The B Vitamins and Choline: Overview and Methods

OVERVIEW

This report focuses on the eight B complex vitamins—thiamin, riboflavin, niacin, vitamin B₆, folate, vitamin B₁₂, pantothenic acid, and biotin—and choline. These water-soluble nutrients fall into two categories: those involved in the reactions of intermediary metabolism related to energy production and redox status and those involved in the transfer of single-carbon units.

Thiamin, riboflavin, niacin, vitamin B₆, and pantothenic acid are required for decarboxylation, transamination, acylation, oxidation, and reduction of substrates that ultimately are used for energy utilization. One or more of these also are important for amino acid, fatty acid, cholesterol, steroid, and glucose synthesis.

Biotin is required for carbon dioxide fixation by four carboxylases. Folate, vitamin B₁₂, choline, and riboflavin are needed for methyl-group transfer. Their metabolism intermingles at the pathway for conversion of homocysteine to methionine. Folate is also important for the supply of single-carbon units for deoxyribonucleic acid (DNA) synthesis.

Both the Système International d’Unités (SI units) and traditional units are used in this report, as described in Appendix C.
METHODOLOGICAL CONSIDERATIONS

Types of Data Used

The scientific data for developing the Dietary Reference Intakes (DRIs) have essentially come from observational and experimental studies in humans. Observational studies include single-case and case-series reports, epidemiological cohort studies, and case-control studies. Experimental studies include randomized and nonrandomized therapeutic or prevention trials and controlled dose-response, balance, turnover, and depletion-repletion physiological studies. Results from animal experiments are generally not applicable to nutritional deficiencies, chronic diseases, and toxic effects in humans, but selected animal studies are considered in the absence of human data. The strategies used for identifying potentially relevant studies are summarized in Appendix D.

As a principle, only studies published in peer-reviewed journals have been used. However, studies published in other scientific journals or readily available reports were considered if they appeared to provide important information not documented elsewhere. To the extent possible, original scientific studies and quantitative meta-analyses have been used to derive the DRIs. A thorough review of the scientific literature resulted in the identification of clinical and functional indicators of nutritional adequacy for each nutrient for each life stage and gender group. Anything that might affect dietary requirements, such as an interaction with other nutrients and the bioavailability of the nutrient, was considered when relevant. For example, the effect of energy intake was considered for thiamin, riboflavin, and niacin; the effect of protein intake was considered for vitamin B₆.

Because of the growing evidence that some B vitamins may prevent the occurrence of developmental abnormalities and chronic degenerative and neoplastic diseases, special consideration was given to the possible use of such indicators as criteria of adequacy. It was beyond the scope of the report to consider the use of nutrients in the treatment of disease or other disorders.

The quality of studies was considered in weighing the evidence. The characteristics examined included the study design and the representativeness of the study population; the validity, reliability, and precision of the methods used for measuring intake and indicators of adequacy; the control of biases and of confounders; and the power of the study to demonstrate a given difference or correlation. When applicable, greatest weight was given to randomized con-
trolled trials and less to nonrandomized trials; prospective cohort, retrospective cohort, and case-control studies; case-series; and single-case reports. Publications solely expressing opinions were not used in setting DRIs.

Statistical association does not imply causation, and this is especially true for relationships between nutrient intake and developmental abnormalities or chronic disease risk reduction as well as for toxic effects. The criteria proposed by Hill (1971) were considered when examining the evidence that a relationship might be causal:

- strength of the association, usually expressed as a relative risk or a correlation coefficient;
- dose-response relationship;
- temporally plausible association, with exposure preceding the effect;
- consistency of association in time and place;
- specificity of cause and effect; and
- biological plausibility.

For example, biological plausibility would not be sufficient in the presence of a weak association and lack of evidence that exposure preceded the effect.

Data were examined to determine whether similar estimates of the requirement resulted from the use of different indicators and different types of studies. For a single nutrient the criterion for setting the Estimated Average Requirement (EAR) may differ from one life stage group to another because the critical function or the risk of disease may be different. When no or very poor data were available for a given life stage group, extrapolation was made from the EAR or Adequate Intake (AI) set for another group based on explicit assumptions on relative requirements.

Method to Determine the Adequate Intake for Infants

The AI for young infants is generally taken to be the average intake by full-term infants who are born to healthy, well-nourished mothers and who are exclusively fed human milk. The extent to which intake of a nutrient from human milk may exceed the actual requirements of infants is not known, and ethics of experimentation preclude testing the levels known to be potentially inadequate. Using the breastfed infant as a model is in keeping with the basis for earlier recommendations for intake (e.g., Health Canada, 1990; IOM, 1991). It also supports the recommendation that exclusive
breastfeeding is the preferred method of feeding for normal full-term infants for the first 4 to 6 months of life, even though most U.S. babies are no longer breastfed by age 6 months. This recommendation has been made by the Canadian Paediatric Society (Health Canada, 1990), the American Academy of Pediatrics (AAP, 1997), the Institute of Medicine (IOM, 1991), and many other expert groups.

In general, this report does not cover possible variations in physiological need during the first month after birth or the variations in intake of nutrients from human milk that result from differences in milk volume and nutrient concentration during early lactation.

In keeping with the decision made by the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, specific DRIs to meet the needs of formula-fed infants have not been proposed in this report. The use of formula introduces a large number of complex issues, one of which is the bioavailability of different forms of the nutrient in different formula types. However, in the section “Special Considerations,” issues related to bioavailability and use of different milk sources are discussed when appropriate.

Ages 0 through 6 Months

To derive the AI value for infants ages 0 through 6 months, the mean intake of a nutrient was calculated based on (1) the average concentration of the nutrient from 2 to 6 months of lactation using consensus values from several reported studies, if possible, and (2) an average volume of milk intake of 780 mL/day. This volume was reported from studies that used test weighing of full-term infants. In this procedure, the infant is weighed before and after each feeding (Allen et al., 1991; Butte et al., 1984; Chandra, 1984; Hofvander et al., 1982; Neville et al., 1988). Because there is variation in both the composition of milk and the volume consumed, the computed value represents the mean. It is expected that infants will consume increased volumes of human milk during growth spurts.

Ages 7 through 12 Months

During the period of infant growth and gradual weaning to a mixed diet of human milk and solid foods from ages 7 through 12 months, there is no evidence for markedly different nutrient needs. The basis of the AI values derived for this age category could be the sum of (1) the specific nutrient provided by 600 mL/day of human
milk, which is the average volume of milk reported from studies of breastfed infants in this age category (Heinig et al., 1993), and (2) that provided by the usual intakes of complementary weaning foods consumed by infants in this age category. Such an approach would be in keeping with the current recommendations of the Canadian Paediatric Society (Health Canada, 1990), American Academy of Pediatrics (AAP, 1997), and Institute of Medicine (IOM, 1991) for continued breastfeeding of infants through 9 to 12 months of age with appropriate introduction of solid foods.

Only one relatively recent published source of information about B vitamin intake from solid foods for infants aged 7 through 12 months was found (Montalto et al., 1985), and it covered only three B vitamins: thiamin, riboflavin, and niacin. These researchers’ estimates are based on data from 24-hour dietary intakes from the 1976–1980 National Health and Nutrition Examination Survey (NHANES II) for infants aged 7 to 12 months. The infants were consuming formula; intake from solid food was reported separately.

For the B vitamins and choline, two other approaches were considered as well: (1) extrapolation upward from the AI for infants ages 0 through 6 months by using the metabolic weight ratio and (2) extrapolation downward from the EAR for young adults by adjusting for metabolic body size and growth and adding a factor for variability or from the AI if the recommended intake for adults was an AI. Both of these methods are described below. The results of these methods are compared in the process of setting the AI.

*Method for Extrapolating Data from Adults to Infants and Children*

*Setting the EAR or AI*

For the B vitamins and choline, if data were not available to set the EAR and Recommended Dietary Allowance (RDA) or an AI for children ages 1 year and older and for adolescents, the EAR or AI has been extrapolated down by using a consistent basic method. The method relies on at least four assumptions:

1. Maintenance needs for the B vitamins and choline expressed with respect to body weight ([kilogram of body weight]$^{0.75}$) are the same for adults and children. Scaling requirements as the 0.75 power of body mass adjusts for metabolic differences demonstrated to be related to body weight, as described by Kleiber (1947) and explored further by West and colleagues (1997). By this scaling a child weighing 22 kg would require 42 percent of what an adult...
weighing 70 kg would require—a higher percentage than that represented by actual weight.

2. The EAR for adults is an estimate of maintenance needs.

3. The percentage of extra B vitamins and choline needed for growth is comparable with the percentage of extra protein needed for growth.

4. On average, total needs do not differ substantially for males and females until age 14, when reference weights differ.

The formula for the extrapolation is

$$\text{EAR}_{\text{child}} = \text{EAR}_{\text{adult}} (F),$$

where $F = (\text{Weight}_{\text{child}}/\text{Weight}_{\text{adult}})^{0.75} (1 + \text{growth factor})$. Reference weights from Table 1-2 are used. If the EAR differs for men and women, the reference weight used for adults differs by gender; otherwise, the average for men and women is used unless the value for women is derived from data on men. The approximate proportional increase in protein requirements for growth (FAO/WHO/UNA, 1985) is used as an estimate of the growth factor as shown in Table 2-1. If only an AI has been set for adults, it is substituted for the EAR in the above formula and an AI is calculated; no RDA will be set.

**Setting the RDA**

To account for variability in requirements because of growth rates and other factors, a 10 percent coefficient of variation (CV) for the

### TABLE 2-1 Growth Factors Used to Extrapolate DRIs

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Growth Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 mo–3 y</td>
<td>0.30</td>
</tr>
<tr>
<td>4–8 y</td>
<td>0.15</td>
</tr>
<tr>
<td>9–13 y</td>
<td>0.15</td>
</tr>
<tr>
<td>14–18 y, Males</td>
<td>0.15</td>
</tr>
<tr>
<td>14–18 y, Females</td>
<td>0.00</td>
</tr>
</tbody>
</table>

**NOTE:** Growth beyond age 13 for females is assumed to represent a negligible increased requirement for vitamins.

**SOURCE:** The proportional increase in protein requirements for growth from FAO/WHO/UNA (1985) was used to estimate the growth factor indicated.
requirement is assumed unless data are available to support another value, as described in Chapter 1.

**Method for Extrapolating Data from Young to Older Infants**

This adjustment, the metabolic weight ratio method, involves metabolic scaling but does not adjust for growth because it is based on a value for a growing infant. To extrapolate from the AI for infants ages 0 through 6 months to an AI for infants ages 7 through 12 months, the following formula is used:

$$AI_{7-12\, \text{mo}} = AI_{0-6\, \text{mo}} \cdot F,$$

where $F = (\text{Weight}_{7-12\, \text{mo}}/\text{Weight}_{0-6\, \text{mo}})^{0.75}$.

**Methods for Determining Increased Needs for Pregnancy**

The placenta actively transports water-soluble vitamins (except biotin) and choline from the mother to the fetus against a concentration gradient (Hytten and Leitch, 1971; Zempleni et al., 1992). Placental transport of biotin is a passive process (Hu et al., 1994; Karl and Fisher, 1992; Schenker et al., 1993). For many of the B vitamins, experimental data that could be used to set an EAR and RDA or an AI for pregnancy are lacking. In these cases the potential for increased need for these nutrients during pregnancy is based on theoretical considerations, including obligatory fetal transfer, if data are available, and increased maternal needs related to increases in energy or protein metabolism, as applicable. With the possible exception of B$_{12}$, vitamin absorption does not appear to improve substantially during pregnancy. For choline, the AI is based on the increase in maternal weight.

**Methods to Determine Increased Needs for Lactation**

For the B vitamins and choline, it is assumed that the total requirement of lactating women equals the requirement for the non-pregnant, nonlactating woman of similar age plus an increment to cover the amount of the nutrient needed for milk production. To allow for inefficiencies in use of certain B vitamins, the increment may be somewhat greater than the amount of the nutrient contained in the milk produced. Details are provided in each nutrient chapter.
Substantial changes in methods have occurred during the 50 years of vitamin B studies considered in this report. Appendix E summarizes information about methods used to determine laboratory values related to B vitamin and choline status. Methodological problems have been documented for folate (see Chapter 8) and other nutrients (see Appendix E).

Interpretation of Results

Caution was used in the interpretation of study results. Some examples of points that were considered follow:

- The method of measurement of urinary excretion of the vitamin (e.g., fasting, random, or 24-hour specimens) introduces different types of errors. The use of creatinine corrections to allow for assay of random fasting urine samples rather than 24-hour collections may need to include considerations of differences in creatinine excretion by age.
- Depletion-repletion studies assess requirements by identifying intakes that return status indicators to the prestudy baseline values. Baseline values have been those of motivated healthy individuals on self-selected diets or on diets containing a recommended level. The assessed requirements based on this approach of returning values to baseline are invariably similar or higher than the baseline vitamin intake. This is addressed in more detail in Chapters 7 and 14.
- Some studies are too short to determine whether a tested level of nutrient intake will be sufficient to stabilize the laboratory test result at a lower but satisfactory value.
- Many laboratory values change during pregnancy (NRC, 1978) and the postpartum period (sometimes with differences between lactating and nonlactating women) (IOM, 1991). A decreased value does not necessarily mean that intake was inadequate.

Sensitivity and Specificity

The terms sensitivity and specificity each have different meanings when applied to laboratory tests as compared with public health applications. Analytic sensitivity is defined as the amount of a nutrient that results in a doubling of background blank in an assay. Analytic
**specificity** is the ability of the assay to discriminate the nutrient of interest from nutrients that might give false positive readings in the assay. In public health usage, sensitivity refers to the ability of a criterion (e.g., a laboratory test and its cutoff point) to identify the individuals who have a particular problem. Specificity, in turn, refers to the ability of a criterion to identify those who do not have the problem. In this report, the terms sensitivity and specificity reflect the public health usage unless preceded by the word *analytic*.

**ESTIMATES OF NUTRIENT INTAKE**

Reliable and valid methods of food composition analysis are crucial in determining the intake of a nutrient needed to meet a requirement. For several B vitamins and choline, analytic methods to determine the content of the nutrient in food have serious limitations (see Appendix E).

**Methodological Considerations**

The quality of nutrient intake data varies widely across studies. The most valid intake data are those collected from the metabolic study protocols in which all food is provided by the researchers, amounts consumed are accurately measured, and the nutrient composition of the food is determined by reliable and valid laboratory analyses. Such protocols are usually possible with only a small number of subjects. Thus, in many studies, intake data are self-reported (e.g., through 24-hour recalls of food intake, diet records, or food frequency questionnaires). Potential sources of error in self-reported intake data include over- or underreporting of portion sizes and frequency of intake, omission of foods, and inaccuracies related to the use of food composition tables. Therefore, the values reported by nationwide surveys or studies that rely on self-report may be somewhat inaccurate and possibly biased.

Food composition databases that are used to calculate nutrient intake from self-reported and observed intake data introduce errors due to random variability, genetic variation in the nutrient content, analytical errors, and missing or imputed data. In general, when nutrient intakes for groups are estimated, the effect of errors in the composition data is probably considerably smaller than the effect of errors in the self-reported intake data (NRC, 1986). However, it is not known to what extent this is true for folate, biotin, pantothenic acid, or choline (see Appendix E).
The accuracy of the food composition data for folate and vitamin B₁₂ is described as “conflicting” (LSRO/FASEB, 1995). Food composition data are not even routinely reported for pantothenic acid, biotin, and choline. Moreover, wide variation in the B vitamin content of similar cooked foods is likely because of susceptibility to cooking losses, especially losses when liquids in which food is cooked are not also consumed.

**Adjusting for Day-to-Day Variation**

Because of day-to-day variation in dietary intakes, the distribution of 1-day (or 2-day) intakes for a group is wider than the distribution of usual intakes even though the mean of the intakes may be the same (for further elaboration, see Chapter 13). To reduce this problem, statistical adjustments were developed (NRC, 1986; Nusser et al., 1996) that require at least 2 days of dietary data from a representative subsample of the population of interest. However, no accepted method is available to adjust for the underreporting of intake, which may average as much as 20 percent for energy (Mertz et al., 1991).

**DIETARY INTAKES IN THE UNITED STATES AND CANADA**

**Sources of Dietary Intake Data**

The major sources of current dietary intake data for the U.S. population are the Third National Health and Nutrition Examination Survey (NHANES III), which was conducted from 1988 to 1994 by the U.S. Department of Health and Human Services, and the Continuing Survey of Food Intakes by Individuals (CSFII), which was conducted by the U.S. Department of Agriculture (USDA). NHANES III examined 30,000 subjects aged 2 months and older. A single 24-hour diet recall was collected for all subjects. A second recall was collected for a 5 percent nonrandom subsample to allow adjustment of intake estimates for day-to-day variation. The 1994 to 1995 CSFII collected two nonconsecutive 24-hour recalls from approximately 5,600 subjects of all ages. Both surveys used the food composition database developed by USDA to calculate nutrient intakes (Perloff et al., 1990). National survey data for Canada are not currently available, but data have been collected from Québec and Nova Scotia. The extent to which these data are applicable nationwide is not known.

Additional data on nearly 700 free-living, elderly persons from the
B VITAMINS AND CHOLINE

Boston Nutritional Status Survey are provided in Appendix F. In this survey, 3-day diet records were kept by participants and subsequently checked by a qualified nutritionist.

Appendix G gives the mean and the first through ninety-ninth percentiles of dietary intakes of six of the B vitamins by age from the first phase of the CSFII, adjusted for day-to-day variation by the method of Nusser et al. (1996). Appendix H provides comparable information from NHANES III, adjusted by methods described by the National Research Council (NRC, 1986) and by Feinleib and colleagues (1993) for persons aged 6 years and older. (There were too few second dietary recalls to do the adjustment for the younger children.) Because food composition data are not readily available for pantothenic acid, biotin, and choline, neither of the U.S. national surveys has estimated intakes for these nutrients. Appendix I provides means and selected percentiles of dietary intakes of seven B vitamins for men and women in Québec and mean daily intake of thiamin, riboflavin, niacin, and folate for men and women in Nova Scotia.

Sources of Data on Supplement Intake

Although subjects in the CSFII were asked about the use of dietary supplements, quantitative information was not collected. Data on supplement intake obtained from NHANES III were reported as a part of total nutrient intake (Appendix H). NHANES III data on overall prevalence of supplement use are also available (LSRO/FASEB, 1995). In 1986, the National Health Interview Survey queried 11,558 adults and 1,877 children on their intake of supplements during the previous 2 weeks (Moss et al., 1989). The composition of the supplement was obtained directly from the product label whenever possible. Table 2-2 shows the percentage of adults, by age, taking at least one of the B vitamins.

Food Sources of Folate and Other B Vitamins

For six of the B vitamins, two types of information are provided about food sources of nutrients: identification of the foods that are the major contributors of the vitamin to diets in the United States and food sources of the nutrient. The determination of foods that are major contributors depends on both nutrient content of a food and the total consumption of the food (amount and frequency). Therefore, a food that has a relatively low concentration of the nutrient might still be a large contributor to total intake if that food
TABLE 2-2  Percentage of Persons Taking Vitamin Supplements, by Sex, Age, and Type of Vitamin Used: National Health Interview Survey, United States, 1986

<table>
<thead>
<tr>
<th>Vitamin Supplement Taken</th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤18 y</td>
<td>18–44 y</td>
<td>45–64 y</td>
<td>≥65 y</td>
</tr>
<tr>
<td>Thiamin</td>
<td>29.5</td>
<td>29.9</td>
<td>30.4</td>
<td>27.2</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>29.5</td>
<td>30.0</td>
<td>30.3</td>
<td>26.7</td>
</tr>
<tr>
<td>Niacin</td>
<td>29.3</td>
<td>29.7</td>
<td>30.3</td>
<td>26.4</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>29.7</td>
<td>30.2</td>
<td>30.5</td>
<td>27.4</td>
</tr>
<tr>
<td>Folate</td>
<td>26.0</td>
<td>27.3</td>
<td>25.8</td>
<td>22.2</td>
</tr>
<tr>
<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>29.3</td>
<td>29.8</td>
<td>29.9</td>
<td>26.9</td>
</tr>
<tr>
<td>Pantothenic Acid</td>
<td>24.9</td>
<td>25.4</td>
<td>25.3</td>
<td>22.9</td>
</tr>
<tr>
<td>Biotin</td>
<td>18.6</td>
<td>19.7</td>
<td>18.5</td>
<td>15.6</td>
</tr>
</tbody>
</table>

NOTE: The high use of supplements by pregnant women is not reflected in this table. SOURCE: Moss et al. (1989).

is consumed in relatively large amounts. Data from the 1995 CSFII were used to identify major contributors. In contrast, the food sources listed are those with the high concentrations of the nutrient; no consideration is given to the amount consumed. Both types of data were provided for this report by USDA (A. Moshfegh, Agricultural Research Service, USDA, personal communication, 1997).

SUMMARY

General methods for examining and interpreting the evidence on requirements for B vitamins and choline are presented in this chapter, with special attention given to infants, children, and pregnant and lactating women; methodological problems; and dietary intake data. Relevant detail is provided in the nutrient chapters.

REFERENCES

B VITAMINS AND CHOLINE

Males

<table>
<thead>
<tr>
<th>Gender</th>
<th>18–44 y</th>
<th>45–64 y</th>
<th>≥65 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤18 y</td>
<td>23.2</td>
<td>23.7</td>
<td>22.1</td>
</tr>
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<td>18–44 y</td>
<td>23.0</td>
<td>23.3</td>
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<td>19.1</td>
</tr>
<tr>
<td>15.8</td>
<td>16.4</td>
<td>14.7</td>
<td>15.1</td>
</tr>
</tbody>
</table>


Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline
http://www.nap.edu/catalog/6015.html


262:C302–C308.


91:1440–1449.

120:1530–1534.

