"A man should look for what is, and not for what he thinks should be."

—Albert Einstein

INSIDE

2 Dietary Factors Associated with Coronary Heart Disease: How Strong is the Evidence?

4 Special Feature: Egg Nutrition Research Presented at Experimental Biology 2009

6 EDITORIAL Choline: An Essential Nutrient for Public Health

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NUTRITION CLOSE-UP

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Higher Choline Levels Associated with Reduced Risk of Neural Tube Defects in Folate-Fortified Population

It is well established that adequate intake of folic acid during pregnancy plays a key role in minimizing the risk of neural tube defects (NTDs)—including spina bifida and anencephaly—in the developing fetus. In fact, the strong protective effect of folic acid sparked what has become perhaps one of the most successful public health campaigns to date. In September 1992, the U.S. Public Health Service recommended that women of childbearing age consume 400 µg (micrograms) of folic acid per day to reduce their risk of NTD-affected pregnancies and the March of Dimes carried the message forward with an aggressive campaign designed to encourage all women of childbearing age to take a daily multivitamin with 400 µg of folic acid before becoming pregnant and during early pregnancy (the developmental window for neural tube formation). Since the U.S. Food and Drug Administration (FDA) began requiring that folic acid be added to enriched breads, cereals, flours, corn meals, pastas, rice, and other grain products in 1998, the U.S. has seen a significant decline in the prevalence of NTDs. Despite these successes with population fortification and individual supplementation, NTDs continue to occur. Researchers question whether there are other nutrients that might play a role in proper fetal neural tube development and have looked specifically at methionine, zinc, vitamin C, vitamin B12, and choline.

During the formation of the neural tube, . . . the fetus is entirely dependent upon maternal choline stores.

Because folic acid plays an integral role in one-carbon metabolism, Shaw et al. chose to expand this area of research by examining other nutrients related to one-carbon metabolism. The research team used midpregnancy (15th-18th week of pregnancy) blood samples obtained as part of the California Expanded AFP (alpha-feto-protein), a program designed to screen pregnant women for NTDs and other abnormalities. Over 180,000 serum samples were obtained from a large population of folate-fortified women in California (including Orange, San Diego, and Central Valley counties) between 2003 and 2005. Of this large sample, there were 80 NTD-affected pregnancies (cases), 31 of which had spina bifida and 49 of which had anencephaly. Controls (n=409) were randomly selected from the group of women with pregnancies not affected by NTDs.

NTDs and structural malformations were assessed by the California Birth Defects Monitoring Program. Serum specimens were analyzed for methylmalonic acid, total homocysteine, cysteine, methionine, total choline, betaine, cystathionine, pyridoxal phosphate, pyridoxal, pyridoxic acid, folate, cobalamin (vitamin B12), riboflavin, and creatinine. Cotinine was also measured to assess cigarette smoking. Lab workers were unaware of the identity of case/control samples during the specimen analysis.

Continued on page 2

As the researchers expected, Hispanic women in this population sample were more likely to have NTD-affected pregnancies than non-Hispanic women. There were also more cases in the 25-29 age group and fewer in the 30-35 age group. The most striking finding was a significantly lower mean total choline level in cases than in controls (at least one-half standard deviation). After comparison of serum levels by quartile, total choline still yielded the strongest associations. Odds ratios were 1.8 for women in the lowest quartile of total choline, vs. 0.4 in the highest quartile (P=0.0003). These results remained the same after adjustment for maternal age and ethnicity (P=0.0006). After dividing total choline levels into deciles, the odds ratio was 2.4 for the lowest vs. 0.14 for the highest decile (odds ratio = 1.0 for the 25th-74th percentile). According to this analysis, the odds ratio associated with a 1-unit increase in total choline would be 0.24. When the same analysis was performed classifying NTDs by phenotype, odds ratios for anencephaly were 1.8 for the lower vs. 0.34 for the upper quartile of total choline. For spina bifida, the odds ratios were 1.8 for the lower vs. 0.60 for the upper quartile.

No association was observed between serum folate levels and NTD incidence, which the authors expected in this population of pregnant women exposed to a food supply fortified with folic acid. The authors also mention that these women were likely to have taken prenatal supplements, although this information was not available.

The strong linear relationship between serum choline and NTD risk observed in this study is supported by previous epidemiological data showing an association between choline intake and NTD incidence. The authors speculate that this association may be linked to the interrelationship between choline, folate, and methionine in one-carbon metabolism and that a deficiency in choline might affect folate and homocysteine metabolism. One reasonable proposed mechanism has to do with phosphatidylcholine, which is vital for the structure of cell membranes. Within embryos, nearly all available choline is converted to phosphatidylcholine for cell development and growth. During the formation of the neural tube, embryos cannot synthesize phosphatidylcholine due to a lack of the required enzyme, thus the fetus is entirely dependent upon maternal choline stores. Other possible mechanisms exist and associations have been observed between NTD risk and elevated homocysteine, low levels of serum folate, and low levels of vitamin B12. The authors emphasize that more research must be completed before these results can be generalized.

Shaw GM, Finnell RH, Blom HJ, et al. Choline and risk of neural tube defects in a folate-fortified population. *Epidemiology* 2009;20(5):714-9.

KEY MESSAGES

- This study showed a strong linear relationship between serum choline and NTD risk, with higher levels demonstrating a protective effect.
- No association was observed between serum folate levels and NTD incidence, which the authors expected in this population of pregnant women exposed to a food supply fortified with folic acid.

Dietary Factors Associated with Coronary Heart Disease: How Strong is the Evidence?

Researchers have sought for decades to clarify how our dietary choices might relate to disease outcomes. Although numerous foods, nutrients, and dietary patterns have been found to be associated with heart disease risk, the strength of these associations has not been evaluated systematically and conflicting data has led to confusion among professionals and consumers. Despite the lack of conclusive evidence for most of these dietary components, strong beliefs persist among health professionals, policy makers, and consumers regarding the relationship between specific dietary choices and heart health. Because statistical associations do not always predict causal relationships, it is critical to evaluate the strength and validity of these relationships before the results can reliably be generalized to large populations for heart disease prevention efforts.

To address this issue, Mente et al. used a systematic approach to gather information from prospective cohort studies and randomized trials evaluating relationships between dietary factors and CHD (coronary heart disease). Their objectives were to 1) evaluate the strength of the evidence supporting each association; 2) determine which dietary factors have been found in randomized controlled trials to support evidence from prospective cohort studies; and 3) to identify associated dietary factors that are not supported by sufficient evidence.

Researchers searched the MEDLINE database to identify eligible prospective cohort studies and randomized controlled trials conducted between 1950 and June 2007. To be eligible for inclusion, these studies had to have followed participants for at least one year. Three distinct dietary patterns were evaluated; 1) the "Mediterranean" dietary pattern (characterized by higher intake of vegetables, legumes, fruits, nuts, whole grains, cheese or yogurt, fish, and monounsaturated fatty acids); 2) the "Prudent" dietary pattern (characterized by higher intake of vegetables, fruits, legumes, whole grains, fish, and other seafood); and 3) the "Western" pattern (characterized by higher intake of processed meat, red meat, butter, high-fat dairy products, eggs, and refined grains).

Bradford Hill guidelines were used to assign causation scores to each food, nutrient, or dietary pattern based on 4 criteria: strength; consistency (whether the finding was replicated in other studies); temporality (dietary exposure preceded the disease outcome); and coherence (finding was not inconsistent with known disease mechanisms). Dietary factors were scored from 1 to 4 based on whether each of these criteria was met. A score of 4 was considered strong evidence of a cause-and-effect relationship, a score of 3 was considered moderate evidence, and a score of 2 or less was considered weak evidence of a causal relationship.

The MEDLINE search resulted in 146 prospective cohort studies (86% of which were primary prevention studies) and 43 randomized controlled trials (74% of which were secondary prevention trials). In the prospective cohort studies, on average, 29,209 participants were included for each dietary factor, 41% of the participants were women, and the median follow-up was 11 years. For the randomized controlled trials, there were 7204 individuals, on average, representing each dietary factor, mean follow-up was 3.7 years, and women made up 36% of the study populations.

Pooled estimates from the cohort studies showed the following factors to be associated with a significantly lower risk of CHD: increased alcohol consumption, dietary beta carotene, fiber, fish, total folate, fruits, marine n-3 fatty acids, monounsaturated fatty acids, nuts, vegetables, total vitamin C, dietary vitamin C, total vitamin E, dietary vitamin E, whole grains, and increased adherence to the Mediterranean diet and other high-quality diet patterns. Increased consumption of trans-fatty acids and foods with a high glycemic index was associated with higher CHD risk. Higher intakes of alpha-linolenic acid, polyunsaturated fats, saturated fats, total fat, vitamin C and vitamin E supplements, eggs, meat, milk, and "prudent" and "western" dietary patterns showed no significant association with CHD risk. When studies of lower methodologic quality were excluded, the "prudent" (RR=0.73 [95% CI, 0.62-0.83]) and "western" (1.55 [1.27-1.83]) diet patterns were significantly associated with CHD. In addition, while fish consumption appeared protective against fatal CHD (RR=0.83 [95% CI, 0.71-0.94]), higher intake of alpha-linolenic acids and marine n-3 fatty acids were not significantly related.

Pooled analysis of the randomized controlled trials demonstrated that higher intakes of marine n-3 fatty acids (RR=0.77 [95% CI, 0.62-0.91]) and adherence to the Mediterranean diet pattern (0.32 [0.15-0.48]) were significantly associated with decreased CHD risk. Intake of folate, beta carotene, vitamin C and vitamin E supplements, fiber, polyunsaturated fats (relative to saturated fats), total fat, fish, fruits, and vegetables did not show a significant association with CHD risk.

Analysis using the Bradford Hill criteria showed strong evidence for a protective role of vegetable and nut intake, Mediterranean and high-quality diet patterns. These criteria also demonstrated strong evidence of a causal relationship between intakes of trans-fats and foods with a high glycemic index or load. Studies of high methodologic quality yielded strong evidence for a protective effect of monounsaturated fat intake and adherence to a "prudent" diet, as well as for a causal relationship between a "western" diet and CHD risk. Scores indicating moderate evidence were given for intake of fish, marine n-3 fatty acids, folate, whole grains, dietary vitamins C and E, beta carotene, alcohol, fruit, and fiber. Scores for vitamin E and ascorbic acid supplementation, polyunsaturated fat, saturated fat, total fat, alpha-linolenic acid, egg, milk, and meat intake were weak, indicating that no significant relationship exists between these dietary factors and CHD risk.

This analysis indicates that strong evidence supporting causal or protective relationships exist for only a few dietary factors, including protective factors such as higher intakes of nuts, vegetables, and monounsaturated fats, and adherence to the Mediterranean, "prudent," and other high-quality dietary patterns, and causal factors including higher intakes of trans-fats, high glycemic index foods, and adherence to a western dietary pattern. At this time, there does not appear to be sufficient evidence to support a causal relationship between other individual foods and nutrients and CHD risk.

The authors indicate that many dietary recommendations, including those encouraged in the Dietary Guidelines for Americans from the US Departments of Health and Human Services and Agriculture are based on epidemiological evidence and cohort studies, with insufficient supportive evidence from randomized controlled trials. They state:

"Despite this lack of information, evidence-based recommendations derived from cohort studies have been advocated. This is cause for concern because dietary advice to limit the intake of a certain nutrient (ie, dietary fat) may result in increased consumption of another (ie, carbohydrates), which can have adverse effects on CHD risk factors. Moreover, without large prospective studies in which multiple health outcomes are evaluated, recommendations to modify a dietary component may decrease the likelihood of one chronic disease (ie, CHD) at the cost of increasing another (ie, cancer)."

The authors suggest that although studies involving individual foods and nutrients may help clarify the mechanisms behind protective or harmful diet patterns, it is not likely that changing these few dietary factors will have a meaningful impact on CHD risk. They recommend an increased focus on dietary patterns in both cohort studies and randomized controlled trials to further our understanding of how diet influences health outcomes. ■

Mente A, de Koning L, Shannon HS, Anand SS. A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch Intern Med* 2009;169(7):659-669.

Special Feature:
Egg Nutrition Research Presented at Experimental Biology 2009

The incorporation of a protein-rich breakfast on appetite sensations and subsequent food intake in "breakfast-skipping" adolescents

Objective: To examine the impact of a protein-rich breakfast (in solid vs. beverage form) on appetite and subsequent food intake in breakfast-skipping adolescents.

Methods: 15 healthy adolescents (age 14 ± 1 y; BMI 79 ± 4 %ile; breakfast 2 ± 1 occasions/wk) randomly consumed (on 4 separate days) a 500 kcal breakfast consisting of a normal protein meal (18 ± 1 g protein) in solid form (PN-S), a protein-rich meal (48 ± 2 g protein) in solid (PR-S) or beverage (PR-B) form, or no meal (NM). Pre and post-meal appetite was measured over 5 hours followed by an ad libitum lunch.

Results: PR-S was the only meal to exhibit significant reductions in post-meal hunger vs. NM ($p<0.01$). Post-meal fullness was greater after each meal vs. NM (all comparisons; $p<0.05$) but not different between meals. All meals led to significant reductions in prospective food consumption vs. NM (all comparisons; $p<0.05$); PR-S led to greater reductions vs. PN-S ($p=0.06$). Fewer calories were consumed at lunch following the PR-S (363 ± 51) vs. NM (490 ± 63; $p< 0.01$), PN-S (504 ± 66; $p<0.01$), and PR-B (464 ± 83 kcal; $p=0.10$).

Conclusion: The incorporation of a protein-rich breakfast beneficially impacts appetite & subsequent food intake in breakfast-skipping adolescents suggesting a potential role in the management of obesity. However, protein-rich beverages may not elicit the same positive effects compared to solid versions.

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Research supported by SAH Research Award, KUMC.

Regular eggs and lutein-enriched eggs increase macular pigment density without changing plasma lipids

Methods: Fourteen subjects (11 women and 5 men) aged 40-70 y old were recruited to evaluate the effects of regular egg and lutein-enriched egg consumption on plasma lipids and macular pigment density (MPOD). All subjects consumed the equivalent of 7 eggs/wk and were randomly assigned to egg substitute (SUB, 0 mg cholesterol, 0 mg lutein per day), regular eggs (EGG, 213 mg cholesterol, 200 µg lutein per day) or lutein-enriched eggs (L-EGG, 213 mg cholesterol, 6 mg lutein per day). Weight, blood pressure, waist circumference, skin carotenoids, MPOD and plasma lipids were measured both at baseline and after 6 wks.

Results: There were no changes in body weight or systolic and diastolic blood pressure for any of the subjects. Plasma triglycerides did not change between baseline and 6 wks. There were also no changes in total cholesterol, LDL-C, HDL-C or skin carotenoids between baseline and 6 wk. However, MPOD was significantly increased for both the EGG (0.27 ± 0.04 at baseline to 0.39 ± 0.09 6 wk) and the L-EGG (0.39 ± 0.10 at baseline and 0.46 ± 0.12 at 6 wk) ($P < 0.01$) while no changes were seen for the egg substitute group (0.41 ± 0.09 at baseline and 0.42 ± 0.07 at week 6).

Conclusion: These results confirm that egg is a good vehicle for dietary carotenoid absorption and that increased dietary lutein correlates with MPOD and not with skin carotenoids. The results also indicate that consuming one egg per day did not raise plasma cholesterol in this group of subjects.

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Research supported by a grant from the American Egg Board.

Macronutrient composition of breakfast influences plasma glucose, satiety hormones and caloric intake in the next 24 h in adult men

Objective: Researchers examined the effect of the macronutrient composition of breakfast on postprandial satiety and caloric intake throughout the day.

Methods: Using a crossover design, 22 men aged 20-70 y consumed two isocaloric test breakfasts in a random order separated by one wk. The macronutrient composition of the breakfasts were: EGG: % CHO:fat:protein = 22:55:23 or BAGEL: % CHO:fat:protein = 72:12:16. Fasting blood samples were drawn at baseline before the test breakfast and at 30, 60, 120, and 180 min after breakfast. After 180 min, subjects were given a standard lunch and asked to eat until satisfied. Subjects filled out visual analog scales (VAS) during each blood draw and recorded food intake the day before and after test days. Plasma glucose and appetite hormones were analyzed at each time point.

Results: Subjects consumed fewer calories following the EGG breakfast compared to the BAGEL breakfast ($P < 0.01$). Additionally, subjects consumed more calories in the 24h period after the BAGEL breakfast compared to the EGG breakfast ($P < 0.05$). Based on VAS, subjects were hungrier and less satisfied 3h after the BAGEL breakfast compared to the EGG breakfast. In addition, subjects had lower plasma glucose 3h post BAGEL ($P < 0.05$) and higher plasma ghrelin concentrations 30 min post BAGEL ($P < 0.05$) compared to post EGG.

Conclusion: These findings suggest that consumption of a protein-rich breakfast (EGG) results in less variation in plasma glucose levels, lower acute ghrelin response at 30 min, and a reduced caloric intake.

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Research supported by a grant from the Egg Nutrition Center.

The Impact of Egg Consumption on Heart Health using the NHANES III Follow-up Survey

Objective: To evaluate the association between egg consumption and heart health using the NHANES III (1988-94) Follow-up Survey and FFQ.

Methods: Egg consumption data was obtained from the 30-day FFQ administered to participants within the NHANES III survey. Adults 17 years and older were followed for mortality from all causes. There were 366 deaths from CHD and 137 deaths due to stroke among 14,946 subjects included in the analysis. Estimated rate ratios (RR) of death from CHD or stroke associated with categories of egg consumption were estimated using Cox proportional hazards regression.

Results: Multivariate models show no increased risk of death from CHD with increased egg consumption (Males: RR = 1.40, 95% CI 0.83-2.85; Females: RR = 0.93, 95% CI 0.30-2.84). There was a slight reduction in risk of mortality from stroke in males among consumers of 1 to 6 eggs/week compared to those reporting consumption of <1 egg/week (RR = 0.93, 95% CI 0.87 - 0.996). Females showed a significant reduction in risk of stroke among consumers of 1 to 6 eggs/week (RR = 0.89, 95% CI 0.83 - 0.95) and 1 or more egg/day (RR = 0.84, 95% CI 0.71 - 0.99).

Conclusion: These results show no association between egg consumption and increased risk of death from CHD or stroke, consistent with the conclusions in previous studies using the NHANES I Follow-up Survey, the Health Professional Follow-Up, and Nurses' Health Study.

Scrafford C, Tran N, Barraj L. Exponent, Inc., Washington, DC. Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

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EDITORIAL

Choline: An Essential Nutrient for Public Health

On September 10, 2009, a group of prominent scientists and researchers gathered in Washington D.C. to present the latest science on choline to an audience of nutrition scientists and health professional educators. The goal of the Choline Science Summit was to elevate awareness of the overlooked, yet potentially serious health consequences of inadequate intake by Americans of choline, a nutrient established to be essential for health by the Institute of Medicine. This initiated a dialogue among nutrition leaders on practical solutions that can help increase Americans' consumption of this essential nutrient. The Choline Science Summit was supported by the Choline Coalition, which includes the Egg Nutrition Center, Balchem Corporation, National Pork Board, and National Cattlemen's Beef Association.

Choline is found in a wide variety of foods; liver, eggs, and wheat germ are among the most concentrated sources. One egg supplies 115-125 mg of choline, about one-third to one-half of the daily recommendation. Other foods rich in choline include beef, pork, poultry, milk, and some types of fish. While the body manufactures some choline, consuming choline-rich foods is necessary to meet the body's daily needs.

Choline was officially recognized as an essential nutrient by the Institute of Medicine (IOM) in 1998. At that time, the Adequate Intake (AI) was established based on estimated dietary intakes and research findings reporting liver damage with lower choline intakes. Advances in the science and understanding of choline reveal that it plays a critical role in human health and development throughout the lifecycle, beginning with fetal brain development. Most recently, studies suggest that significant variations in individual choline requirements exist as a result of genetic differences among individuals and that several subpopulations, including older children, men (especially older men), and women (including pregnant women), may have even higher daily needs than the established adequate intake (AI) level.[1,2]

Choline was not considered in the 2005 revision of the Dietary Guidelines for Americans because of a lack of U.S. consumption data. However,information on the choline content of common foods is now available through a US government database (www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choline.html), making it possible to evaluate the choline content of diets. Since 2005, dietary choline intake data has been included in "What We Eat in America" (NHANES). Survey data from the most recent NHANES (2005-06) revealed that only 10% of individuals had intakes at or above their AI for choline. Among all ages, mean daily intake of choline was 314 mg/d, significantly below the AI of 425-550 mg/d for adults. (See Table)

Table : Dietary Reference Intakes for Choline

Life Stage Group	Adequate Intake (AI) (mg/day)
0-6 months	125
7-12 months	150
1-3 years	200
4-8 years	250
9-13 years	375
Females:	
14-18 years	400
19-70+ years	**425**
Pregnancy	**450**
Lactation	**550**
Males:	
14-70+ years	550

The Role of Choline in Pregnancy and Lactation

Demand for choline is especially high during pregnancy and lactation. Large amounts of choline are delivered to the fetus across the placenta making the choline concentration in amniotic fluid 10-fold greater than that present in maternal blood.[3] Human milk is also a maternal reserve for fetal choline. Because human milk is so rich in choline, lactation further increases maternal demand.[3]

Neural Tube Defects

Choline's role in the conversion of homocysteine to methionine is similar to that of folate, suggesting that choline is also important for neural tube closure.[4] Shaw et al.[5] found that women in the lowest quartile for dietary choline intake had four times the risk of giving birth to a child with a neural tube defect, compared with women in the highest quartile.

Memory Development

Recent studies in animal models show that choline supplementation during critical periods of neonatal development can have long-term beneficial effects on memory.[6-9] In rodents, choline availability during later periods of pregnancy and after birth influences the formation of neural stem cells in the memory center of the brain (hippocampus), causing lifelong changes in brain structure and function. Rodent offspring exposed to extra choline in utero did not show a typical decline in memory with age.[10]

Conclusions and Next Steps

- Choline is an essential nutrient with a critical role in human health and development.
- Intake levels are well below current recommendations while evidence suggests that as much as 50% of the population may have genetic variations that increase their choline needs beyond the established level of an adequate intake (AI).
- Greater attention to consuming choline is warranted, yet current dietary guidance on cholesterol intake, which calls for limited consumption of choline-rich eggs and meats, presents a challenge.
- Moving forward, Summit participants agreed there is a need to increase awareness of the public health consequences of suboptimal choline intakes by providing more nutrition education and guidance to increase consumption of choline-rich foods. ■

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