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CENTRAL SOYA

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May 1, 2001

Gregory L. Paul
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VIA COURIER

Dockets Management Branch
Food and Drug Administration
Room 1061
5630 Fishers Lane
Rockville, MD 20852

Re: Authoritative Statement Nutrient Content Claim Notification

Dear Ladies and Gentlemen:

Central Soya Company, Inc. hereby submits the enclosed Nutrient Content Claim Notification Based on an Authoritative Statement. This notification is submitted pursuant to section 403 (r)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 343(r)(2)(G)).

If you have any questions, please feel free to contact me. Thank you for consideration of this matter.

Sincerely,

Gregory L. Paul

Gregory L. Paul, Ph.D.
Director Nutrition Science

019-0352

HCN1

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CENTRAL SOYA

April 30, 2001

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VIA COURIER

Lynn A. Larsen, Ph.D.
U.S. Food and Drug Administration
CFSAN
200 "C" Street, S.W.
Washington, DC 20204

Re: Authoritative Statement Nutrient Content Claim Notification

Dear Lynn,

For your records, I have enclosed a copy of the notification for making nutrient content claims for choline based on authoritative statements. The notification was sent to Dockets via overnight courier for delivery by 10:00 am CST May 1. We thank you for consideration of this matter, and please contact me if you have any questions.

Sincerely,

Gregory L. Paul, Ph.D.
Director Nutrition Science

NOTIFICATION OF NUTRIENT CONTENT CLAIMS FOR FOODS CONTAINING CHOLINE BASED ON AN AUTHORITATIVE STATEMENT

Before the
Center for Food Safety and Applied Nutrition
Food and Drug Administration
200 C Street, SW
Washington, DC 20204

Submitted by
Central Soya Company, Inc.

May 1, 2001

NOTIFICATION FOR NUTRIENT CONTENT CLAIMS BASED ON AN AUTHORITATIVE STATEMENT

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Notification of Nutrient Content Claims Based on a Authoritative Statement Pursuant to 21 U. S. C. 343 (r) (2) (G)

I. Introduction

Central Soya Company, Inc. (Central Soya) files this notification to use the following nutrient content claims characterizing the level of choline in a food or dietary supplement:

Good source of choline	**Contains choline**
Provides choline	**Excellent source of choline**
Rich in choline	**High in choline**
Added choline	**More choline**
Enriched with choline	**Fortified with choline.**

These claims are authorized by the Food and Drug Administration Modernization Act (FDAMA), 21 U.S.C. 343 (r) (2) (G), as they are based on a current, published authoritative statement of the National Academy of Sciences (NAS), specifically the Food and Nutrition Board (FNB) of the Institute of Medicine, which established a Dietary Reference Intake (DRI), in particular an Adequate Intake (AI), for choline. Specifically, the Summary of the choline chapter (Chapter 12, p 390 - 422) in Dietary Reference

Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (Food and Nutrition Board, 1998) stated, in its entirety:

> **"Choline functions as a precursor for acetylcholine, phospholipids and the methyl donor betaine. The primary criterion used to estimate the Adequate Intake (AI) for choline is the prevention of liver damage as assessed by measuring serum alanine aminotransferase levels. The AI for adults is 550 mg/day of choline for men and 425 mg/day for women. There are no nationally representative estimates of the intake of choline from food or food supplements. Choline in the diet is available as free choline or is bound as esters such as phosphocholine, glycerophoshpocholine, sphingomyelin, or phosphatidylcholine. The critical adverse effect from high intake of choline is hypotension, with corroborative evidence on cholinergic side effects (e.g., sweating and diarrhea) and fishy body odor. The Tolerable Upper Intake Level (UL) for adults is 3.5 g/day."** (page 390)

Finally, this notification meets all of the required elements identified in FDA's "Guidance to Industry: Notification of a Health Claim or Nutrient Content Claim Based on an Authoritative Statement of a Scientific Body" issued June 11, 1998 as described below.

II. Background

Central Soya, located in Fort Wayne, Indiana, is one of the leading producers of soy protein, textured soy protein, refined soybean oil and lecithin in the United States and throughout the world. Central Soya is particularly committed to creating value-added ingredients from soybeans to meet customers' current and future needs.

The Food and Nutrition Board established a DRI, specifically an AI, for choline in 1998 (Food and Nutrition Board, 1998). At the same time, the FNB also established an AI for two other vitamins, pantothenic acid and biotin. Currently, both of these nutrients can be listed on the Nutrition Facts panel. FNB has also established an AI for Vitamin D, calcium, Vitamin K, chromium, and manganese, five other nutrients listed on the Nutrition Facts panel. It appears that choline is the only nutrient that has a DRI but is not allowed to be listed on the Nutrition Facts panel.

Currently, no nutrient content claims are available to characterize the level of choline in a food. However, claims that do not implicitly characterize the level of a nutrient in the food and that are not false or misleading in any respect are allowed (21 C.F.R. § 101.13 (i) (3)). Thus, foods with choline could claim, "contains 100 mg of choline" as long as this claim is not false or misleading.

Thus, each of the nutrient content claims identified above, including "good source of choline," "excellent source of choline," "more choline," and "added choline," qualifies as "a claim … for a nutrient, for which the Secretary has not promulgated a regulation"

described in FDAMA. FDAMA permits new nutrient content claims without prior FDA approval under the following conditions:

1) A scientific body of the United States Government with official responsibility for public health protection or research relating to human nutrition (such as the National Institutes of Health or the Centers for Disease Control and Prevention), or the National Academy of Sciences or any of its subdivisions has published an authoritative statement which identifies the nutrient level to which the claim refers;
2) The authoritative statement is published and is currently in effect;
3) The claim based on the authoritative statement accurately represents the authoritative statement and is presented in a manner that the public can comprehend the information provided in the claim and the relative significance of the information in the context of the total daily diet;
4) The claim and foods eligible to bear the claim use existing terms, meet existing nutrient content claims regulations and the claim is not false or misleading;
5) A submission has been made, at least 120 days before the first introduction into interstate commerce of the food with a label containing the claim, which includes:
    A) a notice of the claim, which shall include the exact words in the claim and shall include a concise description of the basis upon which the

person submitting the claim relied for determining that the requirements of point 1 above have been satisfied;

B) a copy of the authoritative statement that upon which the person submitting the claim relied in making the claim;

C) a balanced representation of the scientific literature relating to the nutrient level to which the claim refers.

The following sections address all the requirements of FDAMA mentioned above and clearly substantiate that the claims set forth in this notification are authorized nutrient content claims under FDAMA.

III. Statements Provided as Basis for the Nutrient Content Claims Are Authoritative

A. The Authoritative Statements

The nutrient content claims set forth in this notification are authorized by FDAMA, 21 U.S.C. 343(r)(2)(G), as they are based on a current, published authoritative statement of a subdivision of the National Academy of Sciences, namely, the Food and Nutrition Board. These statements come directly from the Summary of the Choline section (Chapter 12) of the FNB report:

> **"Choline functions as a precursor for acetylcholine, phospholipids and the methyl donor betaine. The primary criterion used to estimate the Adequate**

Intake (AI) for choline is the prevention of liver damage as assessed by measuring serum alanine aminotransferase levels. The AI for adults is 550 mg/day of choline for men and 425 mg/day for women. There are no nationally representative estimates of the intake of choline from food or food supplements. Choline in the diet is available as free choline or is bound as esters such as phosphocholine, glycerophoshpocholine, sphingomyelin, or phosphatidylcholine. The critical adverse effect from high intake of choline is hypotension, with corroborative evidence on cholinergic side effects (e.g., sweating and diarrhea) and fishy body odor. The Tolerable Upper Intake Level (UL) for adults is 3.5 g/day." (page 390)

A copy of the choline chapter of the NAS report on Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (Food and Nutrition Board, 1998), the bases for these authoritative statements under the meaning of FDAMA, is included in Appendix A.

B. The Authoritative Statements meet FDA Requirements

In Guidance to the Industry provided on June 11, 1998, FDA identified six specific elements that clarify the definition of an authoritative statement for the purposes of a nutrient content claim under FDAMA. FDA indicated that an authoritative statement for nutrient content claims must:

1) Identify the nutrient level to which the claim refers;

2) Be published by a scientific body;

3) Be currently in effect;

4) Not be just a statement of an employee of a scientific body or in the individual capacity of the employee;

5) Reflect a consensus within the scientific body; and

6) Be based on a deliberative review of the scientific body.

The authoritative statement identified above succinctly "identifies the nutrient level to which the claim refers," namely, 550 mg of choline per day for men and 425 mg of choline per day for women. This Report, which was the result of extensive review of the latest scientific literature and numerous meetings discussing science issues, was released in pre-print copy in 1998 and formal Report copies became available in 2000. The Report is the most authoritative and current review of B vitamins and associated compounds and thus meets the requirement to be "currently in effect." This authoritative statement was "published by the scientific body" as an official NAS publication and is not "a statement of an employee of the scientific body made in the individual capacity of the employee" and thus satisfies these requirements of FDAMA. The scientific body is "NAS or one of its subdivisions," which is clearly "a scientific body of the United States with official responsibility for public health protection or research directly related to human nutrition."

Thus, the statements provided in this notification meet all the required elements identified by FDAMA and FDA to be an authoritative statement.

FDA has stated the agency will consult "with the scientific body that is the source of a statement cited as the basis for a claim" and possibly include consultation "with the other federal scientific bodies that have public health responsibility and expertise relative to the claim." In previous communication with NAS, FDA received a response, which indicates that NAS stands behind their published reports. Specifically, via a letter on April 30, 1999, the Director of the Food and Nutrition Board pointed out that in May 1997, the National Research Council (NRC) Governing Board of NAS approved a policy statement regarding authoritative statements made by it or its subdivisions, the NRC and the Institute of Medicine (IOM), noting that authoritative statements "are limited to those that represent the consensus of a duly-appointed committee or views of a duly-appointed principal investigator so that they appear explicitly as findings, conclusions, or recommendations in a report that has completed the institutional report review process" (Yates, 1999). The Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline (Food and Nutrition Board, 1998) has undergone such institutional review. Thus, it appears that NAS would deem the statement provided in this notification as an allowable authoritative statement.

IV. Scientific Literature on Choline Supports the Nutrient Level Used for These Nutrient Content Claims

Choline is important for the structural integrity of cell membranes, cholinergic neurotransmission, transmembrane signaling, methyl-metabolism, and lipid-cholesterol transport and metabolism (Zeisel and Blusztajn, 1994). Choline is a precursor for phosphatidylcholine, sphingomyelin, platelet-activating factor, betaine and other phospholipids (Food and Nutrition Board, 1998). Choline speeds the synthesis and release of acetylcholine, an important neurotransmitter involved in numerous important functions in the body. While it is known that choline is required for cell growth in tissue culture, and FNB stated "... choline is essential to life..." it has been difficult to show that choline is an essential nutrient since there is some de novo synthesis of choline. This biosynthesis of choline is dependent on metabolic methyl exchange, which involves other known nutrients, namely, methionine, folic acid and vitamin B_{12}. In one study, healthy males with normal folate and vitamin B_{12} status were shown to have decreased plasma and phosphatidylcholine concentrations and liver damage, as measured by elevated alanine aminotransferase, when fed a choline deficient diet for three weeks (Zeisel, et al., 1991). Several other studies have indicated that fatty liver and liver damage associated with total parenteral nutrition can be prevented, at least in most individuals with the addition of choline or lecithin (a source of choline). These data led FNB to conclude:

"The data available are supportive of the provisional conclusion that de novo synthesis of choline is not always sufficient to meet human requirements for choline." (page 396)

Based on these data, and other information available, the FNB established various AI for choline based on gender/age groups. Table 1 lists the AI levels established by FNB.

Table 1. Choline Adequate Intake (AI) Summary

INFANTS	CHOLINE
0-6 Months	125 mg/day
7-12 Months	150 mg/day
CHILDREN	
1-3 Years	200 mg/day
4-8 Years	250 mg/day
9-13 Years	375 mg/day
MALES	
14-18 Years	550 mg/day
19 Years and older	550 mg/day
FEMALES	
14-18 Years	400 mg/day
19 Years and older	425 mg/day

The Adequate Intake for pregnancy and lactation were established as 450 mg/day and 550 mg/day, respectively (Food and Nutrition Board, 1998).

Another NAS committee, the Committee on Military Nutrition Research (1994), while reviewing various components which may increase performance in soldiers, reported in the Conclusion and Recommendation section of their Workshop Report: Food Components to Enhance Performance, that:

> **"Diets deficient in choline produce liver dysfunction within 3 weeks, resulting in massive triglyceride accumulation in the liver and abnormalities of liver enzymes."** (page 151)

This section states that **500 mg/day of choline has an impact on plasma levels of choline and phosphatidyl choline**. The Conclusion and Recommendation section also included the following conclusions from the scientific literature regarding choline:

> **"There is evidence that diets low in choline reduce muscle performance. Dietary choline supplementation of individuals with normal intakes during a 20-mile (32-km) run improved the run time by 5 minutes and prevented the drop in choline levels normally associated with the run."** (page 151);

> **"Choline supplementation enhances memory and reaction time in animals particularly aging animals, and enhances memory in humans. Although the mechanisms for this are unclear, there are indications of alterations of the anatomy of brain cells."** (page 151);

"With the diversity of functions of choline in the body, there is ample reason for interest in reviewing its possible value in maintaining or enhancing performance of the soldier." (page 151);

"Free choline and choline-containing esters are present in a wide variety of foods in the human diet. The usual intake is estimated to be in the range of 200-1000 mg per day." (page 150-151)

It should also be noted that in this Workshop Report, NAS concluded choline was one of the few food components or nutrients that offer potential to enhance performance. Choline was included with other well-accepted food components that enhance performance, like carbohydrates and caffeine. The Committee made the following highlighted recommendation:

"The committee recommends that choline should be added to the list of food supplements that have potential to enhance performance and that are being evaluated at the U.S. Army Research Institute of Environmental Medicine (USARIEM)." (page 157)

Additionally, the Committee, based on review of clinical trials in non-military personnel, made several specific recommendations as to the type of studies to be conducted in soldiers:

"1. studies to determine whether choline supplementation enhances endurance and muscle performance, and

2. studies to determine whether choline supplementation enhances intellectual performance and whether this alters performance of soldiers in the field." (page 159)

This report of the Committee on Military Nutrition Research is no longer available from the National Academy Press but can be accessed via the Internet at www.nap.edu/openbook/NI00005/html. Appendix B contains a copy of the Conclusions and Recommendations section of the Report.

A recent publication (Warber, et al., 2000) evaluated the impact of choline supplementation on physical performance in fourteen male soldiers. In this double-blind crossover study, a placebo beverage or a beverage containing choline was administered before and during a treadmill exercise test while soldiers were carrying a 34.1 kg load. The choline beverage supplied 8.4 g choline citrate, which is equivalent to 3 g free choline. Plasma choline increased after consumption of the choline beverage (7.69 μM for placebo and 17.5 μM for choline beverage, $p < 0.001$), however there were no effects of choline supplementation on exercise performance. These researchers concluded there was no need to add supplemental choline to military operational rations to lessen muscle fatigue or to enhance physical performance of soldiers.

Since the publication of the NAS report on Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline, several more research reports on the physiological effects of choline have been published. These additional reports support the conclusion that choline is an essential nutrient that has important physiological functions. Misra, et al., (1999) reported that low plasma free choline was associated with elevated liver aminotransferase and hepatic steatosis in adults on home parenteral nutrition. This group found similar indications for children in a small case-control study. Twenty-one children who required long-term (75 ± 13 months) home parenteral nutrition were compared to 31 normal children. The mean plasma free choline concentration in children on home parenteral nutrition was 6.3 ± 4.3 nmol/ml which was significantly ($p=0.002$) lower than normal children (8.0 ± 2.3 nmol/ml). These researchers also reported a significant negative correlation between plasma free choline concentration and aspartate aminotransferase and alanine aminotransferase ($r = -0.72$, $p=0.04$ and $r= -0.80$, $p=0.02$, respectively). Misra, et al. (1999) concluded that patients on long-term home parenteral nutrition have a significant risk for the development of choline deficiency. Previously, Buchman, et al., (1995) had shown that hepatic steatosis could be ameliorated and possibly prevented by choline supplementation during parenteral feeding. In four patients, which had received total parenteral nutrition for 9.7 ± 4.7 years, supplementation of choline chloride of 1 to 4 g/day increased plasma free choline into the normal range and resolved hepatic steatosis completely. Plasma free choline remained at or above normal range during choline supplementation but decreased to baseline levels after discontinuance of choline

supplementation. These researchers concluded choline might be an essential nutrient for those individuals on long-term parenteral nutrition.

In another report, Jacob, et al. (1999) evaluated the impact of folate (another nutrient involved in methylation pathways) depletion and repletion on choline status – plasma choline and phosphatidylcholine concentrations. In two separate metabolic studies using 21 subjects (11 men and 10 women), total folate intake was varied by supplementing low folate (25 and 56 g/d for men and women, respectively) and low choline (238 and 147 mg/d for men and women, respectively) diets with pterorylglutamic acid for up to six weeks after folate depletion periods of 4-5 weeks. Low folate/low choline diets caused a decrease in mean plasma choline of over 25% in men and women. Additionally, choline status returned to baseline or higher after moderate folate repletion. These researchers concluded that choline is used as a methyl donor when folate intake is low and that de novo synthesis of phosphatidylcholine is insufficient to maintain choline status when intakes of folate and choline are low. These data support the conclusion of FNB in that more than 250 mg/d of choline is needed per day to maintain choline status.

Zeisel (2000a) provides an excellent summary of why choline is considered an essential nutrient by NAS and concludes that choline in the diet is important for several reasons. He was especially interested in recent findings, in animals, on the role of choline in brain development. Several publications on the effects of choline on memory and learning in rats have recently been published (Tees, 1999; Tees, and Mohammadi, 1999; Thomas et al., 2000;) and mice (Ikarashi, et al., 2000). In one study, rats were exposed to choline

both pre-natally, via diet of pregnant rats, and post-natally, via a subcutaneous injection, for 24 days (Tees, and Mohammadi, 1999). At 90 days of age the rats were subjected to series of memory tests. In the first test, a Morris water maze, rats had to first find the hidden platform and then were required to find the platform again in 10 minutes. Animals in the group that received choline neonatally found the platform significantly faster than the animals in the control group. Neonatally treated rats also performed significantly better than control rats in another task, namely, a visual transverse patterning discrimination test. Tees (1999) reported that there was an interaction between early environmental enrichment and choline supplementation. Rats were either exposed to a standard lighted colony or were given exposure to a "complex environment." One-half of each group was given choline pre-natally and post-natally for 24 days. While sex and early diet was not a factor in certain investigative behaviors examined, in the water maze test, choline-treated male rats exposed to the complex environment had an improved ability to locate the hidden platform. He concluded that choline supplementation offers some long-term functional benefits that are probably related to cholinergic basal forebrain function. Ikarashi and colleagues (2000) showed that free choline in plasma decreased dramatically in mice given choline oxidase intraperitoneally. As compared to saline-treated controls, mice given choline oxidase showed significant inhibition to learn avoidance from electric shock but had no effect on impairment in retaining memory once learned. These researchers concluded that choline might be more involved in passive avoidance learning than in maintenance of memory. It has been suggested that comparable studies to those discussed above need to be conducted in humans (Zeisel, 2000a). The improved memory associated with added choline during the neonatal period

has been replicated in two species across several laboratories. These effects appear real and are robust. While human studies are underway to better assess the effects of choline on brain function, the potential for improvement of age-impaired memory in humans is real. This improvement in age-impaired memory, probable positive impact on brain function of choline supplied neonatally through the diet of pregnant women, and the potential sparing effects of folate metabolites by choline all argue for increasing choline in the American diet. Increased choline in the diet could come from increasing consumption of foods already high in choline. However, since most foods with meaningful levels of choline are high in fat, saturated fat and/or cholesterol and Americans are trying to reduce these kinds of foods in their diet, fortification of appropriate foods with choline is warranted.

Thus, while the FNB report contains the best available review of choline metabolism, more recent research continues to support the conclusions in the FNB report that choline is an essential nutrient and suggests that choline may have an important role in brain function, though more research is necessary to understand the role of choline in brain function in humans. Fortification of certain foods with choline seems to be a reasonable and effective way to increase choline intake in America.

V. Foods Eligible to Make the Authorized Nutrient Content Claims

Choline and esters of choline are found in many foods usually in the form of phosphatidylcholine in various membranes (Food and Nutrition Board, 1998). The Committee of Military Nutrition Research (1994) estimated that choline intake ranged from 200-1000 mg/day. FNB estimated intake of dietary choline (as free choline and choline in phosphatidyl choline and other choline esters) in adults to be 730-1040 mg/day. FNB also indicated that older assay procedures for choline were imprecise and some were unreliable since the procedures did not include glycerolphosphocholine or phosphocholine.

A. Defining a Reference Daily Intake (RDI) for Choline as 550 mg/day

FDA determined that for vitamins and minerals "...label reference values (i.e., RDI's) should be based on an approach that selects the highest NAS RDA values from among those for adults and children 4 or more years of age but excludes values for pregnant and lactating females" (58 FR 2211). Adult men have the highest Adequate Intake for choline, namely, 550 mg/day. Using a similar approach to that used by FDA for establishing RDI's for other nutrients with differing recommended intakes for various age, gender or physiological states, we suggest that 550 mg/day be considered the Reference Daily Intake (RDI) for choline. While this will provide more choline than needed by certain other age/gender groups, given the Upper Tolerable Intake is

1000 mg/day in children 4 to 8 years of ages and 3500 mg/day in adults, there should be no safety concerns for this higher value. Thus, similar to the conclusion FDA reached when using this approach for other nutrients, including nutrients with Estimated Safe and Adequate Daily Dietary Intakes (ESADDI) (58 FR 2212), children eating from the general food supply are extremely unlikely to be at risk for any harmful effects with an RDI for choline of 550 mg/day.

B. Defining levels for "good source," "more," and "high" claims

FDA and others have extolled the benefits of helping consumers understand the meaning of the nutrient content of foods. FDA's approach to developing a system of nutrient content claims in response to the Nutrition Labeling and Education Act of 1990 emphasized three objectives:

1) Consistency among definitions;
2) Claims that are in keeping with public health goals; and
3) Claims that can be used by consumers to maintain healthy dietary practices (58 FR 2319 cited from 56 FR 60431).

The authorized claims in this notification are consistent with these objectives. First, the claims use definitions that are all currently in use or authorized for use. Second, NAS has determined 550 mg/day of choline as an Adequate Intake. Third, consumption of choline, an essential vitamin, is consistent with maintaining healthy dietary practices.

FDA has previously authorized certain nutrient content claims for foods containing substances with a defined DRV or RDI. Foods may provide a "good source" claim of a particular nutrient if the food provides at least 10-19% of the established DRV or RDI per reference amount customarily consumed (RACC). FDA has also ruled that synonyms for the "good source" claim include "contains" and "provides" (21 C.F.R. § 101.54 (c)). Foods may also use an "excellent source" claim if the food provides 20% or more of the DRV or RDI per RACC. Synonyms for this claim include "high" or "rich in" (21 C.F.R. § 101.54 (b)). Foods may use relative claims such as "more," "fortified," "enriched," and "added" if the food contains at least 10 percent more of a DRV or RDI per RACC than an appropriate reference food (21 C.F.R. § 101.54 (e)).

As we have noted above, the RDI for choline based on the AI established by NAS should be 550 mg per day. Thus, a food or dietary supplement that contains 55 mg of choline is a "good source of choline" (550 mg/day X 0.10) and a food that contains 110 grams or more of choline is an "excellent source of choline" (550 mg/day X 0.20). Thus, foods that provide 55 mg of choline per RACC would be eligible to claim "good source of choline" and foods providing 110 mg of choline or more per RACC would be allowed to claim "an excellent source of choline." Foods with at least 55 mg more choline/RACC than an appropriate reference food will also be eligible for the relative claim of "more choline." Additionally, modified versions of foods with a standard of identity may also be eligible for certain nutrient content claims authorized in this notification if they meet the requirements of 21 C.F.R. § 130.10.

To further aid consumers, we recommend manufacturers be required to provide information on the amount of choline on a per serving basis using a statement of quantity (e.g., contains 55 mg of choline per serving) immediately adjacent to the nutrient content claim or on the information panel outside the Nutrition Facts panel. Thus, foods and dietary supplements using the nutrient content claims authorized in this notification will be required to provide choline content information on the label thus further helping to educate consumers about the content of foods. Finally, foods and dietary supplements using these choline nutrient content claims would be required to meet all other applicable labeling requirements.

C. Determination of Choline in Foods and Dietary Supplements

Choline content in foods and dietary supplements will be determined by high-pressure liquid chromatography (HPLC) and gas chromatography-mass spectrometry as described by Pomfret, et al., (1989). Briefly, ^{14}C labeled-choline standards were used to determine eluted peaks after separation by HPLC. Internal standards of choline moieties labeled with stable isotopes were used to correct for recovery during the assay. With the use of a microprocessor controlled solvent delivery system with two buffers, Pomfret, et al., (1989) were able to elute betaine and acetylcholine with the first buffer, choline and glycerophosphocholine during transition to the second buffer and phosphocholine with the second buffer. Fractions were collected and hydrolyzed to release free choline. In aliquots of the fractions, the free choline moiety was converted to propionyl esters, methylated and isolated using gas chromatography. Isolated compounds were then fragmented by mass spectrometry.

Recently, the United States Department of Agriculture (USDA) provided a research grant to Dr. Steven Zeisel of the University of North Carolina – Chapel Hill to conduct analysis of choline content of foods. This grant titled, "Choline content of commonly eaten foods" (USDA Agreement # 58-1235-0-059 CRIS # 1235-52000-032-10S) will use methods similar to Pomfret, et al., (1989) and will analyze enough foods to determine the choline intake in the US population via various dietary surveys conducted by USDA (Zeisel, 2000b). Thus, shortly the choline content of a large number of foods will be added to the USDA Nutrient Database and food manufacturers may be able to use these data to determine if their products are eligible for the claims authorized in this notification.

D. Choline Content of Some Common Foods

Based on information provided from Zeisel and Blusztajn (1994), the choline content of various foods are provided in Table 2. Foods eligible for "high" claims are also indicated. As can been seen in Table 2, only a few foods qualify for the claims "excellent source of choline" or "good source of choline." It should also be noted that the foods that do qualify for these claims are typically higher in fat, saturated fat and/or cholesterol. Many Americans, based on recommendations from various private and government public health organizations, are decreasing fat, saturated fat and cholesterol in their diet. Consuming less fat, saturated fat and cholesterol will probably lead to a concomitant decrease in choline intake. Thus, we believe that the addition of choline (fortification) to

certain foods is likely to occur. Any fortification of foods with choline should be consistent with FDA published guidance on food fortification (Sec. 104.20)[1].

Table 2. Choline Content of Some Foods

FOOD	CHOLINE CONTENT, mg/g	RACC[1], g	CHOLINE CONTENT PER RACC[1], mg
Apple	0.022	140	3.06
Banana	0.007	140	0.96
Beef Liver	3.380	85	287.27*
Beef Steak	0.479	85	40.75
Butter	0.164	14	2.29
Cauliflower	0.230	85	19.58
Corn Oil	0.001	14	0.02
Coffee	0.005	240	1.25
Cucumber	0.010	85	0.85
Egg	3.967	50	198.37*
Ginger Ale	0.001	240	0.13
Grape Juice	0.007	240	1.6
Iceberg Lettuce	0.045	85	3.86
Margarine	0.034	14	0.48
Milk (Bovine, whole)	0.019	240	4.48
Orange	0.040	140	5.57
Peanut Butter	0.331	32	10.58
Peanuts	0.418	30	12.53
Potato	0.029	110	3.24
Tomato	0.011	85	0.93
Whole Wheat Bread	0.036	50	1.81

[1] RACC is Reference Amount Customarily Consumed.
* Eligible for "high" claims.
Modified from Zeisel and Blusztajn (1994).

[1] Sec. 104.20 does not directly list choline as one of the nutrients that can be added to foods, though, in that section FDA notes that "(i)it is reasonable to anticipate that the Reference Daily Intakes (RDI's) as delineated in Sec. 101.9 of this chapter and in paragraph (d) of this section will be amended from time to time to list additional nutrients and/or to change the levels of specific RDI's as improved knowledge about human nutrition requirements and allowances develops." Clearly, the report by NAS on choline requirements is the type of "improved knowledge" contemplated by FDA and, thus, choline fortification of appropriate foods is consistent with the Fortification Policy.

VII. Conclusion

We have provided an authoritative statement from NAS regarding the establishment of an Adequate Intake level of choline in the diet. This statement identifies 550 mg of choline per day as the AI for men and is the level recommended to be used as a RDI for labeling and nutrient content claim purposes. We also supplied additional statements from NAS supporting the above conclusion. Thus, "good source," "more," and "high" nutrient content claims are authorized for products with a choline content of 55 mg/RACC, 55 mg/RACC more than an appropriate reference food and 110 mg/RACC, respectively.

Respectfully,

Central Soya Company, Inc.

Gregory L. Paul
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Appendices

Appendix A.
Authoritative Statement from the NAS in support of nutrient content claims for choline

Choline Chapter (pages 390-422) from Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, Vitamin B_{12}, Pantothenic Acid, Biotin, and Choline

12
Choline

SUMMARY

Choline functions as a precursor for acetylcholine, phospholipids, and the methyl donor betaine. The primary criterion used to estimate the Adequate Intake (AI) for choline is the prevention of liver damage as assessed by measuring serum alanine aminotransferase levels. The AI for adults is 550 mg/day of choline for men and 425 mg/day for women. There are no nationally representative estimates of the intake of choline from food or food supplements. Choline in the diet is available as free choline or is bound as esters such as phosphocholine, glycerophosphocholine, sphingomyelin, or phosphatidylcholine. The critical adverse effect from high intake of choline is hypotension, with corroborative evidence on cholinergic side effects (e.g., sweating and diarrhea) and fishy body odor. The Tolerable Upper Intake Level (UL) for adults is 3.5 g/day.

BACKGROUND INFORMATION

Choline is a dietary component that is important for the structural integrity of cell membranes, methyl metabolism, cholinergic neurotransmission, transmembrane signaling, and lipid and cholesterol transport and metabolism. Human cells grown in culture have an absolute requirement for choline (Eagle, 1955). When cells are deprived of choline, they die by apoptosis (Albright et al., 1996; Cui et al., 1996; Holmes-McNary et al., 1997; James et al., 1997; Shin et

al., 1997; Zeisel et al., 1997). There is an endogenous pathway for the de novo biosynthesis of the choline moiety via the sequential methylation of phosphatidylethanolamine using *S*-adenosylmethionine as the methyl donor (Bremer and Greenberg, 1961) (see Figure 12-1). Thus, the demand for dietary choline is modified by metabolic methyl-exchange relationships between choline and three nutrients: methionine, folate, and vitamin B_{12} (lipotropes) (Zeisel and Blusztajn, 1994).

With this type of nutrient interdependence, designation of the essential nature of a nutrient depends on showing that de novo synthesis rates are not adequate to meet the demand for the nutrient when the other nutrients are available in amounts sufficient to sustain normal growth and function. Healthy men with normal folate and vitamin B_{12} status fed a choline-deficient diet have diminished plasma choline and phosphatidylcholine concentrations and develop liver damage (Zeisel et al., 1991). For these individuals, de novo synthesis of choline was not adequate to meet the demand for

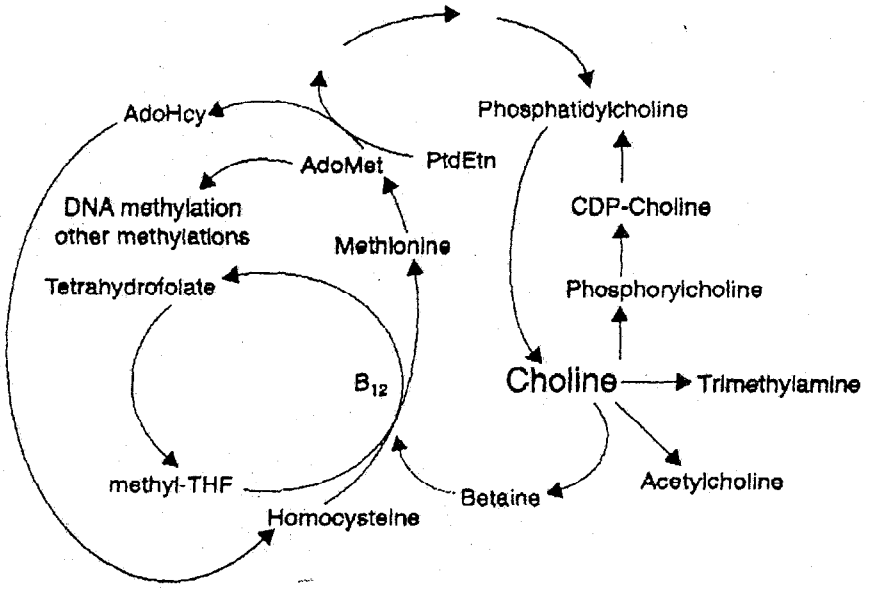

FIGURE 12-1 Choline, folate, and methionine metabolism are closely interrelated. AdoHcy = *S*-adenosylhomocysteine, AdoMet = *S*-adenosylmethionine, B_{12} = vitamin B_{12}, CDP-Choline = cytidine diphosphocholine, PtdEtn = phosphatidylethanolamine, THF = tetrahydrofolate. Reprinted with permission, from Zeisel and Blusztajn (1994). Copyright 1994 by Annual Reviews.

the nutrient. Information about women, infants, children, and older adults is not sufficient to know whether choline is needed in the diet of these groups.

Function

Choline can be acetylated, phosphorylated, oxidized, or hydrolyzed. Several comprehensive reviews of the metabolism and functions of choline have been published (Kuksis and Mookerjea, 1978; Zeisel, 1981; Zeisel and Blusztajn, 1994).

Choline accelerates the synthesis and release of acetylcholine, an important neurotransmitter involved in memory storage, muscle control, and many other functions (Cohen and Wurtman, 1975; Haubrich et al., 1974; Wecker, 1986). It is also a precursor for the synthesis of (1) phospholipids, including phosphatidylcholine (a membrane constituent important for the structure and function of membranes), for intracellular signaling (Exton, 1994; Zeisel, 1993) and hepatic export of very low-density lipoproteins (Yao and Vance, 1988, 1989); (2) sphingomyelin (another membrane constituent) for structural and signaling functions (Hannun, 1994); and (3) platelet activating factor, a potent messenger molecule (Frenkel et al., 1996). Choline is a precursor for the formation of the methyl donor betaine. Betaine is also required by renal glomerular cells, which use betaine and glycerophosphocholine as organic osmolytes to adapt to osmotic stress (Bauernschmitt and Kinne, 1993; Burg, 1995; Garcia-Perez and Burg, 1991; Grossman and Hebert, 1989).

Physiology of Absorption, Metabolism, and Excretion

Dietary choline is absorbed from the lumen of the small intestine via transporter proteins in the enterocyte (Herzberg and Lerner, 1973; Herzberg et al., 1971; Kuczler et al., 1977; Sheard and Zeisel, 1986). Before choline can be absorbed from the gut, some is metabolized by bacteria to form betaine (which may be absorbed and used as a methyl donor) and methylamines (which are not methyl donors) (Zeisel et al., 1983). No other component of the diet has been identified as competing with choline for transport by intestinal carriers. Choline is found in foods as free choline and as esterified forms such as phosphocholine, glycerophosphocholine, sphingomyelin, and phosphatidylcholine. Lecithin is a phosphatidylcholine-rich fraction prepared during commercial purification of phospholipids, and this term is often used interchangeably with phosphatidylcholine. Lecithin is often added to foods as an emulsifying agent.

Pancreatic enzymes can liberate choline from dietary phosphatidylcholine, phosphocholine, and glycerophosphocholine (Zeisel and Blusztajn, 1994). The free choline that is formed enters the portal circulation of the liver (Le Kim and Betzing, 1976) whereas phosphatidylcholine may enter via lymph in chylomicrons.

All tissues accumulate choline by diffusion and mediated transport (Zeisel, 1981). A specific carrier mechanism transports free choline across the blood-brain barrier at a rate that is proportional to the serum choline concentration. In the neonate this choline transporter has an especially high capacity (Cornford and Cornford, 1986). The rate at which the liver takes up choline is sufficient to explain the rapid disappearance of choline injected systemically (Zeisel et al., 1980c). The kidney also accumulates choline (Acara and Rennick, 1973). Some of this choline appears in the urine unchanged but most is oxidized within the kidney to form betaine (Rennick et al., 1977).

In the predominant pathway for phosphatidylcholine biosynthesis, choline is phosphorylated, converted to cytidine diphosphocholine, and then converted to phosphatidylcholine (Kennedy and Weiss, 1956; Vance, 1990) (Figure 12-1). In an alternative pathway, phosphatidylethanolamine is sequentially methylated to form phosphatidylcholine by the enzyme phosphatidylethanolamine-*N*-methyltransferase with *S*-adenosylmethionine as the methyl donor (Bremer and Greenberg, 1961; Vance and Ridgway, 1988). This is the major (perhaps only) pathway for de novo synthesis of the choline moiety in adult mammals. It is most active in the liver but has been identified in many other tissues (Blusztajn et al., 1979; Crews et al., 1981; Yang et al., 1988). Best estimates of in vivo activity of this enzyme, based on in vitro data, are that 15 to 40 percent of the phosphatidylcholine present in the liver is derived from the phosphatidylethanolamine-*N*-methyltransferase pathway, with the remainder coming from the cytidine diphosphocholine pathway (Bjornstad and Bremer, 1966; Sundler and Akesson, 1975). No estimates are available as to the relative extent of choline obtained from cell turnover. Dietary intake of phosphatidylcholine is approximately 6 to 10 g/day (Zeisel et al., 1991).

A significant portion of choline is oxidized to form betaine in the liver and kidney (Bianchi and Azzone, 1964; Weinhold and Sanders, 1973). The methyl groups of betaine can be scavenged and reused in single-carbon metabolism (Finkelstein et al., 1982) (see "Nutrient-Nutrient Interactions").

Clinical Effects of Inadequate Intake

Humans

Although choline is clearly essential to life, there is only one published study examining the effects of inadequate dietary intake in healthy men. That study reported decreased choline stores and liver damage (elevated alanine aminotransferase) when men were fed a choline-deficient diet containing adequate methionine, folate, and vitamin B_{12} for 3 weeks (Zeisel et al., 1991) (Figures 12-2 and 12-3). Another study, in which men were fed a choline- and methyl-deficient diet, reported decreased choline stores but did not report on liver function (Jacob et al., 1995). Individuals fed with total parenteral nutrition (TPN) solutions devoid of choline but adequate for methionine and folate develop fatty liver and liver damage as assessed by elevated alanine aminotransferase; in some individu-

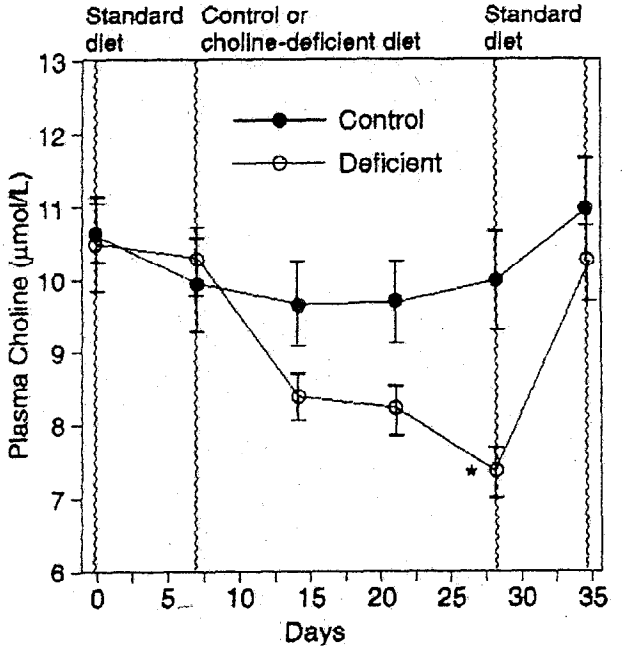

FIGURE 12-2 Plasma choline in healthy men ingesting a control (500 mg/day of choline) or choline-deficient (13 mg/day of choline) diet. *Difference from day 7 value: $p < 0.01$. Reprinted with permission, from Zeisel et al. (1991). Copyright 1991 by the Federation of American Societies for Experimental Biology.

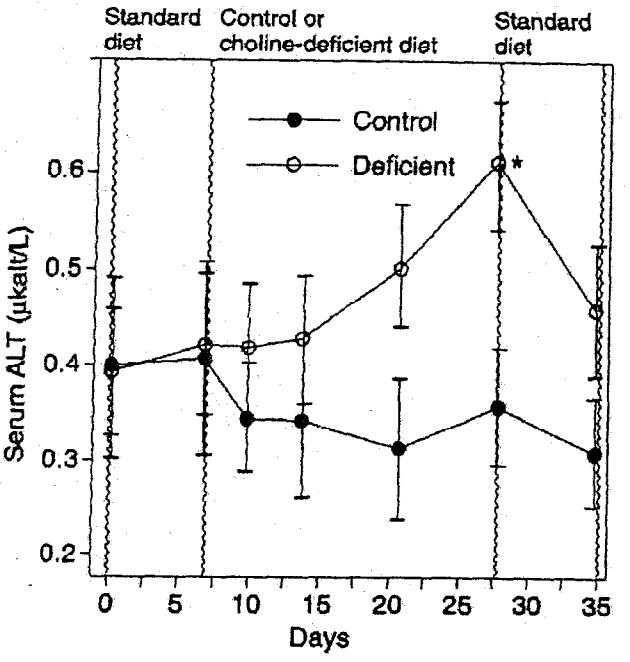

FIGURE 12-3 Serum alanine aminotransferase (ALT) activity in men ingesting a control or choline-deficient diet. Serum ALT was determined by using an automated spectrophotometric assay. Data are expressed as mean activity ± standard error of the mean. *Difference from day 7 value: $p < 0.05$. Reprinted with permission, from Zeisel et al. (1991). Copyright 1991 by the Federation of American Societies for Experimental Biology.

als, this is resolved when a source of dietary choline is provided (Buchman et al., 1992, 1993, 1995; Chawla et al., 1989; Shapira et al., 1986; Sheard et al., 1986). In a double-blind protocol, investigators administered lecithin (30 percent phosphatidylcholine) orally to patients receiving TPN twice daily for 6 weeks. At the end of this time, plasma choline had risen by more than 50 percent in the lecithin group whereas in the placebo group it had decreased by 25 percent. In the treated group, liver fat decreased by 30 percent (Buchman et al., 1992). In another small clinical study (Buchman et al., 1995), four patients who had low plasma concentrations of free choline after treatment with TPN (which contained no additional choline) were given 1 to 4 g/day of choline chloride for 6 weeks. During choline administration, plasma choline concentration

increased into the normal range but decreased back to baseline when choline supplementation was discontinued. Fatty liver was resolved completely during choline supplementation but steatosis (fatty liver) recurred in one patient after 10 weeks of return to choline-free TPN. The available data support the provisional conclusion that de novo synthesis of choline is not always sufficient to meet human requirements for choline.

Animals

Supporting animal studies (in many species, such as the baboon) also found that a choline-deficient diet resulted in decreased choline stores and liver dysfunction (Hoffbauer and Zaki, 1965; Sheard et al., 1986; Tayek et al., 1990; Yao and Vance, 1990). The following animals fed a choline-deficient diet may be susceptible to developing growth retardation, renal dysfunction and hemorrhage, or bone abnormalities: baboon (Hoffbauer and Zaki, 1965), chicken (Blair et al., 1973; Ketola and Nesheim, 1974), dog (Best and Huntsman, 1932; Hershey, 1931), guinea pig (Tani et al., 1967), hamster (Handler, 1949), pig (Blair and Newsome, 1985; Fairbanks and Krider, 1945), quail (Ketola and Young, 1973), rat (Newberne and Rogers, 1986), and trout (Ketola, 1976).

SELECTION OF INDICATORS FOR ESTIMATING THE REQUIREMENT FOR CHOLINE

Markers of Liver Dysfunction

The liver is damaged when humans consume an otherwise adequate diet that is deficient in choline, resulting in elevated alanine aminotransferase levels in blood (Burt et al., 1980; Tayek et al., 1990; Zeisel et al., 1991). Fatty infiltration of liver also occurs in choline deficiency but is difficult to use as a functional marker without special liver imaging techniques (Buchman et al., 1992).

Hepatic choline and choline metabolite concentrations have been shown to decrease during choline deficiency in the rat (Zeisel et al., 1989). Phosphocholine concentration in liver is highly correlated with dietary choline intake, decreasing to 10 to 20 percent of control values after 2 weeks on a diet sufficient in methionine, folate, and vitamin B_{12} but deficient in choline (Pomfret et al., 1990). Hepatic phosphocholine concentration was most sensitive to modest dietary choline deficiency, decreasing to 10 to 20 percent of control values after 2 weeks of a deficient diet (Pomfret et al., 1990). This

measurement is not easily undertaken in humans, although magnetic resonance spectroscopy does makes it possible (Cohen et al., 1995).

Plasma Concentrations

Plasma choline concentration varies in response to diet and is found in the water-soluble fraction as free choline (Buchman et al., 1993; Burt et al., 1980; Chawla et al., 1989; Sheard et al., 1986; Zeisel et al., 1991). It decreases approximately 30 percent in subjects fed a choline-deficient diet for 3 weeks (Zeisel et al., 1991). Plasma choline concentration can increase twofold after a meal high in choline content and three- or fourfold after a supplemental choline dose (Zeisel et al., 1980b). Fasting plasma choline concentrations vary from 7 to 20 μmol/L, with most subjects having concentrations of 10 μmol/L. The disadvantage of using plasma choline as a functional indicator is that these concentrations do not appear to decline below approximately 50 percent of normal, even when subjects fast for more than 1 week (Savendahl et al., 1997). Perhaps this is because membrane phospholipids, which are a large storage pool for choline, are hydrolyzed to maintain plasma choline concentration above this minimal level. Fasting plasma phosphatidylcholine concentrations (mostly as part of plasma lipoproteins) are approximately 1 to 1.5 mmol/L (Aquilonius et al., 1975; Zeisel et al., 1980b, 1991). Plasma phosphatidylcholine concentration also decreases in choline deficiency (Zeisel et al., 1991) but is also influenced by factors that change plasma lipoprotein levels.

Reduction of Risk of Chronic Disease

Dementia

Studies in rodents suggest that dietary intake of choline early in life can diminish the severity of memory deficits in aged animals (Bartus et al., 1980; Meck and Williams, 1997a, b, c). Most available human studies have used choline-containing compounds to treat rather than prevent the symptoms of dementia and therefore did not address whether dementias could be prevented. In the absence of food composition data, epidemiological studies on the association of choline intake with dementia are not available. More human studies are needed to determine whether dietary choline intake is useful in the prevention of dementia.

Cardiovascular Disease

The choline-containing phospholipid phosphatidylcholine (lecithin) has been used as a treatment to lower cholesterol concentrations because lecithin-cholesterol acyltransferase has an important role in the removal of cholesterol from tissues. In humans phosphatidylcholine ingestion is associated with a modest reduction in plasma cholesterol (Hirsch et al., 1978; Wood and Allison, 1982; Zeisel et al., 1991). In addition, choline or betaine treatment has been used to lower high plasma homocysteine concentrations (Anonymous, 1997; Dudman et al., 1987; Wendel and Bremer, 1984; Wilcken et al., 1983, 1985), and choline-deficient rodents have elevated plasma homocysteine concentrations (Varela-Moreiras et al., 1995) (see Chapter 8, "Vascular Disease"). Wendel and Bremer (1984) reported that betaine treatment was more effective than folate treatment in normalizing plasma homocysteine and methionine concentrations of a child with homocystinuria, a genetic disease caused by 5,10-methylenetetrahydrofolate reductase deficiency (choline is the precursor for betaine, which itself is found in sugar beets and wine). Therefore, dietary choline intake might be correlated with cardiovascular disease risk. More human studies are needed before conclusions can be drawn about whether dietary choline intake is useful in preventing cardiovascular disease.

Cancer

In rodents dietary choline deficiency is associated with increased incidence of liver cancer and increased sensitivity to carcinogenic chemicals (Newberne and Rogers, 1986). The mechanisms of the carcinogenic actions of choline deficiency are not known but may be mediated by changes in protein kinase C activity (da Costa et al., 1993, 1995). There are no human data; studies in humans are needed to assess the role of dietary choline in the prevention of cancer.

FACTORS AFFECTING THE CHOLINE REQUIREMENT

Nutrient-Nutrient Interactions

Any consideration of the requirements for choline and methionine needs to include the close interrelationships with other methyl donors. Choline, methionine, and folate metabolism interact at the point that homocysteine is converted to methionine.

Betaine-homocysteine methyltransferase catalyzes the methylation of homocysteine using betaine as the methyl donor (see Figure 12-1) (Finkelstein et al. 1982; Mudd and Poole, 1975; Wong and Thompson, 1972). In an alternative pathway, 5-methyltetrahydrofolate-homocysteine methyltransferase regenerates methionine by using a methyl group derived de novo from the single-carbon pool (Finkelstein et al., 1982, 1988). Methionine adenosyltransferase converts methionine to *S*-adenosylmethionine (the active methylating agent for many enzymatic methylations, including the methylation of phosphatidylethanolamine to form phosphatidylcholine [Ridgway and Vance, 1988]).

Perturbing the metabolism of one of the methyl donors reveals the intermingling of these metabolic pathways. Total hepatic folate content decreased by 31 to 40 percent in rats after 2 weeks on a choline-deficient diet (Selhub et al., 1991; Varela-Moreiras et al., 1995). This effect was reversed by refeeding choline (Varela-Moreiras et al., 1995). Rats fed diets deficient in both methionine and choline for 5 weeks had hepatic folate concentrations that were half of those present in controls (Horne et al., 1989). Tetrahydrofolate deficiency in rats, induced by treatment with methotrexate (Barak and Kemmy, 1982; Barak et al., 1984; Freeman-Narrod et al., 1977; Pomfret et al., 1990; Svardal et al., 1988) or by dietary folate deficiency (Kim et al., 1994) resulted in diminished hepatic total choline, with the greatest decrease occurring in hepatic phosphocholine concentrations. During choline deficiency in rats, hepatic *S*-adenosylmethionine concentrations also decreased by as much as 50 percent (Barak et al., 1982; Poirier et al., 1977; Shivapurkar and Poirier, 1983; Zeisel et al., 1989). In rats choline deficiency for 2 weeks doubled plasma homocysteine levels (Varela-Moreiras et al., 1995). See Chapters 7 and 8 for more information on plasma homocysteine.

Gender

Males may have a higher choline requirement than do females. Female rats are less sensitive to choline deficiency than are male rats (Tessitore et al., 1995), perhaps because of females' enhanced capacity to form the choline moiety de novo. Females rats have greater phosphatidylethanolamine-*N*-methyltransferase activity in liver than do males (Arvidson, 1968; Bjornstad and Bremer, 1966; Lyman et al., 1971). Estimates of the amount of increased activity vary between 10 (Lyman et al., 1971) and 50 percent (Bjornstad and Bremer, 1966). A woman's capacity to form the choline moiety

de novo may decrease after menopause (Lindblad and Schersten, 1976), because estrogens increase hepatic phosphatidylethanolamine-*N*-methyltransferase activity in rats (Drouva et al., 1986; Young, 1971).

Exercise

Strenuous physical activity in trained athletes reduced the plasma choline concentration by approximately 40 percent, from 14.1 to 8.4 μmol/L (Conlay et al., 1986). A choline supplement given to marathon runners modestly enhanced performance (Sandage et al., 1992). In 10 top-level triathletes who were given either a placebo or lecithin at 0.2 g/kg body mass 1 hour before each type of exercise, plasma choline concentrations in all the triathletes decreased on average by 16.9 percent after the bicycle exercise when placebo was taken before the race but did not do so when lecithin was given (Von Allworden et al., 1993).

Bioavailability

No estimates are available for percentage absorption of the various forms of choline in humans. The water-soluble choline-derived compounds (choline, phosphocholine, and glycerophosphocholine) are absorbed via the portal circulation whereas the lipid-soluble compounds (phosphatidylcholine and sphingomyelin) present in foods are absorbed into lymph as chylomicrons via the thoracic duct. This results in differential delivery and kinetics of distribution to tissues (Cheng et al., 1996; Zeisel et al., 1980b).

FINDINGS BY LIFE STAGE AND GENDER GROUP

Data are not sufficient for deriving an Estimated Average Requirement (EAR) for choline. The two published studies in healthy humans used male subjects only and tested a single level of choline intake. For these reasons only an Adequate Intake (AI) can be estimated. This amount will be influenced by the availability of methionine and folate in the diet. It may be influenced by gender, pregnancy, lactation, and stage of development. Although AIs are set for choline, it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

To date, all studies have used choline-free diets and compared them with choline-containing diets; no intermediate levels of defi-

ciency have been reported. Careful dose-response experiments are needed before an EAR can be derived.

Infants Ages 0 through 12 Months

Method Used to Set the AI

An AI is used as the goal for intake by infants.

Ages 0 through 6 Months. The AI reflects the observed mean intake of choline by infants consuming human milk. Thus the choline AI for young infants is based on mean intake data from infants fed human milk exclusively for their first 6 months and uses the choline concentration of milk produced by well-nourished mothers. Human milk contains 160 to 210 mg (1.5 to 2 mmol)/L of choline moiety delivered as choline, phosphocholine, glycerophosphocholine, phosphatidylcholine, and sphingomyelin (Holmes-McNary et al., 1996; Zeisel et al., 1986). The choline phospholipids sphingomyelin and phosphatidylcholine are part of the milk fat-globule membrane (Holmes-McNary et al., 1996; Zeisel et al., 1986).

Rat pups denied access to milk have lower serum choline concentrations than do their fed litter mates (Zeisel and Wurtman, 1981). Thus, milk intake contributes to the maintenance of high serum choline concentrations in the neonate. In the rat, supplemental choline is concentrated in the rat dam's milk (Garner et al., 1995; Zeisel, 1987). In women consuming a low-choline diet, milk choline content is lower than that in women consuming a more adequate diet (Zeisel et al., 1982). Consumption of either a choline-deficient or choline-supplemented diet by lactating rat dams results in significant changes in the phosphocholine concentration of their milk (Holmes-McNary et al., 1996; Zeisel et al., 1986). The concentration of total choline in human milk is 160 mg/L (1.5 mmol/L). For the mean volume of output of human milk of 0.78 L/day and the average choline content of 160 mg/L, the AI for choline is 125 mg/day (1.2 mmol/day) for infants ages 0 through 6 months. For the reference infant weight of 7 kg, this corresponds to an AI of 18 mg/kg of body weight/day (0.17 mmol/kg/day).

Ages 7 through 12 Months. If the reference body weight ratio method described in Chapter 2 to extrapolate from the AI for choline for infants ages 0 through 6 months is used, the AI for choline for the older infants would be 150 mg/day (1.4 mmol/day). The second method (see Chapter 2), extrapolating from the AI for adults, gives

an AI that is essentially the same as that from extrapolating from infants. There are no data estimating choline intake from foods for this age group.

Choline AI Summary, Ages 0 through 12 Months

AI for Infants		
0–6 months	**125 mg/day of choline**	**≈18 mg/kg**
7–12 months	**150 mg/day of choline**	**≈17 mg/kg**

Special Considerations

Although commercially available infant formulas and bovine milk both contain choline and choline-containing compounds (Holmes-McNary et al., 1996; Rohlfs et al., 1993; Zeisel et al., 1986), human milk has a significantly higher phosphocholine concentration (718 μmol/L) than does either cow milk or infant formulas. However, cow milk and cow-milk-derived infant formulas have the same glycerophosphocholine concentration as human milk (400 to 800 μmol/L) (Holmes-McNary et al., 1996) or higher (415 μmol/L) (Holmes-McNary et al., 1996). Soy-derived infant formulas have lower glycerophosphocholine concentration (115 μmol/L or less) (Holmes-McNary et al., 1996). Human milk phosphatidylcholine and sphingomyelin concentrations do not differ significantly from those in cow milk and cow-milk-derived infant formulas (200 μmol/L) (Holmes-McNary et al., 1996). Soy-derived infant formulas contain more phosphatidylcholine than do either human milk or cow-milk-derived formulas but less sphingomyelin than human milk (Holmes-McNary et al., 1996). Unesterified choline concentration in mature human milk is 30 to 80 percent lower than in either cow milk or the infant formulas (Holmes-McNary et al., 1996). The relative bioavailability of choline, phosphocholine, and glycerophosphocholine is similar in a rat model (Cheng et al., 1996) but no information is available for humans. Thus, it is not known whether these differences in milk and formula composition are clinically relevant.

Children and Adolescents Ages 1 through 18 Years

Method Used to Set the AI

No direct data on choline were found on which to base an EAR or AI for children and adolescents. In the absence of additional infor-

mation, AIs for these age groups have been extrapolated from adult values by using the method described in Chapter 2.

Choline AI Summary, Ages 1 through 18 Years

AI for Children	**1–3 years**	**200 mg/day of choline**
	4–8 years	**250 mg/day of choline**
AI for Boys	**9–13 years**	**375 mg/day of choline**
	14–18 years	**550 mg/day of choline**
AI for Girls	**9–13 years**	**375 mg/day of choline**
	14–18 years	**400 mg/day of choline**

Adults Ages 19 Years and Older

Method Used to Set the AI

An intake level of 500 mg/day (4.8 mmol/day; approximately 7 mg/kg/day [0.7mmol/kg/day]) of choline base is the dose that prevented alanine aminotransferase abnormalities in healthy men (Zeisel et al., 1991). This estimate for an AI is uncertain because it is based on a single published study; it may need revision when other data become available. This estimate fits within the bracketing estimates derived from patients on total parenteral nutrition for whom approximately 2 mg/kg/day of choline moiety did not prevent a deficiency syndrome (Sheard et al., 1986) and 31 mg/kg/day of choline moiety restored normal choline status (Buchman et al., 1992, 1993). The amount estimated as adequate for men should be sufficient to prevent an increase in alanine aminotransferase but it resulted in a small decrease in plasma choline in the one study in which it was evaluated, which suggests that dietary intake normally might be slightly higher. Thus the AI is set at approximately 7 mg/kg/day or, for the reference man weighing 76 kg, at 550 mg after rounding.

To arrive at an estimate for AI for women, it is assumed that data from men can be used even though women may use choline more efficiently (see "Gender"). No experimental attempts to make healthy women choline deficient have been reported. However, women on total parenteral nutrition were just as likely as were men to develop low plasma choline concentrations and fatty liver (Buchman et al., 1995).

No experimental data are available from which to calculate an AI for life stage groups other than adults as a whole.

Choline AI Summary, Ages 19 Years and Older

The AI for choline in all forms for men in all age groups is 550 mg and for women is 425 mg. It is not known whether women have the same requirement on a body weight basis as men, but this AI is likely to be adequate on the basis of the earlier discussion on gender. Although there is some evidence that transport across the blood-brain barrier is diminished in the elderly, which suggests the possibility of a higher requirement than for younger adults (Cohen et al., 1995), no adjustment has been made in the AI for the elderly.

AI for Men	**19–30 years**	**550 mg/day of choline**
	31–50 years	**550 mg/day of choline**
	51–70 years	**550 mg/day of choline**
	> 70 years	**550 mg/day of choline**
AI for Women	**19–30 years**	**425 mg/day of choline**
	31–50 years	**425 mg/day of choline**
	51–70 years	**425 mg/day of choline**
	> 70 years	**425 mg/day of choline**

Pregnancy

Evidence Considered in Setting the AI

The need for choline is probably higher for pregnant than for nonpregnant women on the basis of animal data. Pregnancy renders female rats as vulnerable to deficiency as males (Zeisel et al., 1995). During pregnancy in humans (Welsch 1978; Welsch et al., 1981), guinea pigs (Swiery and Yudilevich, 1985; Swiery et al., 1986; Yudilevich and Sweiry, 1985), and rats (Jorswieck, 1974) large amounts of choline are delivered to the fetus through the placenta. Transport of choline from mother to fetus depletes maternal stores of choline; the choline concentration of maternal liver fell from a mean of 130 μmol/L in adult nonpregnant rats to 38 μmol/L in late pregnancy (Gwee and Sim, 1978).

Choline availability during embryogenesis and perinatal development may be especially important. In rats fed adequate diets during pregnancy, postnatally, and at weaning, 1 mmol/day of extra dietary choline results in long-lasting enhancement of spatial memory

(Meck and Williams, 1997a, b, c), altered morphology of septal neurons (Loy et al., 1991; Williams et al., 1998), and enhanced hippocampal long-term potentiation (Pyapali et al., 1998) and cholinergic neurotransmission (Cermak et al., 1998; Holler et al., 1996). The two periods of sensitivity to extra choline occur during embryonic days 12 to 17 and postnatal days 16 to 30 (Loy et al., 1991; Meck et al., 1988, 1989).

In mammals the placenta transports choline to the fetus (Welsch, 1976); choline concentration in amniotic fluid is 10-fold greater than that in maternal blood (S. Zeisel, University of North Carolina School of Public Health, unpublished observations, 1997). At birth, humans and other mammals have plasma choline concentrations that are much higher than those in adults (Zeisel et al., 1980a). It is not known whether de novo synthesis of choline increases during pregnancy.

The AI for pregnant women is greater than that for the adult by the amount needed for the fetus and placenta. Through the use of published values for the choline concentration of various adult rat tissues (Pomfret et al., 1989) and with the assumption of a body organ weight percentage as estimated by Widdowson (1963) for the human fetus, the fetal choline content can be estimated as approximately 5 mmol/kg (520 mg/kg) fetal weight. Human placental tissue has been estimated to average 1.26 ± 0.24 mmol/kg (mean ± standard error) in a small sample ($n = 7$) (Welsch, 1976); a value of approximately 2 mmol of choline per kg of placental tissue should cover almost all pregnant women. If it is thus assumed that the average choline content of fetal and placental tissue combined is approximately 3 mmol/kg (312 mg/kg), that there is no extra synthesis during pregnancy, and that there is no contribution of choline by placental or fetal synthesis, the required dietary amount of choline for the 10 kg of tissue that comprises the fetus (3 kg) and organs of pregnancy (7 kg) is 30 mmol, or 3,000 mg (10 kg tissue × 312 mg), which is approximately 11 mg/day (10 μmol/day) of additional dietary choline throughout pregnancy. This amount would be achieved by increasing the AI (after rounding) to 450 mg/day of choline for pregnancy.

Choline AI Summary, Pregnancy

The increase in the AI to support pregnancy is based on the fetal and placental accumulation of choline.

AI for Pregnancy	**14–18 years**	**450 mg/day of choline**
	19–30 years	**450 mg/day of choline**
	31–50 years	**450 mg/day of choline**

Lactation

Method Used to Set the AI

The need for choline is likely to be increased during lactation because a substantial amount of choline is secreted in human milk, and mechanisms for conserving maternal choline status have not been identified. Lactating rats are more sensitive to choline deficiency than are nonlactating rats (Zeisel et al., 1995).

The AI for women during the first 6 months of lactation should be increased above that in the nonpregnant, nonlactating woman to cover the choline that is transferred into milk. For the assumption of an average volume production of 0.78 L/day (see Chapter 2) and an average choline content of milk of 156 mg/L (1.5 mmol/L), this increase is 125 mg/day (1.2 mmol/day). This increase is based on an assumption of 100 percent efficiency. It is not known whether de novo synthesis of choline increases during lactation. Women who are breastfeeding older infants who are also eating solid foods may need slightly less because of a lower volume of milk production.

Choline AI Summary, Lactation

AI for Lactation	**14–18 years**	**550 mg/day of choline**
	19–30 years	**550 mg/day of choline**
	31–50 years	**550 mg/day of choline**

INTAKE OF CHOLINE

Food Sources

Choline is widely distributed in foods, with most of it in the form of phosphatidylcholine in membranes. Foods that are especially rich in choline compounds are milk, liver, eggs, and peanuts. It is possible to consume a diet of normal foods that delivers 1 g/day of choline (Zeisel et al., 1980b). Lecithins added during food processing may increase the average daily per capita consumption of phosphatidylcholine by 1.5 mg/kg of body weight for adults (this corre-

sponds to 0.225 mg/kg of body weight of choline moiety) (SCOGS/LSRO, 1979).

Dietary Intake

Choline intake is not reported in the Third National Health and Nutrition Examination Survey (Perloff et al., 1990), the Continuing Survey of Food Intake by Individuals (Perloff et al., 1990), or the Boston Nutritional Status Survey (Hartz et al., 1992), and the choline content of foods is not included in major nutrient databases. There are no reports on choline intake from Canada. Estimated average choline dietary intake in adults consuming a typical U.S. or Canadian diet (as free choline and the choline in phosphatidylcholine and other choline esters) is approximately 730 to 1,040 mg/day (7 to 10 mmol/day) (LSRO/FASEB, 1981; Zeisel, 1981). Calculations of dietary choline intake are based on estimates of the free choline and phosphatidylcholine content of foods (Engel, 1943; McIntire et al., 1944; Weihrauch and Son, 1983; Zeisel et al., 1986). Older assay procedures for choline were imprecise and did not always include glycerophosphocholine or phosphocholine content, making many of the available data unreliable. On the basis of a finding of decreased plasma choline and phosphatidylcholine concentrations when humans were switched from a diet of normal foods to a defined diet containing 500 mg/day of choline (Zeisel et al., 1991), the average dietary intake of choline probably exceeds this level in adults. Infant formulas contain approximately 240 mg/L (2.3 mmol/L) of choline in its various forms. (Holmes-McNary et al., 1996).

Intake from Supplements

Choline is available as a dietary supplement as choline chloride or choline bitartrate and as lecithin, which usually contains approximately 25 percent phosphatidylcholine or 3 to 4 percent choline by weight. In the treatment of neurological diseases, large doses (5 to 30 g) of choline and phosphatidylcholine have been administered to humans (LSRO/FASEB, 1981). There are no reliable estimates of the frequency of use or amount of these dietary supplements consumed by individuals in the United States and Canada.

TOLERABLE UPPER INTAKE LEVELS

Hazard Identification

Adverse Effects

Choline doses that are orders of magnitude greater than estimated intake from food have been associated with body odor, sweating, salivation, hypotension, and hepatotoxicity in humans (LSRO/FASEB, 1975, 1981). There are no indications in the literature that excess choline intake produces any additional adverse effects in humans. The animal data provide supportive evidence for a low degree of toxicity of choline. However, some animal studies have indicated growth suppression at high intakes (LSRO/FASEB, 1975). Because of the large doses and routes of administration used (e.g., intravenous and intraperitoneal injection), they were considered not relevant to human intakes from food and supplements (Davis, 1944; Hodge, 1945; Sahu, 1989; Sahu et al., 1986).

Body Odor, Sweating, and Salivation. High doses of choline have been associated with fishy body odor, vomiting, salivation, sweating, and gastrointestinal effects (LSRO/FASEB, 1981). These symptoms were reported in patients with tardive dyskinesia and cerebellar ataxia treated with choline chloride at 150 and 220 mg/kg of body weight/day for 2 to 6 weeks (10 and 16 g/day, respectively) (Davis et al., 1975; Growdon et al., 1977b; Lawrence et al., 1980). Studies of the production of methylamines from ingested choline suggest that fishy odor would have been observed in healthy populations (Zeisel et al., 1983). Fishy body odor results from the excretion of excessive amounts of trimethylamine, a choline metabolite, as the result of bacterial action. Lecithin, a choline-containing phospholipid, does not present a risk of fishy body odor because it generates little methylamine because the bacterial enzyme cannot cleave the ester (Zeisel et al., 1983).

Hypotension. Oral administration of 10 g/day of choline chloride (which is equivalent to 7.5 g [72 mmol] of choline alone) had a slight hypotensive effect in humans (Boyd et al., 1977). Choline could be acting by increasing vagal tone to the heart or by dilating arterioles. Although added choline increases acetylcholine release from in vitro preparations of heart (Loffelholz, 1981), changes in cardiac rate have not been observed in healthy humans treated with choline.

Hepatotoxicity. Mild hepatotoxicity was reported in patients receiving choline magnesium trisalicylate (1,500 mg twice daily for 8 days) (Cersosimo and Matthews, 1987). There is also one reported case of severe hypersensitivity hepatitis with striking tissue and peripheral eosinophilia after ingestion of choline magnesium trisalicylate (Nadkarni et al., 1992). However, it is likely that hepatotoxicity was induced by salicylate rather than by choline (Cersosimo and Matthews, 1987). Humans with and without cirrhosis have been treated with large doses of choline chloride (6 g/day for 4 weeks) with no resultant liver toxicity (Chawla et al., 1989).

Nonspecific Toxicity. Tinnitus and pruritus have been reported in patients treated with doses of 3 g/day of choline magnesium trisalicylate for 6 weeks. These side effects were transient and probably caused by salicylate (Mody et al., 1983). The salicylate effect likely accounts for many of these observations, and the others are likely unusual anomalies, such as the one case of contact dermatitis reported after dermal exposure to choline chloride (Fischer, 1984).

Identification of Sensitive Subpopulations

Individuals with trimethylaminuria (fish odor syndrome), renal disease, liver disease, depression, and Parkinson's disease may have increased susceptibility to the adverse effects of choline. Trimethylaminuria results from a rare genetic deficiency that causes excessive excretion of trimethylamine and, therefore, an increased risk of developing fishy body odor (Al-Waiz et al., 1988, 1989; Humbert et al., 1970; Shelley and Shelley, 1984). Individuals with renal or liver disease may have increased susceptibility because of increased levels of plasma choline (after ingestion of supplemental choline) compared with healthy individuals (Acara and Rennick, 1973; Acara et al., 1983; Chawla et al., 1989; Rennick et al., 1976). In rare cases, consumption of large amounts of choline has been associated with depression (Davis et al., 1979; Tamminga et al., 1976). Finally, mild and transient Parkinsonian signs (bradykinesia, tremor, and rigidity) were observed at high doses (12.7 g/day) of choline as a chloride in people with tardive dyskinesia (Gelenberg et al., 1979), which suggests that supplemental choline intake by Parkinsonian patients may exacerbate symptoms.

Summary

On the basis of considerations of causality, relevance, and the

quality and completeness of the database, hypotension was selected as the critical effect in deriving a Tolerable Upper Intake Level (UL); fishy body odor was selected as the secondary consideration.

Dose-Response Assessment

Adults

Data Selection. The data used to derive the UL for choline include a single case report of hypotension and several other studies involving cholinergic effects and fishy body odor after oral administration of large choline doses.

Identification of a no-observed-adverse-effect level (NOAEL) and a lowest-observed-adverse-effect level (LOAEL). There are no adequate data demonstrating a NOAEL for excess choline intake. A LOAEL of approximately 7.5 g/day of choline can be identified from evaluation of a pilot study that reported hypotension in seven patients treated with choline for Alzheimer senile dementia (Boyd et al., 1977) and reports of fishy body odor in individuals treated with choline for tardive dyskinesia and Huntington's disease (Gelenberg et al., 1979; Growdon et al., 1977a, b; Lawrence et al., 1980). Boyd et al. (1977) treated seven older adult patients with 4 g/day of oral choline as choline chloride for 2 weeks followed by 2 weeks of choline at 7.5 g/day. At 4 g/day of choline, daily blood pressure recordings revealed no hypotension. In addition, there were no reports of nausea or diarrhea or other evidence of cholinergic effects at this dose level. At 7.5 g/day of choline, nausea, diarrhea, and a small decrease in blood pressure were reported in some patients. Other supportive data on cholinergic effects and fishy body odor after excess choline intake are summarized in Table 12-1.

Uncertainty Assessment. An uncertainty factor (UF) of 2 was selected because of the limited data regarding hypotension and the inter-individual variation in response to cholinergic effects.

Derivation of a UL. A LOAEL of 7.5 g/day was divided by an UF of 2 to obtain a UL of 3.75 for adults, which was rounded down to 3.5 g/day.

Choline UL Summary, Adults

Because of the scarcity of data for any adult age group and

TABLE 12-1 Studies Reporting on Cholinergic Effects and Fishy Body Odor after Excess Choline Intake

Study	No. of Subjects	Dose	Duration (wk)	Adverse Effects
Growdon et al., 1977a[a]	20	9 g/d (wk 1); 12 g/d (wk 2)[b,c]	2	Mild cholinergic toxicity: lacrimation, blurred vision, anorexia, and diarrhea.
Growdon et al., 1977b	10	8–20 g/d[d]	2–17	Fishy body odor in all subjects; at 250–300 mg/kg/d, produced lacrimation, anorexia, vomiting, and diarrhea.
Gelenberg et al., 1979[e]	5	8–19 g/d[d]	6–8	100% with fishy body odor after several days; gastrointestinal irritation.[f]
Lawrence et al., 1980[g]	14	0.2 g/d (3 wk); 9 g/d (3 wk)[c,d]	6	At 150 mg/kg/d: 5 of 14 with fishy body odor; 12 of 15 with nausea and diarrhea.

[a] Study involved a double-blind, crossover protocol.

[b] Choline was given as a chloride or bitartrate.

[c] Doses were calculated from data in the report using a reference body weight of 61 kg. Depending on the body weights of the individuals in Lawrence et al. (1980) and Growdon et al. (1977a), the lowest-effect dose may be less than 7.5 g/d.

[d] Choline was given as a chloride.

[e] Nonblinded study; did not include a control group.

[f] Mild, transient Parkinsonian signs (bradykinesia, tremor, and rigidity) were also reported.

[g] Double-blind protocol; included control group.

because no specific physiological function might be expected to affect sensitivity to excess amounts of choline in older persons, no adjustments are proposed for the elderly.

UL for Adults 19 years and older 3.5 g/day of choline

Other Life Stage Groups

For infants, the UL was judged not determinable because of lack of data concerning adverse effects in this age group and concern

about the infant's ability to handle excess amounts. The only source of intake for infants should be from food or formula to prevent high levels of intake. There are no data to suggest that during pregnancy or lactation increased susceptibility to developing cholinergic effects or fishy body odor from excess choline intake would occur. Therefore, the UL of 3.5 g/day is also set for pregnant and lactating women. The UL of 3.5 g/day for adults was adjusted for children and adolescents on the basis of relative body weight as described in Chapter 3, with the use of reference weights from Chapter 1, Table 1-2. Values have been rounded down.

Choline UL Summary, Other Life Stage Groups

UL For Infants		
0–12 months	**Not possible to establish; source of intake should be formula and food only**	
UL for Children	**1–3 years**	**1 g/day of choline**
	4–8 years	**1 g/day of choline**
	9–13 years	**2 g/day of choline**
UL for Adolescents	**14–18 years**	**3 g/day of choline**
UL for Pregnancy	**14–18 years**	**3 g/day of choline**
	19 years and older	**3.5 g/day of choline**
UL for Lactation	**14–18 years**	**3 g/day of choline**
	19 years and older	**3.5 g/day of choline**

Special Considerations

Individuals with the following conditions may be at risk of adverse effects with choline intakes at the UL: trimethylaminuria, renal disease, liver disease, depression, and Parkinson's disease.

Intake Assessment

National surveys do not provide data on the dietary intake of choline. The UL applies to the weight of the choline moiety in the compound; for example, choline chloride contains more choline by weight than does choline bitartrate. Dietary supplements containing choline are available; however, reliable estimates of the

amount of these supplements consumed in the United States and Canada are unavailable.

Risk Characterization

Because there is no information from national surveys on choline intakes or on supplement usage, the risk of adverse effects within the United States or Canada can not be characterized.

RESEARCH RECOMMENDATIONS FOR CHOLINE

High Priority Recommendations

Sufficient human data are not available for determining whether choline is essential in the human diet, how much is required if it is essential, and the public health impact of poor choline nutriture. For this reason, research that could provide such human data is assigned the highest priority:

- Examination of the effects of the use of graded levels of dietary intake of choline on parameters of health. This would include assessing plasma and tissue choline compounds and metabolites; plasma cholesterol and homocysteine concentrations; erythrocyte folate; and liver, renal, brain, and other organ function. To facilitate this process, food composition data are needed for choline, phosphocholine, glycerophosphocholine, sphingomyelin, phosphatidylcholine, and betaine and the analytic sensitivity and specificity of methods for analysis of food composition need to be validated.
- Human studies on interrelationships among requirements for choline, methionine, folate, vitamin B_6, and vitamin B_{12} to compare the homocysteine-lowering effects of combinations of these nutrients.

Other Research Areas

Two additional topics also merit attention:

- The relative effectiveness of different choline-containing compounds in the diet in promoting health and determination of the sparing effect of endogenous synthesis of choline. It will be important to conduct studies on the bioavailability of choline and choline compounds and on the rate of de novo synthesis of choline in vivo.
- Studies using increasing levels of dietary intake designed to assess toxicity for all organ systems, including heart, liver, brain and

kidney; fishy body odor; and possible growth suppression in children from observational data and as determined by experimental studies in animal models.

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Committee on Military Nutrition Research

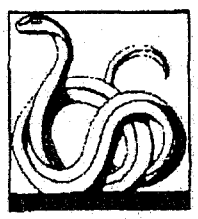
Activity Report

April 1, 1992
through
November 30, 1994

Food and Nutrition Board
INSTITUTE OF MEDICINE

Prepared by

Bernadette M. Marriott and Paul Thomas



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This report presents a summary of activities of the Committee on Military Nutrition Research (CMNR) from April 1, 1992, through November 30, 1994. Many of the activities mentioned here have resulted in reports that were previously published or submitted as letter reports to the sponsor and as such were reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. This activities summary has not been separately reviewed and represents an overview of all activities during the project period as designated.

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Appendix B.
Conclusions of the Committee on Military Nutrition Research regarding choline

Conclusions and Recommendations (pages 145-161) from the Workshop Report: Food Components to Enhance Performance of the Committee on Military Nutrition Research

Appendix I

Conclusions and Recommendations from the Workshop Report: Food Components to Enhance Performance

Submitted May 1994

Preface

The issues addressed in this report as well as in the previous 5 year report, *Committee Military Nutrition Research: Activity Report 1986–1992*, (Marriott and Earl, 1992), illustrate the diversity of activities addressed by the Committee on Military Nutrition Research (CMNR). This diversity has required the use of a broad range of expertise to respond to the issues brought to the CMNR. The range of scientific disciplines represented on the CMNR has been augmented as necessary through the use of workshops or special advisors to enable the CMNR to bring the degree and breadth of expertise necessary to properly respond to the subject under review. The committee has been pleased with and is very appreciative of the willing participation of the invited participants in these sessions and of their providing written papers which have constituted a major part of the CMNR reports. Many of these workshops have included experts from within the military who have shared their research activities and information. They have been excellent representatives of the quality of research that the military has been conducting on many of these problems.

The military is to be commended for continuing to ensure that the nutritional needs of its personnel are adequately met during the stress of military operations through its support of nutrition and related research. There has also been interest and support for modifications of rations of military personnel consistent with the advice provided by the nutrition and public health leadership in the United States. The CMNR is cognizant of the desire

to balance long-term health considerations with the demands of maintaining performance under the environmental extremes of military operations.

The ability of operational rations to help sustain military performance has been the subject of CMNR review since 1982. Field studies have shown the adequacy of nutrient intake other than calories sufficient to maintain the weight and performance of troops in the field. Complex interactions involving palatability of the ration components, convenience, fluid intake, socialization, and physical and psychological stresses that influence the consumption of operational rations are discussed in the publication, *Not Eating Enough, Overcoming Underconsumption of Operational Rations* (CMNR, in press). Further evaluation of these complex factors will undoubtedly continue to be of interest to the military and the CMNR.

We have appreciated the close working relationships with COL David Schnakenberg and Colonel Wayne Askew, who have now retired, and the excellent liaison they provided between the military and the Committee. They greatly assisted the work of the Committee by bringing issues forward for consideration and helping to identify expertise familiar with these problems, particularly from within the armed forces. We look forward to continued close association and guidance from Dr. James A. Vogel and his group at USARIEM.

As Committee Chair, I express my deep appreciation to all of the Committee members who have given their time, dedication, and expertise to the careful analysis of the issues and to developing the conclusions and recommendations of the Committee. I also thank all participants in the many workshops who have greatly aided our activities and assured that the appropriate expertise has been available to the Committee. Finally I wish to express my appreciation to the staff of the Food and Nutrition Board assigned to this activity over the past 3 years.

In particular I acknowledge for myself and the entire committee the outstanding support presently provided to this activity by Bernadette Marriott, Ph.D., Associate Director, Food and Nutrition Board, Institute of Medicine, and her assistant, Donna Allen. They have worked with extreme dedication to update and complete publication of several pending CMNR reports and to assure a timely response to the issues currently under consideration by the Committee. The additional assistance of Paul Thomas and Susan Knasiak with this activity report is gratefully acknowledged.

Robert O. Nesheim, Ph.D.
Chair

Contents

Conclusions and Recommendations

CONCLUSIONS

As stated in Chapter 1, the Committee on Military Nutrition Research (CMNR) was asked to respond to six specific questions dealing with the potential for food components to enhance performance for military personnel in combat settings. The committee's responses to these questions appear below. The committee further reviewed the current knowledge base regarding specific categories of food components that were identified by Army scientists as having potential to enhance performance in light of the classification of ergogenic aids and the mechanisms of action as discussed by John Ivy (Chapter 12). Substances that may optimize physical performance are frequently referred to as ergogenic aids (Chapter 12). These may be divided into five categories: (1) mechanical, (2) psychological, (3) physiological, (4) pharmacological, and (5) nutritional. The mechanisms by which foods or food components may act as ergogenic aids as discussed by Ivy are (1) acting as central or peripheral stimulants, (2) increasing the storage or availability of limiting substrates, (3) acting as a supplemental fuel source, (4) reducing or neutralizing metabolic by-products, and (5) enhancing recovery. Each food component was also reviewed in light of the time frames and military scenarios drawn up by Army scientists (see Appendix A). The recommendations and conclusions drawn about the potential for these food components to enhance performance are included in the specific committee recommendations that follow.

GENERAL CONCLUSIONS

General Concepts of Performance Enhancement

The first consideration in maintaining or enhancing performance is to endeavor to insure that troops are in a well-hydrated, rested and well-nourished state-including optimal amounts of all essential micronutrients, plus the best in military training, both physical and mental, in advance of anticipated periods of stress. Under these circumstances performance is unlikely to be improved in the absence of the imposition of military operations which impose physical or mental stress.

Obviously battlefield situations are not free of stress. Under these conditions troops are frequently deprived of sleep, apprehensive, haven't eaten sufficient food to meet their energy expenditures, dehydrated to varying degrees and exposed to environmental extremes of heat, cold, altitude, etc. which impacts on their physical and mental state. Given these conditions, enhancement of performance is more likely to be restoring performance to non-stressed baseline than to improvement over that expected from well-nourished and well-rested troops. The military Science and Technology Objective (STO) of enhancing performance by 10-15 percent is more realistic in short term enhancement of performance under stress than to obtain super performance from troops in a well-fed, well-rested state.

While some of the food components considered in this report may be used at usual dietary levels (caffeine, carbohydrate) others are likely to be at levels of intake that may be considered pharmacological. These components may be provided in operational ration items designed to be used at specific times and provide short-term enhancement through increased vigilance, reduced feeling of fatigue, improved mental state, etc. The enhancement capability of a component likely will have a threshold which must be met to have a benefit and will also likely have a "wear out" when the stimulus can no longer overcome the adverse effect of the stress. In researching the effectiveness and safety of these pharmacological components it will be important to determine these levels and time periods to evaluate both safety and efficacy.

It is also noted that some of these helpful nutritional effects may be maximized by the additional use of conventional over-the-counter drugs that block the intracellular formation of stress-induced prostaglandins, which contribute importantly to many symptoms and the ill effects of stress.

Food Components or Nutrients that Offer Potential to Enhance Performance

The following food components have potential for enhancing performance under certain circumstances that may be encountered in military operations.

- **Carbohydrates.** The role of carbohydrate as a fuel source for extended physical activity is well-known. Increased storage of glycogen prior to extended physical performance through consumption of high-carbohydrate meals and consuming carbohydrates during an extended physical activity as a means of increasing performance is well established. Studies with soldiers in military activities are less clear but likely relate to the more intermittent nature of the physical activity, in comparison with the extended moderate-to high-level physical activities of athletic competition. The value of carbohydrate supplementation in extending physical performance is usually demonstrated after 60–90 minutes of continuous activity at 60 to 70 percent of maximal oxygen uptake ($\dot{V}_{O_2\,max}$). Moderate to heavy physical activity of a lesser time period followed by rest or reduced activity does not usually demonstrate a value for carbohydrate supplementation during the activity.

The potential role for carbohydrates in affecting such behaviors as mood, performance, and satiety, with emphasis placed on sensorimotor and cognitive performance as discussed in Chapter 18, is worthy of further consideration. Mood changes that may affect motivation to operate under stressful conditions are an important consideration. These stressful situations, such as combat, may unmask performance deficits that are not apparent under nonstressful conditions. It also should be emphasized that meals containing protein and carbohydrate demonstrate more beneficial effects than meals that are nearly protein-free. The behavioral effects seen are usually time context dependent. Snacks (providing combinations of protein and carbohydrate) may have utility in enhancing performance between meals. Research in evaluating the benefits of supplemental carbohydrates on performance should include the more subtle evaluations of motivation and coping in addition to the simple cognitive and sensorimotor measures.

Evaluation should be made of the potential performance-enhancing benefits of supplemental carbohydrate and carbohydrate-containing snacks on physical and cognitive performance, including mood and motivational effects.

- **Caffeine.** Caffeine exerts its central nervous system-mediating effects by blocking adenosine receptors. Its stimulant effects when compared with those of other drugs such as amphetamines are weak, but most studies to date suggest that caffeine tends to delay sleep and reduce the deterioration of performance associated with fatigue and boredom. Caffeine at higher doses

reverses sleep deprivation-induced degradation in cognitive performance, mood, and alertness—important considerations in extended military operations in subjects who report low levels of caffeine intake. The principal side effects include nervousness/jitteryness and decreased sleepiness, which may persist for several hours.

Caffeine definitely should be considered in developing performance-enhancing rations or ration components. Caffeine is safe as a component of food at doses required to overcome sleep deprivation and has been included in diets in coffee and many soft drinks. Since many soldiers may not normally drink coffee, a mechanism for including caffeine in another ration component that can be selectively used when the situation requires should be evaluated. It appears that doses of 300–600 mg/70-kg person will achieve the desired stimulus in those nonhabituated to caffeine; additional research needs to be conducted to determine the effects of this level of caffeine in those with higher habitual intakes.

- **Tyrosine.** The amino acid tyrosine is the precursor of the neurotransmitters dopamine, norepinephrine, and epinephrine. Under highly stressful conditions, the availability of tyrosine may be rate limiting for the synthesis of these neurotransmitter products. The observation that the functioning of catecholaminergic neurons can be precursor dependent is the basis for the hypothesis that tyrosine will mitigate the adverse effects of acute stress, because such neurons regulate, in part, the behavioral, cardiovascular, and neuroendocrine consequences of stress.

A series of studies in animals has demonstrated that the performance decrement observed in highly stressed animals can be restored by tyrosine supplementation. Studies in humans as well as animals suggest that the amino acid tyrosine may have beneficial effects on humans that are subject to acute strossors. The adverse effects of hypoxia, cold, body negative pressure, and psychological stress have been reduced by treatment with tyrosine. Research is needed to define methods of administration and the effective and safe levels of tyrosine required.

- **Choline.** Choline and choline-containing compounds are critical for a wide variety of processes within the body, including acting as a messenger within the cells and as neurotransmitters in the nervous system controlling muscle contraction, providing methyl groups in a variety of intracellular reactions, acting as a component of triglyceride transport, and participating in the immune response. The best-known function of choline is as a component of acetylcholine, an important neurotransmitter.

Free choline and choline-containing esters are present in a wide variety of foods in the human diet. The usual intake is estimated to be in the range of

200–1,000 mg per day. There is no Recommended Dietary Allowance (RDA) for choline in humans, but intake of 500 mg/day results in decreased plasma choline and phosphatidylcholine concentrations. Diets deficient in choline produce liver dysfunction within 3 weeks, resulting in massive triglyceride accumulation in the liver and abnormalities of plasma levels of liver enzymes.

There is evidence that diets low in choline reduce muscle performance. Dietary choline supplementation of individuals with normal intakes during a 20-mile (32-km) run improved the run time by 5 minutes and prevented the drop in plasma choline levels normally associated with the run. Placebo-controlled, randomized, double-blind trials are needed to determine whether choline supplementation will enhance performance of military personnel undergoing rigorous activity in the field.

Choline supplementation enhances memory and reaction time in animals, particularly aging animals, and enhances memory in humans. Although the mechanisms for this are unclear, there are indications of alterations of the anatomy of brain cells. Carefully controlled laboratory studies with human subjects may suggest field studies to evaluate cognitive performance enhancement in stressful field situations.

With the diversity of functions of choline in the body, there is ample reason for interest in reviewing its possible value in maintaining or enhancing performance of the soldier. Since choline is a normal constituent of many foods and can safely be used at the high usual levels of intake, it is worthy of evaluation to determine whether it may enhance either the physical or the cognitive performance of soldiers who are functioning in a stressful environment.

Other Food Components of Theoretical Importance but Low Probability of Improving Performance

On the basis of a review of information presented at the workshop and review of background materials, it is concluded that the following materials have some theoretical importance but offer a very low probability of demonstrating an improvement in performance under conditions anticipated in military operations.

- **Carnitine.** Carnitine is important metabolically in exercising muscle. Carnitine functions as a transportable high-energy compound that can be reformed without the use of ATP. It acts as a storehouse of high-energy compounds, stimulates fatty acid oxidation, transports acylcoenzyme A (acyl-CoA) across membranes, prevents the accumulation of lactate, and stimulates carbohydrate and amino acid utilization. These functions have led to the

hypotheses that supplementation of free carnitine, acetylcarnitine, or propionylcarnitine theoretically might enhance the oxidation of fatty acids during exercise, thus sparing the use of muscle glycogen, delaying the onset of fatigue, and enhancing exercise performance.

Most Americans consume 50–100 mg of carnitine per day, with some consuming three times that amount. Carnitine appears to be safe, but there is little evidence to suggest that higher amounts are beneficial to healthy individuals. Carnitine has been extensively researched, and at this time there is no conclusive evidence that carnitine supplementation is helpful in enhancing physical performance during exercise.

Its importance metabolically in exercising muscle indicates that research on its use should be followed. It is not recommended for consideration in military ration development at this time.

• **Structured lipids.** Structured lipids are defined as fats that are synthesized from mixtures of long- and medium-chain fatty acids. Therefore, they are differentiated from typical dietary fats by the presence of medium-chain fatty acids (5–10 carbon atoms). Their potential as a performance-enhancing ingredient is based on the hypothesis that glycogen utilization during exercise may be spared by the rapid oxidation of the medium-chain fatty acids. Since the medium-chain fatty acids in the diet are delivered directly and rapidly to the liver via the portal circulation, their metabolism in the liver produces the ketone bodies acetoacetate and ß-hydroxybutyrate, which would circulate to the muscle and be oxidized, sparing glycogen.

The nutritional advantages of structured lipids have been demonstrated mostly in individuals with such stresses as burns, trauma, and infection. Research to date has not supported the hypothesis that the supplements of structured lipids will spare glycogen utilization during exercise, which is more closely related to the objective of enhancing physical or mental performance during military operations. In the absence of new data that demonstrate potential in this area, the inclusion of structured lipids in rations or food components for improving performance is not recommended.

ANSWERS TO THE QUESTIONS POSED TO THE COMMITTEE

The committee has answered the six questions posed by the Army in light of the general conclusions described above. These answers are further elaborated in the recommendations that follow.

1. Is enhancement of physical and mental performance in "normal," healthy, young adult soldiers by diet or supplements a potentially fruitful approach, or are there other methods of enhancing performance that have greater potential?

Emphasis should be given to making sure that troops are adequately hydrated and fed prior to military operations. There is little evidence from current nutrition research to suggest that soldiers already consuming nutritionally adequate rations as specified in the Military Recommended Dietary Allowances (MRDAs) will show significantly improved performance when nutritional supplements are added (as differentiated from pharmaceutical levels of some food components). Troops going into operational situations are presumably in good physical condition and have been consuming adequate amounts of military rations to meet their nutrient needs. Individual vitamin and mineral supplements are unlikely to improve performance under these circumstances. Soldiers who have been deprived of adequate food intake for a period under the pressure of military operations would likely benefit from receiving additional food to overcome the caloric deficit before entering another operation. Similarly, if they have been deprived of adequate sleep or rest because of extended physical activity, an opportunity to sleep or physically rest would help restore performance to normal levels.

Stimulants such as caffeine may help in the short term to overcome the effects of physical and mental fatigue when continuous operations are required.

2. The Army Science and Technology Objective (STO) states: By FY98 demonstrate a 10–15 percent enhancement of soldier performance in selected combat situations through the use of rations/nutrients that enhance caloric utilization and/or optimize the physiological levels of neurotransmitters. (Army Science Board, 1991).

Is the level of enhancement identified in this STO reasonable with the current scientific knowledge?

The Army Science and Technology Objective (STO) of demonstrating a 10–15 percent enhancement of performance through specific ration or nutrient consumption by Fiscal Year 1998 is overly optimistic, particularly if this is expected as enhancement over the level achieved by normal, well-fed, physically fit soldiers. However, if enhanced performance is defined as restoring or preventing all or part of the decrease in performance that is usually encountered over extended field operations, then there may be opportunities to achieve this objective.

Current studies of troops in extended field operations show that troops tend to reduce food intake, lose weight, and in some instances dehydrate. Overcoming these deficits is more likely to maintain performance. Since only modest dehydration will result in reduced performance, ensuring adequate fluid

intake offers the best opportunity to overcome potential performance deficits. Adequate food intake to meet caloric needs also will help maintain high levels of performance. Under conditions of extended moderate physical activity, carbohydrate supplementation to maintain muscle glycogen levels can extend the ability to perform at this activity level. Simply eating frequent meals may accomplish this. Stimulants such as caffeine may also temporarily maintain physical and cognitive performance.

3. Which food components, if any, would be the best candidates to enhance military physical and mental performance?

Food components that would help provide energy sources to large muscles would be most likely to enhance or maintain performance. The proper use of carbohydrate supplements for persons engaged in continuous, moderate physical activity over at least 1.5 to 2 hours has the ability to extend the time to exhaustion. Caffeine has also been demonstrated to improve physical and cognitive performance. Tyrosine may also benefit cognitive performance under certain circumstances. Choline has shown some possible benefit in improving performance over extended periods of physical activity. Studies with marathon athletes need to be carefully reviewed relative to these applications to military operations. Soldiers in military operations seldom are required to perform at a similar continuous level of physical activity and over the extended time period as athletes in marathon events.

4. Should the mode of administration be via fortification of the food in rations, supplemented via a separate food bar or beverage component, or administered in a "vitamin pill mode"? Is palatability a significant issue in this type of supplementation?

The answer to this question depends not only on what food component or individual nutrient is under consideration but also on issues of safety and efficacy that have not yet been addressed. Depending on the circumstances, carbohydrate supplements can be delivered effectively in either beverages or snack bars. Caffeine is currently widely consumed either in beverages or in pill form, as a means of enhancing wakefulness and alertness. It could easily be added to snack bars or food items, but because of adverse reactions to caffeine in some individuals as well as religious proscriptions, this would be less desirable. It is premature to answer the question for individual nutrients such as tyrosine, tryptophan, and choline. Their effectiveness depends on large increases in plasma levels and is reduced when consumed as part of a normal meal containing protein and carbohydrate. Conversely, their safety is likely to be highest when these substances are consumed as supplements to a meal. The safety of these substances as single supplements when given in large enough doses to be effective has not yet been demonstrated.

5. Are there specific ethical issues that need to be considered with this type of research?

The ethical issues depend upon the nature of the enhancement. When the safety of the use of the ration is not an issue, informing the soldiers about the ration and its purpose should suffice.

If the component(s) is used at a pharmacological level, the criteria for evaluating the safety of the component as a drug should be met. Soldiers should be informed of its benefits, and possible side effects and should be educated concerning its condition of use. Research needs to proceed through proper stages of safety and efficacy evaluation before trials on large numbers of troops are conducted. Issues related to ethnicity, gender, and religious beliefs need to be considered, and evaluation and follow-up on any reported adverse or side effects must be conducted.

The best guidelines for this research would be U.S. Food and Drug Administration (FDA) guidelines for research on proposed new drugs.

6. What regulatory issues must be considered with the types of food components that are being evaluated by the Army?

The considerations for the approval of food additives are well developed by John E. Vanderveen in Chapter 23. The most important consideration is the demonstrated safety of the material in question. The general approach to demonstrating safety is well spelled out in the FDA's Red Book (Food and Drug Administration, 1982). A further consideration is the matter of whether the uses considered during this workshop represent usage as a "food" or as a "drug." Different regulations control each class of materials. Further, if a substance is classified as a "drug," then not only must safety be demonstrated but data showing efficacy must also be presented.

It would seem critical for the military to follow the same requirements that the FDA would require for general use of a component in the civilian population. Therefore in considering the components other than caffeine and carbohydrates that have been discussed as agents capable of enhancing performance, it is important to recognize that none of these materials has been demonstrated to be "safe," notwithstanding the fact that all of these agents exist in natural foods at levels required for potential effects. Importantly, the proposed uses (to enhance performance) require exposure levels that are in excess of what would be consumed in foods.

It would seem that the intended uses as performance enhancers would classify the compounds in question in the drug category. The testing requirements are not necessarily more stringent for a drug; in fact, as noted by Dr. Vanderveen, a drug classification permits a benefit-risk consideration that is not possible for a food category consideration. Thus, it would be necessary

to generate data demonstrating minimal risk from the exposures expected and data clearly demonstrating a benefit from the proposed doses.

RECOMMENDATIONS

General

1. On the basis of data presented at the workshop, the Army's prior selection of carbohydrate, caffeine, and tyrosine as food supplements that may enhance performance is fully justified. It is recommended that research with all three should continue.

2. The utility of caffeine in reversing the degradation in cognitive performance, mood, and alertness associated with sleep deprivation that has been widely explored at USARIEM and elsewhere is well understood. It is recommended that future research with this compound explore and attempt to categorize individual differences in responses to caffeine as well as the issue of expectancy and placebo effects.

Recommendations Regarding Food Components Proposed by the Army

On the basis of the papers presented by the invited speakers, discussion at the workshop, and subsequent committee deliberations, the Committee on Military Nutrition Research recommends the following:

1. The following components have clearly demonstrated their ability to enhance performance under appropriate simulated conditions and should be evaluated in appropriate delivery systems.

Caffeine. Caffeine functions as a weak stimulant that, in low doses, tends to delay sleep and reduce the deterioration of performance associated with fatigue and boredom. At higher doses caffeine reverses the sleep deprivation-induced degradation in cognitive performance, mood, and alertness. The long experience with the use of coffee suggests that caffeine is *safe* at levels required to achieve the desired effects, and its effects are reversible over time. **The primary issues that need to be answered in providing caffeine are the appropriate carrier that should be used to provide the supplement and the amount required to achieve the desired benefit in those both habituated and nonhabituated to it.** Since it would not be desirable to inhibit

sleep when operations permit, the timing and availability of the caffeine-containing food component should be evaluated.

Carbohydrate. Carbohydrate is an important fuel source and is particularly important for enhancing extended continuous physical activity. The potential role for carbohydrate in affecting such behaviors as mood, performance, and satiety relating to sensorimotor and cognitive performance has not been as thoroughly evaluated. Many studies have been carried out with carbohydrate supplements, with the major emphasis on physical performance. **The committee recommends that this line of research at USARIEM should be continued. However, emphasis should be shifted to the effect of the macronutrient composition of meals and supplements on the affective domain, including such aspects as mood, perceived fatigue, and motivation.** Hedonic properties and the timing and setting of meals and supplements are important variables to be considered, as are food preferences and aversions related to race, ethnicity, geography, and gender. Carbohydrate-containing snacks, which also provide sufficient protein, should be evaluated as a means of overcoming fatigue and improving mood and performance. Research to evaluate the performance-enhancing potentials of such products should be conducted not only as a means of potentially improving performance in the short term but also as an aid in overcoming some of the caloric deficits usually noted for troops in field operations. It is also suggested that the possibility of providing caffeine in such a product may define a product that could be used in a particularly stressful time to enhance performance.

2. The following components are suggested for further research on the basis of their importance in energy metabolism and/or neurotransmitter actions in the body.

Choline. On the basis of its diverse functions in the body, both in physical performance and in cognitive function, and limited studies demonstrating potentially improved performance in extended physical activity, in cognitive function in animals and humans, and its relative safety, the committee believes that choline should be evaluated for its performance enhancement potential. **The committee recommends that choline should be added to the list of food supplements that have potential to enhance performance and that are being evaluated at the U.S. Army Research Institute of Environmental Medicine (USARIEM).** It is suggested that carefully controlled laboratory studies with human subjects be conducted initially, the results of which may suggest field studies that could be used to evaluate enhanced physical and/or cognitive performance under stressful field conditions.

Tyrosine. Research has demonstrated that tyrosine may be rate limiting for the synthesis of neurotransmitter products under highly stressful conditions. Animal studies and limited human studies have demonstrated that tyrosine may have beneficial effects in overcoming the adverse effects of acute stressors. These data are encouraging and demonstrate that additional research should be conducted under carefully controlled conditions to further define when tyrosine may be beneficial in reversing acute stress. The research with tyrosine currently being carried out at both USARIEM and the Naval Medical Research Institute is exciting. **The committee recommends that this research be expanded, with more emphasis placed on safety, interactions with ration consumption, stress, and field studies.** Data are required on the safety of tyrosine use at levels required for efficacy. Since the effect of tyrosine appears to be pharmacological, the FDA protocols for demonstrating safety and efficacy should be considered. Evaluation of the proper method of delivering an effective dose of tyrosine to affected troops would also be required.

3. The following compounds have a low probability of enhancing performance through their use in military rations.

Carnitine. Because of its importance metabolically in exercising muscle, research in the exercise physiology literature should be monitored, but carnitine is not recommended for consideration in performance enhancement ration development and evaluation by the military until it is demonstrated that carnitine supplementation over that normally supplied in usual military rations has some value.

Structured lipids. There are no data to support the fact that structured lipids spare glycogen utilization during exercise and therefore support improved performance. It is recommended that structured lipids not be further evaluated as a performance-enhancing component of operational rations.

Specific Recommendations

Tyrosine. Although tyrosine has been demonstrated to reverse the effects of certain acute stressors, some critical issues remain to be addressed before it can be recommended for use in enhancing the performance of acutely stressed military personnel. These issues, as outlined by Harris R. Leiberman (Chapter 15), are as follows:

1. demonstrating the generalizability of tyrosine effects across a wider range of stressors,

2. establishing a dose-response function for tyrosine's beneficial effects,
3. determining whether tyrosine has efficacy in chronic stress paradigms,
4. determining the safety of tyrosine administration,
5. assessing the risks and benefits of acute versus chronic administration of tyrosine, and
6. determining the most appropriate method for providing tyrosine supplementation.

Choline. Both clinical and basic research into choline and its effects on the body may have relevance for the military. Several clinical studies are obvious:

1. studies to determine whether choline supplementation enhances endurance and muscle performance, and
2. studies to determine whether choline supplementation enhances intellectual performance and whether this alters performance of soldiers in the field.

Carbohydrate supplements. Since carbohydrate supplements have been shown to enhance performance in athletes performing at moderate to heavy levels of physical activity for extended periods of time, it is desirable to evaluate various military operational scenarios to determine whether and when a carbohydrate supplement would be advantageous. Suggested areas are:

1. continuous load carrying at 50–70 percent maximal oxygen uptake for 1–2 hours without resting, and
2. sleep-deprived states when moving into simulated-combat situations.

Another possible area of research would be to determine the amount of protein needed in relation to carbohydrate to prevent the "perceived fatigue" effect reported with carbohydrate intake.

Other Areas that Offer Research Potential

- While tryptophan was extensively used by many individuals, serious safety concerns led to its being banned from use. Depending upon federal regulatory guidelines, tryptophan may at some point offer research potential in the area of sleep promotion. Issues of mode of administration and dose would be areas of significant concern for military research with tryptophan.
- Laboratory research indicates heightened self report of fatigue after ingestion of high-carbohydrate, low-protein supplements. Studies of carbohy-

drate/protein ratios in supplements also offer research potential for sleep promotion.

- Limited data from laboratory studies suggest that the buffering effects of sodium bicarbonate ingestion on muscle pH changes during physical exercise offer potential for further research.
- Glycerol is another substance that, although not specifically covered in this workshop, may warrant further investigation as a dietary supplement to enhance performance.
- Likewise, while not specifically discussed in the CMNR workshop, there are reports that carbohydrate supplementation is beneficial in improving performance at high altitude.
- Although this report has emphasized the specific isolated food components identified by the U.S. Army, and thereby focused recommendations regarding these components on a component-by-component basis, further research would need to include careful investigations of the interactions among any components as well as the interactions of regular dietary levels of caffeine and carbohydrates with performance-enhancing food components.
- Symptoms that frequently occur during stress (including headaches, myalgias, somnolence, and reduction in food intake) contribute importantly to decrements in performance. Carefully controlled studies should be considered during military-type stresses of the ancillary use, prophylactic and/or therapeutic, of common, symptom-treating, over-the-counter drugs that block the cytokine-induced intracellular production of prostaglandins, that is, drugs such as aspirin or ibuprofen. Prostaglandin blockade with such drugs could not only reduce symptoms to improve performance but could also have the ancillary nutritional benefits of improving appetite and reducing the hypermetabolic loss of body nutrients and muscle protein known to be associated with prostaglandin release.

AREAS FOR FUTURE RESEARCH

The Committee on Military Nutrition Research recognizes the potential value for performance enhancement in combat settings and suggests a number of areas for future research within the military. The CMNR believes that the military services, through their pool of volunteer personnel, offer an excellent and often unique opportunity to generate research data and statistics on the nutrition, health, and stress reduction in service personnel. These findings can be directly applied to improving both the health and the performance of military personnel and those of the general U.S. population.

1. Much of the research needed to establish the safety of large doses of tyrosine and potentially choline needs to be carried out with rats. Amino acid, neurotransmitter, and metabolite levels need to be measured in specific brain nuclei, and many other animal studies are needed including gross and microscopic pathologies in both short-and long-term experiments. Possibly this could be accomplished through the Army funded neuroscience research at the Pennington Biomedical Research Center, Baton Rouge, Louisiana, in support of the human studies at USARIEM.

2. Performance, including cognitive, emotional, and physical aspects, is of crucial importance to all service branches. **It is recommended that an interservice committee be established to coordinate and facilitate research and development activities in this area.**

3. **A final general recommendation is to focus nutrition/performance research on diet/stress/immune function relationships in both acute and chronic situations.** It would be desirable to relate the research, at least in part, to researchable issues raised by the two Ranger studies. Immunological studies should include studies of humoral immunity, cellular immunity, and plasma cytokine concentrations before, during, and after the period of stress.

The Committee on Military Nutrition Research is pleased to participate with the Division of Nutrition, U.S. Army Research Institute of Environmental Medicine, U.S. Army Medical Research and Development Command, in programs related to the nutrition and health of U.S. military personnel. The CMNR hopes that this information will be useful and helpful to the U.S. Department of Defense in developing programs that continue to improve the lifetime health and well-being of service personnel.

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