factor. In this scenario there is no cysteine to bind with mercury and other heavy metals. The buildup of brain acidity from the NO intensifies both the metal's reactivity and the brain's susceptibility to damage. In addition, because of the weakened interactions of apo E-4 for tau-protein (which helps prevent Alzheimer's disease by regulating the stability of microtubulin), apo E-4 further promotes Alzheimer's disease by destabilizing the microtubulin.

Apo E-2, being heavily endowed with the sulfhydryl-rich cysteine, binds readily with heavy metals but (lacking the arginine substrate) fails to produce sufficient NO production to help fight infection and to allow for proper smooth muscle relaxation. The lack of NO

may also fail to stimulate sufficient lipase needed to metabolize triglyceride fats, which may largely account for the heightened risk for glucose intolerance seen in people who express the apo E-2/2 phenotype and who consume eggs in quantity. For these people to receive the magic bullet effect, they need to have a program tailored for them.

Apo E-3, carrying one molecule each of both cysteine and arginine, tends to produce the best overall protection against a variety of health conditions where cysteine and arginine may play a role.

Apo E-4, as stated earlier, offers no protection against heavy metals, which have been implicated as an initiating factor not only in Alzheimer's and CAD but also in many toxicities and other diseases as well. Fortunately, it occurs in only 3–10 percent of the population. Yet people who carry one or more apo E-4 alleles account for more than 70 percent of the people with Alzheimer's, making it a very high risk factor.[25,26,27] While apo E-2/4 and apo E-3/4 slightly raise the risk of Alzheimer's, people with E-4/4 are at the highest risk, especially after experiencing a head injury. This makes sense since apo E activity is known to rise in response to macrophages that naturally accompany nerve and brain injuries. While the purpose is to restore health to the injured site, the increase in apo E activity in people who express arginine-rich apo E-4 results in an excess of NO. The acidic nature of NO causes a serious drop in the threshold for when the "tangles" of microtubulin familiar to Alzheimer's patients become evident.

For People Who Express the Apo E-2/2, E-2/4, E-3/4, or E-4/4 Phenotype, What Chance Do They Have of Experiencing the Magic Bullet Effect?

For those lucky ones who've inherited E-2/3 or E-3/3, they have the best chance to experience the magic bullet effect. People in the other groups will have more difficulty. Supplementation will improve their odds.

While this question begs for a quick and easy answer, the research in this area is lacking. Yet the facts suggest that a strategy can be implemented with some anticipated success. If you have inherited E-2/2, E-2/4, E-3/4, or E-4/4, the following steps might be considered to improve outcome.

Apo E-2/2

Focus on foods that are rich in arginine, which is lacking with this phenotype and is necessary to provide NO. You might also consider supplementing with arginine in accordance with the following conservative dosage schedule of 500 mg at bedtime for a 150-pound person.

Apo E-2/4, E-3/4, and E-4/4

Focus on foods that are rich in glutathione, such as asparagus, lamb, veal, parsley, and avocado.[29] Otherwise, you might consider supplementing L-glutathione in accordance

with the dosage schedule of 300 mg per day for a 150-pound person, per egg per day. (Glutathione is safer than supplementing with cysteine because cysteine given as an amino acid may be neurotoxic.) Some intestinal absorption occurs, but maximum absorption occurs in the oral cavity across oral mucosa.[28] Thus, you might mix glutathione powder in water and drink it slowly.

Conclusion

While the average person may have difficulty altering their long-held perceptions, whole eggs, by stimulating a rise in apo E activity, possess the potential for serving as the magic bullet for not only preventing heart disease but also reversing it. The outcome, however, whether good or bad, depends first and foremost on whether the person has earned the right to eat eggs. If this person is not following the basic steps required for good cholesterol turnover, then he or she certainly hasn't earned the right and is not likely to get the magic bullet effect no matter his or her apo E phenotype. This person should probably limit egg intake to no more than one per day.

For those who comply with the rules for good cholesterol turnover as outlined in the Elliott study, the clinician might then award them with a prescription to test for both an apo E phenotype and an apo E activity level. With this data in hand and a cohort of highly cooperative test subjects, a pilot study could then be conducted with relative safety regarding the effects on the arteries of a free-living group of men and women expressing either the apo E-2/3 or E-3/3 phenotype who volunteer to eat whole eggs daily and in quantity. They would predictably experience the magic bullet effect.

There are other caveats that might be considered before doing any study that could skew results. At the Institute for Health Realities, for instance, we do a very extensive blood analysis before our health coach team offers any advice because there are so many other factors that need to be addressed if health is the ultimate goal. If this is done, the information provided in this chapter begs for a second study of people who bear either the apo E-2/2, E-2/4, E-3/4, or E-4/4 phenotype, who would predictably have difficulty in experiencing the magic bullet effect. The findings would help determine just how many eggs they can eat safely and to what extent that they too can participate in gaining the benefits.

For the person with the apo E-2/2 phenotype, who has difficulty metabolizing fatty acids due to reduced lipase activity, the research team might make certain beforehand that he or she qualifies as per the requirements for apo E biosynthesis (see Figure 14.1) and perhaps even supplement with the lipase enzyme. Having screened the subjects adequately, researchers might further supplement their program with a nominal daily dosage of arginine for the purpose of supplying the missing nitric oxide.

For the person bearing an apo E-4 phenotype the project could be expanded to include supplementation with reduced L-glutathione to provide a readily functional source of intracellular, sulfhydryl binding.

It seems logical, given the evidence, that whole eggs offer the perfect magic bullet not only for unclogging arteries and handling the infections that often associate with arterial lesions but also for removing heavy metals from the brain and nervous system, for reducing the risk for Alzheimer's disease, and for reducing smooth muscle spasms in people

with angina and high blood pressure. Even people with the most troublesome apo E phenotypes can benefit. The key to this level of success from eating whole eggs, however, would largely rest on the relationship between the health care provider, whose role it is to test and advise, and the person who seeks the magic bullet, whose role it is to work diligently toward earning the right to eat them.

Dedication

This chapter is dedicated to three people who have played an important role in our understanding of egg nutrition.

- H.B. Wallace, my mentor and close friend who pioneered the egg industry and who now administers the Wallace Research Foundation.
- The late Blanton Smith of Nashville, Tennessee, who bravely and openly placed a full page ad in the Wall Street Journal stating, "There is no scientific evidence, whatsoever, that eating eggs increases your risk to heart disease." While a retraction to the statement was forced in a U.S. district court, I was impressed that those who testified to the contrary could never prove that the statement was wrong.
- Fred Kummerow, Ph.D., of the Burnside Research Laboratory, University of Illinois, Urbana/Champaign, who provided the initial key testimony on behalf of eggs in the now famous Egg Trial and who continues to look at the many causal factors in heart disease.

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15

Enriched Eggs for Human Consumption and the and Feeding Pattern of Layers

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Demographic and social changes have produced an increase in life expectancy and a shift in deaths from children to adults. Currently, the main causes of death are cardiovascular diseases, cancer, injuries, and congenital and metabolic conditions.[1] The high incidence of these diseases is related to malnutrition and sedentary and stressful lifestyles.[2] The rate of death attributable to ischemic heart disease has become the number one cause of adult death in the world, accounting for 7,200,000 deaths in 1997.[3]

As a consequence of urbanization, work becoming industrialized, and other factors, food habits have evolved to the consumption of highly refined, nutritionally concentrated products, increased levels of fat intake and saturated fatty acids, low intake of n-3 fatty acids, and other practices that hinder health. In developed countries, deaths due to ischemic heart disease have decreased from 51 percent in 1985 to 46 percent in 1997 due to a growing concern about the relationship between ischemic heart disease and nutrition, while in developing countries such deaths have increased from 16 to 24 percent, respectively.[3] All of these dietary practices are inherent to affluent, developed societies, which favor consumption of animal products (mainly red meats and milk products) that are a clear sign of "status."

When the United States is compared with east Asian Pacific developed societies like Japan, whose population lives under similar environmental and economic conditions, deaths from coronary heart disease in the United States and other developed Western countries exhibit a higher age adjusted rate (106.5/100,000 in the United States versus 30/100,000 in Japan, 1997).[4] Studies concerning dietary practices that can explain these epidemiological profiles in both societies show that the main sources of animal protein in typical east Asian diets are marine products like fish and seafood; terrestrial animals like cattle, pigs, and poultry provide animal protein (and fat) in the West.

In 1998, average per capita fish and seafood intakes were 66.5, 20.7, and 19.7 kg/year for Japan, the United States, and Europe, respectively; these figures have been virtually constant in Japan since 1978 and slightly higher in the United States and Europe. For the same period, meat per capita consumption in the United States has inceased from 106 to

122 kg/year, decreased from 76 to 72 kg/year in Europe, and increased from 28 to 42 kg/year in Japan.[5]

The reasons for the low fish and seafood consumption in Western societies are multicausal and lay in historical cultural patterns, reinforced by modern lifestyle; as such, these patterns are difficult to modify, at least in the short term.

As a consequence of the growing concern of consumers about risk factors for chronic diseases (including the cholesterol content of eggs and its possible adverse effects on health[6]), per capita egg consumption in the United States has decreased in the last decades: 290 to 235 eggs/year in 1970 and 1998, respectively. In the United Kingdom these figures were 275 and 225, respectively.[5] A similar trend has been observed in many other parts of the world, except for a few Asian and African countries whose intakes have been historically very low.

The avian industry is one of the most successful examples of the proper application of scientific and technological knowledge to improve the efficiency of animal production. Thirty years ago, under initial intensive industrial conditions of production, a broiler chicken took about 70 days to reach its market weight (1.4 and 1.8 kg for female and male chickens, respectively) with a feed conversion rate of 2.4 to 2.6 kg feed/kg live weight gain produced. Currently, it takes just 35 to 42 days and 1.8 to 1.9 kg feed for similar indexes of production. Similarly, eggs were produced with about 3.1 to 3.4 kg feed/kg egg and presently are produced with values ranging between 1.95 to 2.45; moreover, these products present better market and organoleptic characteristics, a greater diversity of alternatives for commercialization, relatively lower production costs per unit, and lower prices for consumers.

Eggs contain an almost ideal amino acid profile and significant levels of other nutrients like fat, vitamins, and minerals (Table 15.1); eggs can be produced under many management conditions ranging from simple domestic henhouses to highly industrialized laying enterprises that can lodge flocks of thousands of hens; eggs can be used in many culinary preparations and can be stored with just the common and ordinary precautions of refrigeration accorded to any fresh animal food, especially in relation to basic biosecurity rules. These characteristics make them an ideal source of nutrients, particularly for populations whose animal protein consumption is low because of the high prices of meat and milk products. Furthermore, it can be hypothesized that the phospholipids (PLs) that eggs contain may minimize the deleterious effects of cholesterol consumption. Moreover, the highly polyunsaturated fatty acid (PUFAs) esterified in those PLs are responsible for the decrease in the endogenous cholesterol synthesis under normal metabolic conditions.[7–9] In spite of this, nutritional and some genetic research efforts to decrease the cholesterol content of the egg have not been very successful, so we have to live with this characteristic inherent in a food of the highest biological value for the population.[10]

Fish, seafood, and their derivates exhibit a typical profile of fatty acids (FAs) in their fat: long-chain PUFAs of the n-3 series ranging from 18 to 22 atoms of carbon, mainly eicosapentanoic acid (EPA, C20:5 n-3), docosapentanoic acid (DPA, C22:5 n-3), and docosahexanoic acid (DHA, C22:6 n-3), comprise up to 30 percent of their fat's FAs; about 25 percent correspond to monounsaturated fatty acids (MUFAs), and the remaining

Table 15.1. Nutritional composition of yolk of large egg

Total weight	Energy	Water	Fat	Protein	Cholesterol
17 g	61 kcal	8.3 g	5.2 g	2.8 g	0.3 g

Fatty acids (weight and percentage of fat content)

Saturated	Monounsaturated	Polyunsaturated	Cholesterol
1.6 g (30.8)	2.0 g (38.5)	0.7 g (13.5)	218 mg

Minerals

Ca	P	Na	K	Mg	Fe	Zn	Cu	Mn
23 mg	83 mg	7 mg	16 mg	2 mg	0.6 mg	0.53 mg	0.004 mg	0.012 mg

Vitamins

A	C	B_1	B_2	Niacin	B_6	B_{12}	Folic acid	Pantothenic acid
331 IU	0	0.03 mg	0.11 mg	0	0.07 mg	0.53 mcg	25 mcg	0.65 mg

Source. Reference 14.

FAs are saturated (SFAs) (Table 15.2).[11–12] This profile gives their fats polyunsaturation indexes higher than those of other animal fats (0.9 to 1.5 versus 0.04 for lamb fat to 0.7 for the fat of chickens fed corn-based diets) and makes them foodstuffs with unique functional and nutritional properties.[13] Fish oils and fish meal lipids have high digestibilities and energy values in all animals tested and have been proved very succesful ingredients for enhancing growth and production indexes in all types of farm animals.[12] Some vegetable oils, like flaxseed oil, cottonseed oil, sunflower oil, canola oil, and others, exhibit very high polyunsaturation indexes and also contain n-3 FAs,[14] but in a lesser quantity than the marine lipids (Table 15.3).

PUFAs are essential nutrients. They are important components of cellular membranes and exert outstanding roles in many biological functions, as in the platelet aggregation mechanisms, the function of receptors of neurotransmitters and insulin, and the immune system of all known animals. n-3 FAs and their relationship with n-6 FAs have been extensively studied in avian experiments. Almost all of the studies concluded that these nutrients exert a beneficial influence upon the humoral and cellular immunity of the birds by different mechanisms: in some cases decreasing the cytotoxicity of some eicosanoids normally produced, in other cases increasing the mitotic rate of specialized cell populations related to the production of specific antibodies, and in many cases modulating the balance of enzyme pathways that control prostaglandins, tromboxanes, and leukotrienes.[15–18]

All the functions listed here depend on the proper relationship between the n-6 and n-3 series of FAs, as well as their chain length.[19] It is estimated that a balanced diet with respect to the supply of n-6:n-3 FAs must present a ratio of 4:1 to 5:1. Relationships of higher magnitude may imply a serious deficiency of the long-chain PUFAs of the n-3 series. Metabolically, the biochemical transformations within the three families of FAs

Table 15.2. Iodine values, weight percentage of saturated fatty acids, monounsaturated fatty acids and EPA + DHA of some major marine oils

		Iodine values	SFA (%)	MUFA (%) C20:1 + C22:1)	PUFA (%) (EPA + DHA)
Body oils	Herring (Atlantic)	125	19	35	14
	Capelin	125	18	36	11
	Redfish	125	21	36	9
	Herring (Pacific)	140	34	10	7
	Sand launce	140	24	27	17
	Mackerel	150	27	38	15
	Salmon (Pacific)	150	36	17	19
	Sardine	160	30	8	24
	Menhaden	162	32	2	20
	Anchovy	181	30	3	26
	Pilchard	185	28	5	26
Liver oils	Cod (Atlantic)	165	21	13	24
	Pollock (Alaska)	160	18	30	17
	Squid (Pacific)	180	21	17	28
Other	Salmon egg (Pacific)	210	¿?	¿?	38
	Seal (Atlantic)	150	14	17	14

Source. References 11 and 12.

Note. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; EPA = eicosapentanoic acid; DHA = docosahexanoic acid.

Table 15.3. Composition of some fish and vegetable oils (%)

	SFA (%)	MUFA (%)	PUFA (%)	PUFA:SFA
Herring oil	21.4	56.4	15.7	0.73
Menhaden oil (hydrogenated)	88.6	0.0	0.0	—
Salmon oil	14.3	29.3	40.0	2.790
Sardine oil	30.0	33.6	32.1	1.070
Canola oil	7.1	58.6	29.3	41.300
Coconut oil	86.4	5.7	2.1	0.024
Corn oil	12.9	24.3	58.6	4.540
Cottonseed oil	25.7	17.9	52.1	2.030
Olive oil	13.6	73.6	8.6	0.630
Palm kernel oil	6.9	5.2	1.3	0.190
Peanut oil	17.7	46.4	32.1	1.810
Safflower oil	9.3	12.1	74.3	7.990
Soybean oil	15.0	42.9	37.9	2.530
Sunflower oil	10.0	19.3	65.7	6.570

Source. Reference 14.

Note. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

(n-3, n-6, and n-9) depend on the mutual competition that these FAs exert upon the Δ-6 and Δ-5 desaturase enzymes, resulting in the importance of an appropriate relationship between the FA series in the fats and oils consumed.[20]

Marine fats and oils, due to their long-chain PUFA content, have important cardio-protective effects by being able to lower low-density lipoprotein (LDL) cholesterol, promote vasodilation, and inhibit platelet aggregation.[21] This association was virtually demonstrated when the relation of eskimo diets (high in marine FAs) and low rates of cardiovascular diseases was reported.[22]

As already discussed, many people in Western societies are reluctant to increase their consumption of fish and other marine products and prefer those foods that contain terrestrial animal meat, eggs, and refined carbohydrates. Consequently, an interesting strategy has been developed in the last 20 years: those "desired" foodstuffs have been enriched with "desirable" nutrients to increase the consumption of the nutrients without altering deeply rooted food habits that, despite the efforts that have already been made, will not be modified without much more education.

The products of nonruminant species (meat, eggs, and milk) very closely reflect their feeding pattern of fat and amino acids. This condition is a useful tool for the development of the following strategy: if nonruminant species are fed "desirable" nutrients, the "desired" products can be obtained for human use.

Nutrition can influence many of the quality characteristics of eggs, such as their size and the proportion of their main constituents: yolk and albumen. Dietary changes have little effect on the overall composition of albumen and yolk in terms of dry matter content and amino acid composition; however, micronutrient and FA composition can be modified.[23]

Recently, these changes have taken a new significance in relation to human health, particularly in the area of n-3 FAs,[23] and the modification of the lipid profile of egg yolk by dietary manipulations has been studied by many authors that have established that enriched eggs would be a source of FAs with beneficial health effects;[24–30] currently, there are market eggs enriched with n-3 FAs—both from marine and vegetable sources—in Europe, the United States, and South America, mainly in Brazil and Chile.

Corn, milo, barley, and oats are the major coarse grains used for feeding poultry, mainly for energy supply in the diets. Occasionally surplus or frost-damaged wheat is sufficiently low priced to compete with the coarse cereals used for poultry feeds. In some areas of the world, broken rice is a low-cost energy source. Some cereal byproducts like those resulting from dry and wet milling of corn (hominy feed, corn bran, corn germ cake, and corn germ meal), those from the milling process of wheat (wheat bran, wheat middlings, wheat shorts, etc.), and screenings (the residual material obtained in the process of cleaning grain and seeds) are also used in hens' diets.

Soybean meal, corn gluten meal, cottonseed meal, and peanut meal represent the major plant protein supplements used in poultry feeds. Other plant protein supplements used in hens' diets are decorticated safflower seed meal, sesame meal, sunflower seed meal, rapeseed meal, linseed meal, and coconut oil meal.

Protein sources derived from animal products have been prized for many years for animal feeding. Fish meal, meat meal, and tankage and poultry industry byproducts have been used in hen diets.

The most concentrated sources of energy used in poultry feeding are fats and oils that are available from the meat-processing industry, from byproducts of the manufacture of soaps and refining of vegetable oils, or vegetable or fish oils themselves. For a long time these materials were included in hen diets only for energetic purposes because of their high energy concentration and the desirable qualities that they add to feeds (less dustiness and improved palatability). Because there is more concern about the quality of fats, their fatty acid composition, and the beneficial effects that they can transfer to other animal products, they must be defined to enable prediction of the nutritional value of any particular product.

Some products resulting from fermentation of various grains have been used in poultry diets; examples of these products are dried or irradiated yeasts from the brewing or distilling industry. Other byproducts, such as molasses, result from the sugar industry based on cane or beet; they are a cheap source of carbohydrates and can be used in up to 4–5 percent of diets because of their high mineral content.

Research to modify the lipid profile of yolk fat has been focused on the utilization of both vegetable and marine souces of FAs, the single n-3 FA, and, to a lesser extent, marine algae. Such modifications, directed essentially to increasing n-3 FAs, began with the incorporation of fish oils and meals. The frequent appearance of undesirable organoleptic characters led to the development of alternative diet integration approaches (utilizing, for example, marine algae–based feed integrations and microencapsulation) and to the reconsideration of the use of plant α-linolenic acid (α-LNA, C:3, n-3) sources such as flax, colza, and soy in layers' feeding.

The production of eggs requires the deposition of large amounts of yolk lipids, mostly during the days prior to ovulation. Yolk lipids and proteins are synthesized in the liver under the influence of estrogen and progesterone and are transferred through the blood to the ovarian follicles. Lipids in the yolk are of two main types: lipoproteins (LPs) and vitellogenins (VGs). In the chicken, LPs contribute about 95 percent of the yolk lipids. The liver packages and secretes triglycerides (TGs) and phospholipids (PLs) in a special yolk-targeted VLDL (VLDLy), which has unique structural and biochemical properties for targeting it to the ovary. VLDLy is half the size of normal VLDL and has apolipoprotein VLDL II on its surface, making it a poor substrate for lipoprotein lipase. Consequently, the TGs in VLDLy are not well used by skeletal muscle or adipose tissue. Its small size permits it to pass through the granulosa basal lamina of the ovarian follicle and bind to the apolipoprotein B receptor of the oolemma. It is endocytosed intact to form the yolk. The sievinglike action of the ovarian-follicle basal lamina prevents the uptake of portomicrons arriving from the diet. This combination of follicular ultrastructure and VLDLy size allows dietary fat to be modified by the liver prior to inclusion into the yolk of eggs, permitting better control of yolk lipid characteristics by the female.[18] This hepatic modification is not complete, however, and yolk lipid composition still reflects that of the diet, especially in content of PUFAs.[31]

Hargis et al.[30] studied the effect of a standard laying hen diet enriched with 3 percent menhaden oil, versus an isocaloric control diet with no added fat, on the lipid profile of shell egg yolk. Menhaden oil did not affect any productive parameter, total yolk fat, or

yolk cholesterol contents. However, yolk contents of n-6 and n-3 FAs were influenced by the diet. Arachidonic acid (AA, C20:4, n-6) decreased and eicosapentanoic acid (EPA) increased after 1 week with the menhaden diet, and 1 and 2 weeks later linoleic acid (LA, C:18:2, n-6) and DHA acid increased respectively. These changes resulted in a decrease in the ratio of n-6 to n-3 FAs from 18 to 3 for eggs from hens fed the control and experimental diets respectively.

García and Albala[32] analyzed eggs from hens fed marine (fish meal) and vegetable (soy meal) sources of FAs. Their results showed that eggs from hens fed marine products, an ordinary practice in Chile where fish meal is relatively cheap and is used on average in 3–4 percent of layers' diets, contain significantly less cholesterol (0.92 versus 1.29 g/100 g yolk) and fewer TGs and PLs than those of hens fed only vegetable feeds. Even though there were no significant differences in the total proportions of SFAs, MUFAs, and PUFAs in the yolk, n-3 FAs—mainly EPA and DHA—were significantly higher (1.7 and 4 times, respectively) and n-6 were 30 percent lower in the eggs of hens fed marine products (Table 15.4).

Grashorn and Steinhilber[33] studied the effect of different n-6:n-3 relations in the diets of hens. These relations were obtained by means of the addition of fats with different fatty acid compositions to a basal diet to make a diet with 17 percent crude protein and 11.6 MJ metabolizable energy/kg. The fats used were mixtures of soybean oil, linseed oil, rapeseed oil, sunflower oil and /or fish oil at a level of fat addition of 2.5 percent. The n-6:n-3 relations studied were 10:1, 5:1, 3:1, 1:1, 1:3, and 1:5. In the yolks the percentage of total n-3 FAs increased with increasing levels of n-3 in the diets, and the relation between n-6 and n-3 decreased, corresponding quite well to the relations in the diets. Nevertheless, it seems to be hardly possible to increase n-3 FAs over the proportion 3:1 because there may exist a threshold for the accumulation of n-3 in the yolks. This may be due to the relation between LA and α-LA in the diets used. Even in the diet with the highest amount of n-3 FAs, the proportion of LA is higher by the factor 1.5 than the proportion of α-LA. This

Table 15.4. Lipid profile of egg yolk and its relation to the feeding pattern of hens

Fatty acids in yolk lipids (g/100 g)	Feeding pattern of hens based on	
	Marine products	Vegetable products
Saturated	43.26 ± 1.11	43.80 ± 2.15
Monounsaturated	31.67 ± 2.13	32.23 ± 1.08
Polyunsaturated	23.07 ± 1.82	22.98 ± 2.88
Total n-3	7.13 ± 0.83[a]	1.77 ± 0.55[b]
C20:5 n-3 (EPA)	0.57 ± 0.25[a]	0.33 ± 0.02[b]
C22:6 n-3 (DHA)	5.96 ± 0.59[a]	1.40 ± 0.44[b]
Total n-6	15.71 ± 2.51[a]	20.88 ± 2.32[b]

Source. Reference 32.
Note. EPA = eicosapentanoic acid; DHA = docosahexanoic acid.
[a]Significantly different from saturated ($P < 0.05$).
[b]Significantly different from marine product fed ($P < 0.05$).

means the n-6 pathway is favored in the metabolism of the hen, limiting the transformation of LA to EPA and DHA. Therefore, the proportion of LA is 4.4 times higher in yolks than the proportion of α-LA. In general, the use of linseed oil in the diet will result in higher proportions of n-3 FAs in the yolks than the use of fish oil due to linseed oil's higher content of n-3 FAs. TBAR values were very low, so the oxidative processes may still be of no importance; probably the content of α-tocopherol in the yolk was high enough to compensate for oxidative processes. These authors suggested that a distinct enrichment of eggs with EPA and DHA and/or other n-3 FAs may only be achieved by using pure FAs instead of oils.

Other authors have used mixtures of cereals varying in their FA composition to modify the yolk lipid profile; Kaminska,[34] used different mixtures of maize, barley, dehulled oats, wheat, and fish meal. These mixtures had varying levels of LA. Including 20–40 percent dehulled oats in the diet decreased the content of MUFAs and elevated the content of PUFAs. Eggs from hens fed diets in which maize was fortified with 20–40 percent of dehulled oats contained more LA than eggs from hens fed other diets. The increased content of LA acompanying an increased proportion of dietary oats was reported previously by other authors.[35] Feeding oats, especially barley, caused a highly significant increase in α-LA and DHA compared with maize. The highest level of DHA was found when a barley-wheat diet was supplemented with 2 percent fish meal. The addition of fish meal decreased PUFA n-6 and increased PUFA n-3. Inclusion of 30 percent barley in the diet caused the n-6:n-3 ratio to decrease very significantly. This ratio (5.11:1) is close to the ideal ratio suggested by Sincler, quoted by Farrell.[36] The narrowest ratio (4.69:1) was achieved after supplementing the barley-wheat diet with fish meal.

Evans et al.[37] studied the effect of the addition of a vegetable source of fat by means of 2.5 percent cottonseed oil to the diet of laying hens; their results showed that there were no changes in yolk VLDL and LDL and the fatty acid composition of those lipid fractions did not differ from those of the control group. Lipids of all the different LPs isolated from egg yolk contained more stearic and less oleic and palmitoleic acids than did those of control hens. The increased content of stearic acid increased the density of the lipoproteins so that a larger proportion of the LPs were in the LDL and a smaller portion in the VLDL fractions of yolk lipids.

The effect of the addition of saturated and unsaturated FAs and cholesterol to the diet of laying hens was studied by Sim and Bragg.[38] The sources of fat used were 8 percent hydrogenated coconut oil or safflower oil with or without either cholesterol or soysterols or both. Both cholesterol and soysterol decreased oleic acid and decreased palmitic and/or stearic acid of the yolk. These changes were significantly greater upon feeding of cholesterol than soysterol. However, when both sterols were added simultaneously, the effect decreased greatly.

Mixtures of steam-pelleted barley and full-fat canola seed at different proportions (80:20; 70:30; 60:40; 50:50) in pullet diets were evaluated.[39] These mixtures were used at the 40 percent dietary level in isonitrogenous, isocaloric diets. Yolk color index and contents of LA, α-LA, and DHA increased linearly as full-fat canola seed content of the diets increased, suggesting that this source of n-3 FAs may provide an alternative for the

enrichment of shell eggs with these healthful nutrient components.

Results by Ferrier et al.[29] showed that feeding diets containing 0, 10, or 20 percent flaxseed to Leghorn pullets provided a marked progressive increase in n-3 yolk FAs. The n-3 acids were α-LA (28, 261, and 527 mg/egg, respectively) and DHA (51, 81, and 87 mg/egg, respectively). These authors, after a clinical trial with male volunteers, reported that the inclusion of flaxseed in the diets of laying hens, and its correlate in the eggs produced, represents an important nutritional source of n-3 FAs.

Jiang et al.[40] fed hens diets high in oleic acid (OA, C18:1, n-9), α-LA, or LA prepared by incorporation of high-oleic sunflower seed, full-fat flax seed, or regular high-LA sunflower seed, respectively, to study the effects of dietary fats on the FA profile of major lipid classes of poultry eggs. After 3 weeks of feeding, the FA composition of yolk total lipids, TGs, phosphatidylcholine (PC), and phosphatidylethanolamine (PE) were measured by gas chromatography. Dietary treatments had no effects on yolk total lipid content. Feeding high-OA sunflower seed increased yolk OA by 17 percent, and this change affected only the TGs. The increases of yolk LA and AA upon high-LA sunflower seed were distributed evenly among TGs and PC with moderate effect on the PE fraction. The enrichment of LA in eggs from the flax regime was mainly in TGs. The longer-chain FAs, such as EPA, DPA, and DHA, were deposited exclusively in PLs, particularly in PE. The contents of the longer-chain n-3 FAs in PE were three to seven times those in PC, indicating a preferential incorporation in that phospholipid fraction.

Ground and whole flaxseed at levels of 5 or 15 percent were used by Aymond[41] to enrich egg yolk fat with n-3 FAs; the author compared the lipid profile and yolk oxidation quality with those of eggs enriched by means of 1.5 percent menhaden oil. All the treatments increased n-3 FA content in the yolk (α-LA, EPA, and DHA for flaxseed and EPA and DHA for menhaden oil), but at the 15 percent flaxseed level, the increase in n-3 FAs was higher when the seed was ground than when the whole seed was used. The oxidative quality of the yolk lipids wasn't affected by the incorporation of the n-3 saturated FAs as reflected by the determination of TBAR.

Collins[42] investigated whether pearl millet, when substituted for corn in laying hen diets, would enrich egg yolks with n-3 FAs. Diets were isoenergetic and isocalorically formulated with corn, equal amounts of corn and pearl millet, and pearl millet as grain sources. The substitution of pearl millet for corn decreased the ratio of n-6 to n-3 FAs (13.1:1; 10.1:1; and 8.3:1 respectively), showing clearly that pearl millet inclusion produces eggs significantly enriched in n-3 FAs.

In another study, the addition of 3 percent α-LA to the diet of hens increased the amount of n-3 FAs of the yolk by 6.5 percent—70 percent as α-LA, 20 to 25 percent as DHA, and the remaining 5 to 10 percent as DPA.[43]

Some authors have studied the supplementation of hen diets with α-tocopherol in order to prevent oxidation of unsatutated FAs when these FAs are increased to enrich eggs. These authors reported that the content of α-tocopherol in eggs increases in a dose-dependent manner.[44–46] Galobart et al.[47] studied the effect of the addition of increasing levels of α-tocopheryl acetate to a basal diet containing 5 percent linseed oil (65 percent PUFAs, 34.3 percent n-3) and how it affects storage stability. Their results showed that the

vitamin E content of eggs decreases with processing and storage time; thus, the level required in hens' diets should be adjusted depending not only on the fatty acid composition of the eggs but also on the conditions of processing and conditions and time of storage.

These same authors[48] investigated the effect of vitamin E and canthaxanthin and their relation with n-3 or n-6 FA supplementation. They found that n-3 FA–enriched eggs are more susceptible to oxidation than those enriched with n-6 FAs and that the addition of 200 ppm of vitamin E reduces the oxidation induced by the spray-drying process, but supplementation with 5 ppm of canthaxanthin had no significant effect as an antioxidant.

The enrichment of eggs with a vegetable fatty acid blend, linseed oil, and fish oil supplemented with crushed and spray-dried aplanospores of the green micro alga *Hematococcus pluvialis* to provide 0.2 or 4 mg of astaxanthin per kg feed was studied by Elwinger and Inborr.[49] The deposition rates of carotenoids and astaxanthin in the egg yolk were 14 and 10 percent, respectively. Peroxide values of the feed containing linseed oil without added algal meal tended to be elevated, whereas addition of algal meal to the diets decreased the peroxide value. The anisidine value of egg yolks followed the same pattern, indicating antioxidant activity of the algal meal. The content of n-3 FAs was highest in eggs from hens fed linseed oil. The content of EPA plus DHA was calculated to be 223, 106, and 85 mg per egg in eggs from hens fed fish oil, linseed oil, and vegetable fatty acid blend, respectively. Stored eggs from hens fed fish oil were categorized as having a "stronger egg taste" by a taste panel. Otherwise there were no remarkable gastronomic differences, either in fresh or in stored eggs, due to fat or astaxanthin supplementation.

An experiment carried out by Mozzon et al.[50] was aimed at the evaluation of the efficiency of the transfer of n-3 PUFAs (EPA and DHA) to the eggs of hens given diets integrated with these components. The integrators utilized were made up of an appropriate mixture of fats from different sources, supplied as microspheres obtained through a technology analogous to spray-drying. Fish oils represented the source of n-3 FAs: their mixing with silica and fractionated palm oil appeared to allow a proportional decrease in the microsphere structure of the surface-exposed PUFAs. This, together with the addition of appropriate concentrations of tocopherols, allowed sufficient antioxidant protection of PUFAs, preventing oxidation and consequently preventing health problems and the appearance of negative organoleptic characters in the final product. The total amount of PUFAs in the eggs was higher than that of a control group. Regarding the individual PUFA content, enriched eggs containing EPA—not present in control nonenriched eggs—had higher content of DHA and DPA and lower content of AA.

The "enrichment" strategy already commented on, with marine oils or meals, overcomes the "fishy" taste or smell transmitted to the enriched products. This condition has been evaluated in different experiments carried out by submitting the different avian products obtained (eggs and meats) to sensorial evaluations made by trained panels of experts. In general, when the marine sources of lipids did not exceed 5–8 percent of the diets, the different panelists could not distinguish between egg types.[9,51–52]

Fish taste doesn't seem to be a problem when fish FAs are used to enrich eggs, provid-

ing that all the technological processes involved in the production of marine meals and oils have been adequately controlled and that the eggs are conveniently stored and commercialized within a reasonable time. When other sources of FAs are used, mainly from vegetable oil seeds, this "fishy taste" problem is absent.

Dried-fermented algae can contain over 7 percent DHA in naturally encapsulated form. Herber[53] investigated the use of a natural golden marine algae in laying hen diets to incorporate n-3 FAs into yolk lipids. This algae is unique in its FA profile due to its high content of DHA and the absence of other n-3 FAs normally present in marine oils such as menhaden oil. In two experiments, conducted with different levels of incorporation of the algae and menhaden oil (compared with the typical corn-soybean diets fed to hens), the supplementation with marine products significantly increased yolk total n-3 FAs with concomitant reductions in yolk n-6 FAs. Although menhaden oil diets contained predominantly EPA, the main FA deposited was DHA. Marine algae also promoted an efficient deposition of DHA, the highest concentrations being attained when it was fed at 4.8 percent of the diet (180 mg total n-3 FAs). This data indicates that the use of this algae as a direct source of dietary n-3 FAs may be an efficient alternative to current sources of n-3 FAs for the supplementation of poultry diets for the enrichment of eggs.

Herber and Van Elswyk[54] utilized a microalgal product enriched with DHA as a source of n-3 FAs. The diets were supplemented at the 2.4 or 4.8 percent level. The results showed that the n-3 FA content of yolk lipids increased with the supplementation and that consumer acceptability of the eggs remained unaffected by the enrichment with n-3 FAs.

These marine algal products provide perhaps one of the most effective ways of producing n-3–enriched eggs.

The use of some crustaceans (*Mexican langostilla*) as meals in the diets of white layer hens was reported to provide an excellent way to incorporate adequate amounts—and proper proportions—of n-3 and n-6 FAs into eggs, without any adverse effect on the productivity of the hens.[55]

The effect of supplementation of hen diets with different products to decrease the cholesterol content of shell eggs has been studied. Beyer and Jensen[56] studied the effect of orotic acid in the diet on the cholesterol content of eggs. There were no effects from its addition on egg weight, yolk weight, percentage of yolk, or egg production. However, the data indicated that orotic acid is ineffective for reducing egg cholesterol levels.

The effect of supplemental niacin on laying hen performance and egg cholesterol content was investigated by Leeson et al.[57] Dietary niacin level had no effect on cholesterol content of eggs, even when levels as high as 1.022 mg/kg, which closely simulates therapeutic levels used for humans, were added.

Beyer and Jensen[58] used α-ketoisocaproic acid and leucine in laying hen diets to test whether these substances at different levels could modify egg cholesterol and layer performance. They found that 0.09 percent α-ketoisocaproic acid and 0.09 percent leucine significantly reduced egg cholesterol after 4 weeks' intervention. After 8 weeks 0.27 percent α-ketoisocaproic reduced egg cholesterol significantly below controls.

Cholesterol content of eggs is difficult to manipulate by nutrition or other genetic or

pharmacological methods; it has been suggested that the hen will cease laying when egg cholesterol content is lowered below the amount necessary to sustain embryonic development.[59] Clinical studies with normolipaemic individuals showed that they can eat two eggs per day[60–61] or up to four n-3–enriched eggs per day[62] as part of a moderate fat diet not exceeding 30 percent of calories from fat and 10 percent as SFAs without alteration in their lipid profiles.

Finally, it appears very clear that the nutritional enrichment of eggs is not only possible but necessary. This can be done in different ways depending on the local availability of foodstuffs and their costs. In the southern Pacific hemisphere, fish products are probably the most adequate source of n-3 FAs because they are normally available at convenient prices, whereas in other parts of the world, especially on the Atlantic coast of South America and in the northern hemisphere, cereals, oil seeds, and algae are the best resources. In the near future structured lipids with a desired chemical configuration will be widely available, and their incorporation in layer diets will most likely represent the most adequate way to enrich eggs and other foodstuffs with specific FAs.

Another fact that must be taken into account when deciding the best strategy for enrichment of eggs is the additional benefit in the birds that the egg producers have when using appropriate n-3:n-6 ratios in the layers' diets. It has already been proven that they will improve the cellular as well as the humoral immune responses of the birds. This sanitary "plus" must be taken into account when comparing the economic profit of the enrichment strategy with the direct costs of the enrichment.

Eggs have been stigmatized because of their cholesterol content; this has masked their nutritional advantages: quality protein, vitamin and mineral supplies, energetic content, and, in the case of enriched eggs, lipid profile. According to the American Dietary Guidelines,[63] healthy people can eat up to 300 mg/day of cholesterol, so when their diets are adjusted to these nutrient recommendations, eating three to four eggs per week won't be dangerous at all; moreover, if eggs are higher in n-3 FAs and the proportion of SFAs recommended is not exceeded, they can represent a source of beneficial nutrients.

The price of enriched shell eggs will depend on the source of FAs incorporated and the technological processes needed for modification. Their cost and consumer concerns about their benefits will determine the convenience and feasibility of their production.

The enrichment of eggs may increase their market price, but once consumers are aware of their health benefits, they should be well disposed to spend more to obtain their extra advantages.

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16

Predictability of Respiratory Atopy from Egg Hypersensitivity in Children

Kelly Blackstock

Basic Mechanisms of an Allergic Reaction

The body's ability to combat disease comes from the immune functions of the lymphatic system. Resistance to pathogens is divided into specific and nonspecific. Nonspecific resistance is made up of general mechanisms of attack directed toward a variety of foreign invaders. Specific resistance involves specialized lymphocytes recognizing a particular pathogen. This branch of immunity mediates allergic responses. Typical lymphocytes associated with specific resistance are B-cells and T-cells, although mast cells and basophils are synergists in subsequent reactivity.

Substances that elicit reactivity are called antigens. Antigens become introduced into the body through the respiratory tract or gastrointestinal tract or through contact of the integument. Initial contact of an antigen to a predisposed person will not manifest symptoms; rather, the immune system will become *sensitized*. Sensitization begins with recognition of a specific antigen by a matching B-cell. Antigen protein will be presented on the plasma membrane of the B-cell by the major histocompatibility complex (MHC). Corresponding T-cells, once bound to the MHC, will release chemicals, stimulating the B-cell to divide. The final result of B-cell division is the production of antibodies, or immunoglobins (Igs). The binding sites of the Igs will be specific for the initial antigen. Several isotopes of Igs exist, but it is IgE that is responsible for allergic hypersensitivity. Once the immune system has become sensitized, it is primed to react to a second exposure. Circulating IgEs will bind to new antigens and then to the F_c receptors of both mast cells and basophils. Following binding, mast cells and basophils are stimulated to produce histamine and prostaglandin, whose actions include promoting inflammation and other clinical allergic symptoms.

All true allergies are manifested through this mechanism. However, it should be noted that a variety of IgEs exist for the array of possible antigens. For this reason differences arise between allergies in their expression. Factors including age, gender, ethnicity, stress, and seasonal changes can affect when and if an individual will be sensitive to an antigen.[2,15] For example, aeroallergens typically affect individuals later in life, while food allergens prevail in infancy.[9,15]

Food Allergy

Food allergies are generically described as adverse reactions to food generated by an immune response. Food antigens are water-soluble, heat- and acid-stable glycoproteins with molecular weights between 15 and 60 kDa.[1] Their admittance to the body is through the GI tract (although studies have shown reactivity to food as aeroallergens).[9] For any immunologic reaction to occur, there must first be penetration of molecules through a barrier, in this case the mucosal membrane of the intestines. It has been demonstrated in studies as early as the 1930s that antigenically intact macromolecules are transmitted across the mammalian gut. Wilson and Walzer intradurally injected IgE antibodies specific for hen eggs in both adult and juvenile subjects. Upon ingestion of eggs, an average of 79.7 percent of subjects had positive reactions, proving significant intestinal absorption.[16]

Though antigen absorption does exist, it should be minimal, making reactivity almost nonexistent. It is the role of the mucosa, specifically a protein called secretory immunoglobin A (sIgA), to act as a barrier and prevent a majority of macromolecule absorption from occurring. Transient deficiencies in sIgA have been linked to reagenic manifestations.[14] Injury, inheritance, or immaturity typically causes defects in the barrier.[2] In the case of children, immaturity appears to be the most prominent contributor to food allergy. It is hypothesized that immature sIgA in children allows more antigenic protein to cross over, compounding any predisposition.[2,15] This assumption is validated by the fact that food allergy is the most common allergen in the first years of life[10,11,15] and decreases in occurrence with age.

Egg Hypersensitivity

Of all food allergens, egg appears to be the most common.[1,9,10,12,15,17,18] Egg protein, or albumen, was found to be more allergenic than the yolk.[15] Albumen constituents include ovalbumin (54 percent), ovatransferrin (12 percent), and ovomucoid (11 percent). Ovomucoid is most often associated with allergic responses because of its thermostability and resistance to trypsin digestion. Severity of reaction is dependent on the individual. Minute exposure to egg protein can be mild or result in life-threatening anaphylaxis.[1]

Treatment of egg allergy, as with all food allergies, is a restrictive diet. Concerns do arise involving nutrient deficiencies, especially with children, but eggs are not a primary source of any one nutrient. Their exclusion, then, poses no nutritional threat.[4] Egg-sensitive individuals usually have greater difficulties isolating the hidden sources of eggs. Binders, emulsifiers, coagulants, and clarifying agents are just a few roles that egg components play in food preparation. For this reason eggs can be found in items as innocuous as root beer, salad dressing, soups, candy, and meat products.[1,4,15] In addition, cross contamination of foods during restaurant preparation is common. Cooking rarely helps because egg allergens are recognized by human serum IgE even after pasteurization.[8] Furthermore, many vaccines, including influenza, yellow fever, and typhus, are harvested in chick embryo tissues, posing yet another concern.

Connection of Food Allergy to Respiratory Atopy

Prevalence of atopic disorders, including atopic dermatitis, urticaria, and asthma, has been steadily rising in children.[5] For this reason, increasing interest has been directed toward isolating predictive measures so that primary and secondary interventions can be initiated. Though controversial, some researchers believe early sensitization to any antigen results in an increased risk of developing subsequent allergic disease.[5,6,13,17] Prediction, then, would be most valuable during gestation or in early infancy. Attempts at finding a prenatal marker using umbilical cord levels of total IgE, however, proved unfounded.[6,10,12,17] New theories have surfaced using indicator markers that develop promptly postnatally, the most common being total serum IgE and specific IgE to food antigens.

The majority of investigation into total serum IgE has been done by Kulig et al. In a recent study involving 4,082 German children, these researchers evaluated serum IgE levels in atopic versus nonatopic children annually from birth until 6 years of age. As was hypothesized, IgE percentiles were markedly higher in atopic versus nonatopic subjects ($p < 0.001$). Distributions were graphed and found to overlap considerably, making a discernable division between atopic and nonatopic children impossible. The inability to discriminate between atopic and nonatopic children reflects the moderate capacity of total IgE as a reliable predictor.[7] Similar findings were reported by Zeiger and Heller and again by Kulig et al.[18,5]

Another shift in investigation is toward the use of food allergens as predictors. IgE antibodies against inhalant allergies usually appear later in life among allergic individuals in comparison with IgE antibodies against food allergens, which appear in infancy. The apparent inverse proportionality between time of development of respiratory and food allergens might suggest a physiological link. Early theories connecting the two are predominantly circumstantial. The first hypothesized connection is that ingested allergens are found to circulate quickly to nasal mucosa, stimulating mast cells to increase airway reactivity. Also, asthma can be exacerbated by inhalation of airborne food proteins.[15] The relation of reactivity could possibly indicate that IgE antibodies are not entirely specific to only one antigen, suggesting that cross-reactivity is occurring. Secondly, food-sensitized children are more prone to aeroallergens,[2,5,6,10] and aeroallergens are linked to atopy such as asthma.[5,12] At least one study has confirmed the connection, showing children with food allergies at age 7 years to have almost two times the prevalence of both allergic rhinitis and asthma compared with those without food allergies.[18]

Of all food allergens studied, specific IgE to egg protein had the highest correlation to atopic disease.[6,5,10,12,18] Sigurs et al. showed that of 135 subjects with at least one food allergen, 46 had significant levels (0.35 kU/L) of IgE to eggs. Of those egg-sensitive individuals, 57 percent had developed aeroallergens by 2 years of age and 76 percent by 15 years of age.[13] Additional studies found that not only was egg sensitivity significantly linked to atopy including asthma but also risk of development of atopic disease in those high-risk infants was at least doubled by age 7.[2,17]

Evidence regarding degree of sensitization has compounded the issue. Findings showed early sensitization leading to higher concentrations of serum IgE to egg protein.[6,10] Consequently, varying levels of concentration were evaluated as risk predictors. It appears that as serum levels of IgE increase so does risk. Going one step further, studies showed children with long-lasting sensitization developed allergic rhinitis and allergic asthma significantly more often than children just transiently sensitized to egg protein. ($p < .001$), increasing the risk of allergic rhinitis 3.4 times and asthma 5.5 times.[6] However, these findings are controversial. Prophylactic procedures done by Zeiger and Heller showed no relationship of food sensitization to development of atopy.[18]

Is Egg IgE a Valuable Predictor?

Correlation tied to egg IgE was high in all studies, but to be an accurate predictor, clinical sensitivity and positive predictive values (PPVs) also need to be high. In most cases use of specific IgE to eggs increased both the sensitivity and PPV;[5,6,10,12] however, the highest recorded sensitivity was 56 percent (Table 16.1). This means that, of all individuals that will develop atopy, the IgE egg marker will only detect 56 percent of them. The highest PPV (71 percent) still leaves a 29 percent chance of false-positives. The strength of the IgE egg marker lies in the clinical specificity, ranging from 92 to 97 percent. Unfortunately, this information is of little use to prevention.

Lack of validity for egg IgE as a predictor led some researchers to cross-examine the marker with other variables. Family history and risk profiles were evaluated most often.[5,9,12,18] By themselves, they too were poor predictors of atopy, but together with egg IgE, they drastically increased sensitivity and PPV. One study showed PPV rising from 37 to 70 percent, and another listed sensitivity as high as 86 percent with the inclusion of a positive family history.[5,10]

The appeal of using food-specific antibody to determine risks of atopic development is understandable. The tests are easy to perform and cost-effective. The greater challenge is obtaining high enough results for clinical sensitivity and PPV. As of yet, egg-specific IgE tests are not sufficient for predicting atopic disorders. Furthermore, critics contend that previously obtained results were biased, arising from homogeneous cohorts of genetically high-risk subjects. Inclusion of additional members of a more general population could warrant a decrease in predictability percentages, although of the articles that were reviewed most implemented large cohorts. Also, allergenicity was determined and compared by both skin-prick tests and radioallergosorbent test (RAST), neither of which is without limitations. Sensitization, however, is a prerequisite for development of allergic airway disease.[6,5,9] Sensitized children must be regarded as high risk for subsequent development of allergic disease, a fact noted by strong correlation and relatively high sensitivity. For these reasons, the IgE egg marker for respiratory atopy should not be abandoned. Rather, further study should be implemented not only for specific egg IgE as a predictor but also to unravel the physiological mechanism that might link the two.

Table 16.1. Capacity of egg sensitization to predict the development of respiratory atopy

Study	Risk factor	Sensitivity (%)	Specificity (%)	PPV (%)
Hattevig et al.[a,*]	8-mo egg-IgE ≥ 0.35 kU/L	56	96	63
Nickel et al.[b,*]	1-yr egg-IgE ≥ 0.35 kU/L	20	93	36
Zeiger and Heller[c,*]	Egg-IgE skin-prick test (1-yr)	21	93	80
Kulig et al.[d,*]	1-yr egg-IgE ≥ 0.35 kU/L	35	94	71

Note. PPV = positive predictive value.
[a]Reference 3.
[b]Reference 10.
[c]Reference 18.
[d]Reference 6.
[*]$P < .001$

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17

Effects of Cooking and Storage on the Nutritional Value of Eggs

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Introduction

> "And to this research and discovery which name more appropriate than Medicine could be given? Because this discovery was made for the health of man, for his salvation and feeding, in place of that regimen from which troubles, diseases and death were coming":[1] this is how the author of *De Antica Medicina*—the most famous treatise of the *Corpus Hippocraticum*—heralds the discovery of cooking, which, in his account, marked the beginning of medicine. Even if nowadays such an assertion is considered exaggerated, there is some truth in it. Apart from making foods more digestible, cooking actually increases the availability of some minerals (e.g., iron).[2] However, cooking may also reduce the content of some nutrients, especially vitamins.[3,4] Storage is another "discovery" of humans that has substantially improved their food intake. However, storage may also produce loss of nutrients.[3] This chapter offers a brief critical review of the effects of cooking and storage on the nutritional value of the hen egg.

Eggs in Nutrition

We start by recalling the main nutritional properties of eggs since this will allow us to better understand the effects of cooking and storage on their nutritional value. First, eggs are sources of high-quality proteins (and are in fact the standard against which the protein quality of other foods is judged). Second, eggs are rich in unsaturated fatty acids. Third, eggs are rich in cholesterol and are sources of many vitamins and minerals (Table 17.1).

Effects of Cooking on the Nutrient Content of Eggs

To explain the effects of cooking, we compared the nutrient content of raw eggs with that of boiled, fried, poached, and scrambled eggs using the data provided by the Royal Chemistry Society.[6] The egg samples and the recipes employed to produce these data are described in Table 17.2.

Table 17.1. Contribution of a 100 g hen egg to the recommended daily allowance (RDA) of vitamins and minerals for an adult man (25–50 years)

	Qty. / 100 g	RDA (%)
Vitamin B_{12} (μg)	2.50	125
Biotin (μg)	20.00	20–67[a]
Panthotenic acid (mg)	1.77	25–44[a]
Iodine (μg)	53.00	35
Vitamin D (μg)	1.75	35
Riboflavin (mg)	0.47	28
Folic acid (μg)	50.00	25
Retinol (μg)	190.00	19
Iron (mg)	1.90	19
Phosphorus (mg)	200.00	17
Vitamin E (mg)	1.11	11

Source. Values were calculated from the egg composition provided by the Royal Chemistry Society[6] and the RDA for Americans.[5]

Note. Only micronutrients with a contribution > 10 percent are given.

[a]Variability within the recommended range.

Table 17.2. Egg samples and recipes employed to develop the egg composition tables of the Royal Chemistry Society

Raw (whole) egg	Analysis of battery, deep litter and free range
Boiled egg	10 eggs
Fried egg (vegetable oil)	12 eggs, shallow fried
Poached egg	10 eggs, no fat added
Scrambled egg (milk)	2 eggs, 20 g butter, 15 ml milk, ½ level tsp. salt; melt butter in pan, stir in beaten egg, milk, and seasoning. Cook over gentle heat until moisture thickens.

Source. Reference 6.

Note. Allowances were made for any water loss or fat uptake in cases where eggs were cooked with fat.

Macronutrients

Boiling and poaching have virtually no effect on eggs' macronutrient content (Table 17.3). However, both frying and scrambling substantially increase their energy value through the addition of fats. Scrambling may be responsible for a modest loss of proteins. However, the intestinal absorption of egg proteins and their accumulation in body tissues is greater for cooked than for raw eggs. For instance, Evenepoel et al. recently showed that 94 percent (25 g) of cooked egg proteins are assimilated as compared with 64 percent of (the same amount of) raw proteins.[7]

Boiling and poaching were found not to be associated with any change in lipid composition (Table 17.4), which was confirmed by previous observations.[8] Of course, the addition of oil for frying does increase fatty acids and that of butter is responsible for a substantial increase in saturated fatty acids in scrambled eggs.

Micronutrients

Vitamins

Owing to their susceptibility to thermal degradation,[9] water-soluble vitamins are the micronutrients of egg most prone to loss during cooking (Table 17.5).

According to data provided by the Royal Chemistry Society, vitamin B_{12} undergoes the greatest average loss (42 percent) (Fig. 17.1), with a substantial difference, however, between scrambling (16 percent) and boiling (56 percent). Riboflavin, pantothenic acid, folic acid, thiamin, and biotin are lost in amounts between 18 and 28 percent, while pyridoxine and niacin are virtually unmodified. As noted by Everson and Souders[10] and reviewed by Froning,[11] the loss of thiamin is independent of the cooking method employed. However, contrary to what Hanning reported,[11,12] similar losses of riboflavin take place in scrambling and boiling. Moreover, according to these data, the susceptibility of folic acid to undergo loss appears to be lower than reputed in the past.[11] As far as folic acid is concerned, it should be noted that egg (yolk) folates are the most stable and bioavailable among many foods.[13]

Finally, the data of the Royal Chemistry Society show that among liposoluble vitamins (A, E, and D) only vitamin D undergoes a modest loss in scrambled eggs.

Table 17.3. Effect of cooking on the macronutrient and energy content of eggs

	Raw	Boiled (% change)	Fried (% change)	Poached (% change)	Scrambled (% change)
Proteins (g%)	12.5	0	+ 9	0	−14
Fats (g%)	10.8	0	+29	0	+109
Carbohydrates (g%)	Tr	Tr	Tr	Tr	Tr
Energy (kJ)	612.0	0	+22	0	+67

Source. Percent changes were calculated from the data of the Royal Chemistry Society.
Note. Tr = tracks.

Table 17.4. Effect of cooking on the fatty acid profile of eggs

	Raw	Boiled (% change)	Fried (% change)	Poached (% change)	Scrambled (% change)
SFA (g / 100 g)	3.1	0	+ 29	0	+274
MUFA (g / 100 g)	4.7	0	+ 28	0	+ 53
PUFA (g / 100 g)	1.2	0	+ 25	0	+ 17

Source. Percent changes were calculated from the data of the Royal Chemistry Society.[6]
Note. SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

Table 17.5. Effect of cooking on the vitamin content of eggs

	Raw	Boiled (% change)	Fried (% change)	Poached (% change)	Scrambled (% change)
Thiamin (mg / 100 g)	0.09	–22	–22	–22	–22
Riboflavin (mg / 100 g)	0.47	–25	–34	–23	–30
Niacin (mg / 100 g)	0.10	0	0	0	0
Pyridoxine (mg / 100 g)	0.12	0	+17	0	–25
Biotin (μg / 100 g)	20.00	–20	–10	–25	–17
Pantothenic acid (mg / 100 g)	1.77	–26	–26	–26	–27
Folic acid (mg / 100 g)	50.00	–22	–20[a]	–10	–44
Vitamin B_{12} (μg / 100 g)	2.50	–56	–36	–60	–16
Vitamin C (mg / 100 g)	0	0	0	0	Tr
Vitamin A (μg / 100 g)	190.00	Tr	+13	0	+55
Carotene (μg / 100 g)[b]	Tr	Tr	Tr	Tr	Tr
Vitamin D (μg / 100 g)[c]	1.75	0	+14	0[a]	–11
Vitamin E (μg / 100 g)	1.11	0	N/A	0	+11

Source. Percent changes were calculated from the data of the Royal Chemistry Society.[6]
Note. Tr = tracks; N/A = not available.
[a]Estimated value.
[b]Given as β-carotene equivalents.
[c]Values may be higher for hens fed a supplement.

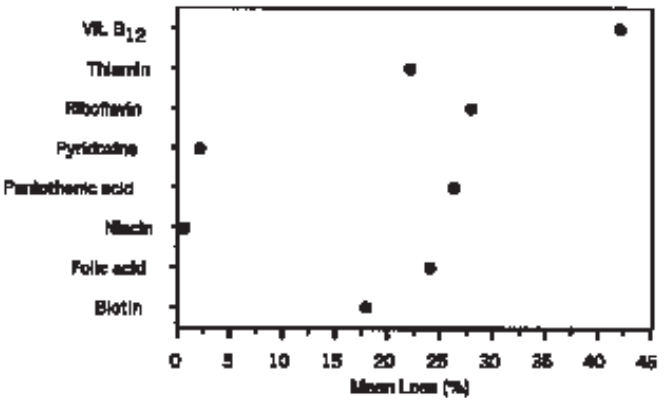

Figure 17.1. Mean losses of egg water-soluble vitamins during cooking. The graph was drawn from the data presented in Table 17.5.

Minerals

Boiling and poaching do not affect the mineral content of eggs (Table 17.6). Many minerals undergo a modest increase in percentage as a result of frying, and the addition of salt for scrambled eggs does substantially increase the quantity of sodium and chloride. Modest losses of phosphorus, iron, copper, zinc, selenium, and iodine occur with scrambling. However, it should be noted that iron's bioavailability actually increases with cooking.[14]

Table 17.6. Effect of cooking on the mineral content of eggs

	Raw	Boiled (% change)	Fried (% change)	Poached (% change)	Scrambled (% change)
Sodium (mg / 100 g)	140	0	+14	0	+636
Potassium (mg / 100 g)	130	0	+15	0	0
Calcium (mg / 100 g)	57	0	+14	0	+11
Magnesium (mg / 100 g)	12	0	+17	0	+42
Phosphorus (mg / 100 g)	200	0	+15	0	−10
Iron (mg / 100 g)	1.9	0	+16	0	−16
Copper (mg / 100 g)	0.08	0	+12	0	−12
Zinc (mg / 100 g)	1.3	0	+15	0	−15
Chloride (mg / 100 g)	160	0	+12	0	+869
Manganese (mg / 100 g)	Tr	Tr	Tr	Tr	Tr
Selenium (µg / 100 g)	11	0	+ 9	0	−18
Iodine (µg / 100 g)	53	0	+13	0	− 2

Source. Percent changes were calculated from the data of the Royal Chemistry Society.[6]
Note. Tr = tracks.

Effects of Storage on the Nutrient Content of Eggs

Cold storage is reported not to produce substantial modifications of the protein content of eggs.[11] As far as lipids are concerned, no consistent changes in fatty acid content of shell eggs were seen after cold storage at 0°C for 6 to 12 months.[8] Studies of cold storage of eggs have shown significant losses of pyridoxine already at 3 months; increasing losses of riboflavin, niacin, pyridoxine, and folic acid at 6 months; and more significant losses of pyridoxine, folic acid, and vitamin B_{12} at 12 months (Table 17.7).[11] Apparently, pantothenic acid and biotin are minimally affected by cold storage. For most vitamins, however, losses are relevant only after 3 months of storage, and since eggs are presently marketed within a few days, these losses are probably of no practical relevance.

Table 17.7. Effect of cold storage on the vitamin content of eggs

	Raw	3 months (% change)	6 months (% change)	12 months (% change)
Riboflavin (µg / g)	3.49	5	16	12
Niacin (mg / g)	0.66	9	18	N/A
Pyridoxine (µg / g)	2.52	18	29	47
Biotin (ng / g)	225.00	0 (−8)[a]	0 (2)[a]	0 (−1)[a]
Pantothenic acid (µg / g)	12.50	6	6	6
Folic acid (ng / g)	94.00	1	15	21
Vit. B_{12} (ng / g)	6.54	7	6	23

Source. Data were adapted from Froning[11] with minor modifications (percent values were recalculated from raw data).
Note. N/A = not available.
[a]The values between parentheses were considered equivalent to zero.

Dehydrated eggs do not generally show protein losses, and their content of riboflavin and niacin is stable.[11] However, significant losses in retinol have been described. These losses are due to oxidation and are more significant at high storage temperatures. For example, 9 months of storage at –9.4°C resulted in a 60 percent loss; at 21.1°C, a 75 percent loss; and at 37.0°C, an 80 percent loss.[11] Dehydration does not appear to influence substantially the baseline vitamin D content of eggs.[11]

Conclusion

Based on this review, the following conclusions can be drawn about the effects of cooking and storage on the nutritional values of eggs:

- Among cooking methods for eggs, scrambling may be responsible for a loss of proteins. However, this loss is modest and does not change the fact that the hen is a rich source of proteins.
- Boiling and poaching are preferable to scrambling because they do not add fats, which increase the energy value of eggs and alter the fatty acid profile of the hen egg. This is not to say that the consumption of scrambled eggs should be avoided by healthy individuals but, simply, that scrambling should be less frequently employed than other forms of cooking to better safeguard the nutritional value of eggs as far as their lipid composition is concerned.
- The fact that vitamin B_{12} may be lost at values as high as 60 percent (poaching) when eggs are cooked does not change the fact that eggs are a rich source of this vitamin. In fact, even with a loss of 60 percent, a 100 g poached egg would still be able to contribute 50 percent of the adult Recommended Daily Allowance for vitamin B_{12}. Losses of other water-soluble vitamins are lower and are expected to be counteracted by eating a balanced diet rich in fruits and vegetables. Among liposoluble vitamins only vitamin D undergoes a modest loss.
- Losses in minerals due to cooking are generally low and are expected to be counteracted by eating a balanced diet rich in fruits and vegetables.
- Cold storage affects the vitamin content of eggs only after long periods (≥3 months), a circumstance that is quite uncommon with actual marketing channels.

Thus, the nutritional value of the cooked egg, especially when boiled or fried with vegetable oil, is high and confirms that hen eggs are a nutritious and healthy food.

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18

The Potential Use of Eggs for the Protein Requirements of Endurance Exercise

Jaclyn Maurer

Introduction

Athletic excellence is a must for any serious athlete. Attaining this excellence is a complicated process that includes proper training, adequate rest, and a balanced diet. Numerous studies have been conducted exploring the carbohydrate requirements of and optimal carbohydrate sources for endurance athletes, leaving other nutrients like protein in the background. Existing and emerging research, however, is introducing the possibility of increasing protein requirements for endurance athletes based on the body's changing needs for protein during endurance exercise. The best sources of protein to satisfy these needs are still unclear; however, egg protein lends itself as a potentially optimal choice of protein for endurance athletes.

Our Basic Need for Protein

Before one can understand why exercise may increase the body's need for dietary protein, it is critical to understand why the body needs dietary protein to begin with. Essentially, protein is responsible for regulation of gene expression; biochemical catalysts; formation of major cellular structural elements: muscle, nails, teeth, hair, ligaments; and formation of antibodies and hormones.[1] The building blocks of protein are amino acids. Twenty amino acids exist; some are nonessential (our body can synthesize them), while nine are essential and can only be obtained from dietary protein intake.[2] When ingested in the diet, synthesized via excess carbohydrates or fat and ammonia, or released from the breakdown of our own body proteins, these amino acids will enter the body's pools of free amino acids.[3] The amino acids in these pools are then utilized for protein synthesis and called upon to compensate for the breakdown of body protein. The breakdown of body protein happens readily with exercise and is discussed in the following sections.

Adequate dietary protein intake is necessary to keep the pools full of free amino acids (both essential and nonessential) for the body to utilize as needed. When dietary protein intake is compromised or poor, the body starts to deplete the free amino acids in its pools. As the pools empty and do not get replenished, losses in muscle size and strength can occur, since amino acids are being drawn from the muscles to refill these pools.[3] These

decreases will eventually lead to fatigue and weaker performance in physically active people. Clearly, adequate protein intake is necessary for our bodies to perform at their optimum physically.

Physical Activity and Our Need for Protein

Muscle activity over a prolonged time (i.e., endurance exercise) causes exercise-induced muscle injury (structural damage to muscle) and increases in mitochondrial protein synthesis. This structural damage to muscle invokes protein synthesis as a means of repair, while an increase in mitochondrial protein synthesis promotes amino acid oxidation.[2,4,5] As the body demands more ATP production to fuel working muscles during endurance exercise, the muscles start to utilize amino acids for this needed fuel.[2]

Initially, the liver regulates amino acids from ingested protein; however, the liver does not control the fate of branch chain amino acids (BCAAs). Instead BCAAs travel to the adipose and muscle tissue and are metabolized as needed. The presence of enzymes specific to the catabolism of BCAA—like the enzyme branch chain α-keto acid dehydrogenase (BCKA dehydrogenase), which controls the irreversible decarboxylation of BCAAs, primarily in the muscle—supports the hypothesis that amino acids may be used as a direct fuel source for muscle.[1,2] Both amino acid oxidation and exercise-induced muscle injury increase protein metabolism. This ties back to the body's need for ample pools of free amino acids to pull from in order to offset the protein degradation and amino acid oxidation that endurance exercise induces. It is important to note, however, that the extent of amino acid oxidation and muscle structural damage is suggested to be dependent on the intensity and duration of the exercise.[2–5] According to findings from Lemon, the limiting enzyme BCKA dehydrogenase is dependent on the intensity and duration of exercise.[4] Researchers Kasperek and Snider supported Lemon's finding with their research that discovered the activity of BCKA dehydrogenase increased as the intensity of exercise increased.[6] Kasperek and Snider linked this increase to an increased requirement for citric acid cycle intermediates.[6] (Note that intermediates for the citric acid cycle are readily utilized during endurance exercise as the need for the generation of ATP increases.) Additional research has documented that mild-intensity endurance exercise will increase amino acid oxidation, but this increase is balanced throughout the day via times of reduced oxidation.[2] Thus, while endurance exercise does appear to increase protein requirements, the extent of this increased need is related to the intensity of the exercise.[4] Therefore, going for a leisurely jog will promote amino acid oxidation but less than a prolonged, intense tempo run. This is an important point for an endurance athlete to consider since it requires the athlete to monitor and adjust protein intake according to training duration and intensity.

Interestingly, research of amino acid oxidation and endurance exercise led to the discovery that the synthesis of the amino acid alanine in the muscle greatly increased with increased exercise intensity. It was found that as both exercise intensity and amino acid oxidation increased, the synthesis of alanine in the muscle greatly increased to help minimize the accumulation of muscle pyruvate. (Alanine is synthesized via the transfer of the

amino group from BCAAs to the carbon of pyruvate, and thus the amount of pyruvate produced in the muscle is decreased.[5] Alanine also serves as a means for transporting the nitrogen from the catabolism of amino acids to the liver for disposal. Once in the liver, this alanine can be converted to glucose and released into the bloodstream where it is picked up and utilized by muscle for energy.[1]) By minimizing the production of muscle pyruvate, the synthesis of alanine in intense working muscles helps offset the production of lactate and consequently wards off fatigue.[5] This finding suggests that branch chain amino acids could potentially be useful as fuel for endurance exercise.

Furthermore, a study done by Hayward et al. examining the effects in rats of dietary protein on enzyme activity after exercise showed that the amount of dietary protein consumed greatly affected both muscle and serum enzyme activity.[7] The enzymes creatine kinase, lactate dehydrogenase, pyruvate kinase, and aspartate aminotransferase come from the skeletal muscle. An increase of these enzymes in the serum following exercise indicates muscle injury. Ideally, an athlete would want to reduce the release of these enzymes after exercise to reduce muscle injury, and Hayward et al. hypothesized that an increase in dietary protein would do that. However, all they found was that rats fed high-protein diets and engaging in exercise had significantly higher serum aspartate aminotransferase and creatine kinase levels than rats fed low-protein diets and exercised. This means that while additional protein in the diet influences serum enzyme activity, further studies need to be done to assess how this increase in dietary protein affects the specific processes of muscle enzymes. (Note that prior exercise could influence the rate of increase in serum concentrations of enzymes after exercise independent of increases from greater intake of protein.[7])

How Much Protein?

As far back as 1840, the debate on how much protein a physically active person needs was in full swing. Researcher von Liebig in 1842[3,4,8] proposed that protein was the main nutrient for fuel for exercise; however, his idea was dismissed with the turn of the century as knowledge expanded about exercise metabolism and researchers discovered the roles of carbohydrate and fat in providing fuel during exercise. So, with almost a full century focusing on carbohydrate and fat, protein was left behind. Perhaps with the role of protein in the physically active out of sight and out of mind for the most part, the Recommended Daily Allowance for protein was (and still is) set at 0.8 g/kg body weight/day.[4] This value is based on data from mostly sedentary people; therefore, it does not account for the potentially greater needs of physically active people. Luckily for protein's sake, not all researchers pushed it aside. Lemon particularly took interest in examining protein in relation to physical activity and reported some interesting findings.[3–5,8]

It has already been established, from exploring how the body utilizes protein during endurance exercise, that as duration and intensity of physical activity increase so does the requirement for protein. Lemon proposed that the requirement for protein for endurance-training athletes be increased from the RDA of 0.8 g/kg body weight/day to 1.2–1.4 g/kg body weight/day,[3] while Meredith et al. found that the protein requirement in endurance-

trained males averaged out to 1.26 g/kg body weight/day.[9] Furthermore, research from Burke et al. suggested that elite male endurance athletes may even need up to 1.5 g/kg body weight/day to satisfy their protein requirements,[10] and still other studies reported that moderate to high-intensity endurance exercise increases protein requirements to 1 g/kg body weight/day.[2] With all these proposed requirements, the American Dietetic Association revised its protein RDA in 1987, increasing it to 1 g/kg body weight/day for the physically active.[11]

Why all the discrepancies? The level of intensity, duration, point of exercise training, and possibly gender can all contribute to the varying need for protein for endurance athletes. Level of intensity as well as duration have already been addressed: the higher the intensity and longer the duration of exercise, the higher the requirement for protein (to a point). While increasing the protein requirement for endurance athletes is supported by research, benefits of protein intakes higher than 2 g/kg body weight/day have not been supported.[2] The stage of exercise training also factors into the protein requirement. Research supports that protein needs are higher for athletes in the beginning of their exercise program, and as the body trains longer and becomes adept at conserving protein, this need levels off.[11] Gender, too, appears to play a role in protein requirement. While exploring the nutritional needs of the female athlete, Manore concluded that the female endurance athlete has elevated protein needs in the range of 1.2–1.4 g/kg body weight/day.[12] However, both Tarnopolsky[11] and Lemon[3] reported research supporting that female endurance athletes may require a smaller protein increase than their male counterparts, since limited results find that women may utilize less protein during endurance exercise. Further studies on this matter need to be conducted.

The quality of the protein consumed, as discussed later, has the potential to affect its requirement. Few of the studies reviewed thoroughly accounted for the source(s) of protein that the endurance athletes consumed; therefore, this potentially contributes to the varying recommendations for the protein requirement.

Additionally, there are other reports stating positive effects of increased protein in the diet of endurance athletes. Researchers found that increasing protein intake at the start of an endurance exercise program reduced the development of anemia.[11] Also, further research found that increasing protein in the diets of female endurance athletes might reduce the occurrence of amenorrhea.[11]

Diet Composition and Its Effect on Protein Requirements

The composition of the diet has interesting effects on protein utilization and requirements. To begin with, total energy intake greatly influences the body's use of protein. When energy intake is excessive (promoting positive energy balance), protein is stored, whereas when energy intake is compromised (causing negative energy balance), degradation of body protein increases. This breakdown of body protein increases the body's overall need for protein.[2] Likewise, a demonstrated need for increased protein in the diet of an endurance athlete may be attributed to caloric deficiency and not necessarily a need for additional protein.[11]

When an endurance athlete consumes inadequate amounts of carbohydrate, this leads to rapid depletion of liver and muscle glycogen stores during endurance exercise. These depleted glycogen stores then lead to an increase in the utilization of protein by the body during exercise.[8] Exercise in conjunction with low-carbohydrate body stores stimulates BCKA dehydrogenase activity and promotes the breakdown of BCAAs to supply the liver with carbon skeletons for gluconeogenesis.[2] Important to understand, though, is that protein is likely to account for only 10 percent of total energy expenditure even when carbohydrate stores are low.[2] Thus, protein appears to serve an auxiliary role to carbohydrates, the main fuel source utilized during endurance exercise.

A study on the food intake and energy expenditure of participants during the Tour de France concluded that a high-protein intake might adversely affect carbohydrate intake (reduce it in some cases) and consequently reduce endurance. A diet insufficient in carbohydrates will lead to poor glycogen stores and a reduced energy supply for the endurance athlete's body to call upon during prolonged exercise.[13]

Clearly there needs to be a balance between carbohydrate and protein intake. While endurance exercise does appear to increase the athlete's need for protein, the athlete's need for carbohydrates should not be adversely affected by this increase in protein. For most of these studies concerning increased intakes of protein, the percentage of total calories from carbohydrates remained high, at around 55–65 percent, reinforcing the idea that carbohydrates are the most essential source of fuel during exercise, and protein is an auxiliary fuel source.

The Role of Protein in the Postexercise Diet

The role of postexercise protein consumption on protein metabolism is an interesting area of research.[14] One study exploring the value of protein in the postexercise meal found that consumption of a combined carbohydrate and protein meal after exercise increased the levels of the anabolic hormone insulin (which influences protein metabolism) higher than postexercise meals made up of only carbohydrates or only protein.[2] This result of increased insulin may be a consequence of the carbohydrate and protein meal having one-third more calories. Other research examining protein consumption after endurance exercise concluded that carbohydrate-protein mixtures increased levels of plasma insulin and growth hormone more than carbohydrates or protein alone.[2] These findings proposed that adding protein to carbohydrates for postexercise recovery may reduce breakdown of muscle and/or improve recovery. A possible reason for these findings is that since exercise promotes protein synthesis and oxidation of amino acids that lead to body protein breakdown and depletion of the amino acid pools, protein intake postexercise may spare muscle catabolism.[2] More research needs to be conducted in order to come to a final conclusion.

Where to Find Protein: Eggs?

Biological value (BV) is the gold standard for determining the quality of a protein. The higher quality the protein, the better source it is for our bodies. Why? The biological value

of a protein is the extent to which the amino acid composition of the particular protein matches the amino acid composition of mammalian tissue.[15] Therefore, a protein with a high biological value is essential to an endurance athlete who oxidizes amino acids during exercise. The consumption of protein with high BV potentially supplies an athlete's body with enough essential and nonessential amino acids to fill the body's internal pools, which are continually drawn from to repair damaged muscles and oxidize amino acids during exercise. A diet of poor quality protein, lacking in some of the essential amino acids, will put the endurance athlete's body at risk of early fatigue and compromised performance. Remember that endurance exercise increases oxidation of the amino acid alanine in the muscle (proportional to exercise intensity), so consuming a protein source with adequate amounts of alanine, along with other complimentary amino acids, is essential for any endurance athlete desiring to perform optimally and ward off fatigue.

Eggs contain the highest quality of protein known; in fact, the egg is often the standard against which all other sources of protein are judged. The only superior source of protein is human milk because of the quantity of essential amino acids human milk supplies.[15] However, since human milk is not a readily available source, and because its acceptability as a protein source for post–breast-feeding age humans has not been evaluated, athletes do not consume it.

The RDA for protein is based on the assumption that the average mixed protein diet has a biological value of 70.[16] Therein lies the possibility that if an endurance athlete consumes a diet with an overall average biological value higher than 70, he or she may not need the additional protein that is recommended by current research. A diet composed of protein sources with a combined biological value higher than the biological value of 70 potentially provides an endurance athlete with the appropriate amounts of amino acids at a lower protein requirement than some of the proposed requirements. The egg has a biological value of 100.[16] This leads to the question of whether eggs as the main dietary source of protein would have potential benefits for an endurance athlete. This is still an area of speculation, and research needs to be done to determine if the amount of high-quality protein in the diet would affect the overall requirement for protein.

Eggs are an excellent source of the high-quality protein that an endurance athlete's body demands, but many athletes may restrict consumption of eggs due to the misconception that eating eggs causes increases in cholesterol. Although egg yolk is a concentrated source of cholesterol, studies show that intakes of one egg per day appear to have little or no effect on cholesterol levels and risk for coronary heart disease or stroke in healthy people.[17] Eggs do not need to be the sole source of dietary protein in an endurance athlete's diet, but consumption of eggs regularly along with a balanced diet of adequate energy and other high-quality protein sources has the potential to fuel an endurance athlete to perform at his or her best.

Are There Adverse Effects to Consuming More Protein?

While Lemon's research on protein requirements and athletes suggested that protein intake between 1.2 and 2 g/kg body weight/day does not cause problems in athletes,[17]

excess protein and amino acid intake has been associated with adverse affects on renal function. Researchers Poortmans and Dellalieux studied whether these same adverse effects were apparent in healthy athletes who consumed a higher-protein diet than the current recommended RDA. The endurance athletes Poortmans and Dellalieux studied consumed 1.35 g protein/kg body weight (+/−0.12) daily. Findings showed no impairment of renal function based on the glomerular filtration rate and rate of calcium excretion on intakes up to 2.8 g/kg body weight/day in healthy athletes.[17]

Furthermore, Colombani et al. fed marathon runners a protein-supplemented drink (providing 1.8 g protein/kg body weight) during their race and found that the protein-and-carbohydrate–supplemented drink, compared with the control carbohydrate-only drink, did not affect myofibrillar protein breakdown as speculated, but importantly the added protein was proven to be absorbed and mostly oxidized with no apparent adverse effects on metabolism.[18] What this research revealed is that increasing the protein, to an extent, in the diets of endurance athletes has no apparent adverse metabolic effects. This along with Poortmans and Dellalieux's finding suggests that increasing protein intake in an endurance athlete's diet will not have adverse effects on either renal or metabolic function.

Conclusion

Our bodies need protein; there is no doubt about that. How much protein our bodies need is another question. The current RDA for protein is set at 0.8 g/kg body weight per day, but this is based on research of mostly sedentary people. A physically active person, especially an athlete participating in endurance activity, needs more protein than even the RDA for athletes of 1 g/kg body weight per day. Endurance activity causes muscle damage and oxidation of amino acids and stimulates protein synthesis. All these consequences of endurance activity lead to an increased need for protein to offset protein losses the body endures with the breakdown of body tissue and muscle. How much extra protein is needed is debatable and appears to depend upon point of training, intensity of training, diet composition, and gender. Recommendations, though, call for protein consumption in endurance athletes to be 1.2–1.5 g/kg body weight per day but not to exceed 2 g/kg body weight per day. The source of protein an athlete consumes should be of high biological value, making egg protein a potentially prime source of protein for athletes. Also, to fully benefit from increasing protein, athletes should not let protein intake interfere with total caloric intake or intake of carbohydrates. Finally, an increased amount of protein in a healthy athlete's diet has not been shown to have adverse effects on metabolism and/or renal function.

Increasing the protein requirement for endurance athletes is supported by significant evidence; however, further research needs to be conducted to explore how specific sources of protein affect this increased protein requirement. In theory, eggs, with their optimal biological value, appear to be the ultimate protein source for any serious athlete; therefore, further research is warranted of the effects of this high–biological value protein source on the protein metabolism and physical performance of endurance athletes.

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