Serum Total Homocysteine Concentrations in the Third National Health and Nutrition Examination Survey (1991–1994): Population Reference Ranges and Contribution of Vitamin Status to High Serum Concentrations

Jacob Selhub, PhD; Paul F. Jacques, ScD; Irwin H. Rosenberg, MD; Gail Rogers, BA; Barbara A. Bowman, PhD; Elaine W. Gunter, MT; Jacqueline D. Wright, MPH; and Clifford L. Johnson, MSPH

Background: The concentration of circulating total homocysteine is a sensitive marker of inadequate folate and vitamin B₁₂ status. Elevated homocysteine concentrations are associated with an increased risk for vascular disease.

Objective: To identify reference ranges for serum total homocysteine concentration in U.S. residents and quantify the contribution of circulating vitamin concentrations to high homocysteine concentrations.

Design: Cross-sectional prevalence study.

Setting: United States.

Patients: A nationally representative sample of 3563 male participants and 4523 female participants 12 years of age or older who participated in the third National Health and Nutrition Examination Survey.

Measurements: Reference ranges (5th and 95th percentiles) for the total homocysteine concentration were defined among participants who were folate- and vitamin B₁₂–replete and had normal creatinine concentrations. A high total homocysteine concentration was defined as one that exceeded the sex-specific 95th percentile for the reference sample (participants 20 to 39 years of age). The population attributable risk percentage was calculated to determine the contribution of low folate (<11 nmol/L) and vitamin B₁₂ (<185 pmol/L) concentrations to a high homocysteine concentration.

Results: Reference ranges for serum total homocysteine concentration increased with age; these ranges were 4.3 to 9.9 μmol/L for male participants and 3.3 to 7.2 μmol/L for female participants 12 to 19 years of age and from 5.9 to 15.3 μmol/L for men and 4.9 to 11.6 μmol/L for women 60 years of age or older. A high homocysteine concentration was defined as at least 11.4 μmol/L for male participants and at least 10.4 μmol/L for female participants. Approximately two thirds of the cases of high homocysteine concentrations were associated with low vitamin concentrations.

Conclusions: Upper reference limits for the serum total homocysteine concentration increased with age and were higher for male participants than for female participants at all ages. In most cases, high homocysteine concentrations were associated with low serum vitamin concentrations.

Homocysteine, a non–protein-forming sulfur amino acid, has attracted attention because elevated concentrations of circulating total homocysteine are associated with an increased risk for vascular disease (1, 2). Homocysteine is also a sensitive functional marker of inadequate cellular folate and vitamin B₁₂ concentrations (3). Inadequate status of these vitamins has important health consequences that may be independent of their role in homocysteine metabolism. Low folate concentrations increase a woman’s risk for having a baby with a neural tube defect (4, 5), and an inadequate vitamin B₁₂ concentration is known to produce various neurologic and cognitive effects (6, 7).

Persons with low circulating folate or vitamin B₁₂ concentrations have higher fasting total homocysteine concentrations (8–10), and elevated fasting total homocysteine concentrations are usually normalized by treatment with folic acid and vitamin B₁₂ (6, 11–14). However, less is known about the importance of these vitamins as risk factors for high homocysteine concentration in the general population. Only three studies have examined the relation between homocysteine concentration and its vitamin determinants in samples that were designed to be representative of U.S. national (8) or regional (9, 10) populations. One of these studies (9) reported that approximately two thirds of all cases of moderately elevated total homocysteine concentrations were potentially attributable to low vitamin concentrations, but estimation of the proportion of cases with high homocysteine concentrations that can be attributed to inadequate vitamin status is complicated by the lack of a standard definition of a high total homocysteine concentration. In the absence of a definition based on increased risk for an adverse health outcome, such as vascular disease, upper reference limits from samples of healthy persons without established risk factors for high homocysteine

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For author affiliations and current addresses, see end of text.
concentrations have been used to define a high total homocysteine concentration (10, 15–17).

We previously described the distribution of total serum homocysteine concentrations in participants 12 years of age or older from the third National Health and Nutrition Examination Survey (NHANES III), a population-based sample of U.S. residents (18). These data present a unique opportunity to develop population reference ranges for serum total homocysteine concentration and to determine the extent to which elevated homocysteine concentrations are associated with low circulating vitamin concentrations in a representative sample of U.S. residents.

### Methods

#### Participants

The NHANES III was developed to obtain nationally representative information on the health and nutritional status of the civilian, noninstitutionalized U.S. population (19, 20). Homocysteine concentrations were measured as part of an NHANES III surplus sera project on serum samples from participants 12 years of age or older who were seen during phase II of this survey (1991–1994). This project is described in greater detail elsewhere (18). Homocysteine concentrations were measured at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University by using the high-performance liquid chromatography method of Araki and Sako (21). The interassay coefficient of variation for this assay was 6%. Folate and vitamin B12 values were determined for phase 2 specimens at the Centers for Disease Control and Prevention central laboratory by using a Quanta Phase II radioassay kit (Bio-Rad Laboratories, Hercules, California), and analyses are described in detail in the NHANES III Laboratory Procedures Manual (22). The coefficients of variation for folate and vitamin B12 were 6% and 7%, respectively.

Informed consent was obtained from all respondents. The NHANES III protocol was approved by the National Center for Health Statistics NHANES Institutional Review Board, and measurement of serum homocysteine was approved by the Human Investigations Review Committee at the New England Medical Center.

We used the following search strategy, combining Medical Subject Headings (MeSH terms) and text words, to identify all population-based studies relating vitamin status to circulating homocysteine concentrations: ((homocysteine [MeSH] OR homocysteine [Text Word]) AND (vitamins [MeSH] OR vitamin [Text Word])) AND (epidemiologic studies [MeSH] OR data collection [MeSH] OR survey [Text Word]). This search identified 137 citations, of which 16 were reviews. We selected original studies that 1) were designed to be representative of national, regional, or local populations and 2) described the relation between circulating homocysteine concentrations and either intake or circulating concentrations of folate or vitamin B12. As of 1 March 1999, 3 articles met our criteria (8–10).

#### Statistical Analysis

We used sample weights in analyses to account for unequal probability of selection and nonresponse and to produce estimates of means and percentiles that were representative of the noninstitutionalized, civilian U.S. population. We used SUDAAN statistical software (23) to account for the complex survey design in the variance estimates.

Because total homocysteine, folate, and vitamin B12 values were skewed, logarithmic transformations were applied. To show the relations between total homocysteine concentrations and vitamin concentrations, we classified participants into age- and sex-specific vitamin decile categories and estimated the geometric mean of the serum total homocysteine concentration within each decile. Analyses were adjusted for ethnicity and serum creatinine concentration. In addition, the relation between total homocysteine and folate concentrations was adjusted for vitamin B12 concentrations, and the relation be-

| Characteristic       | Male Participants | Female Participants |
|----------------------|-------------------|---------------------|
|                      | Non-Hispanic White | Non-Hispanic Black  | Mexican American | Non-Hispanic White | Non-Hispanic Black  | Mexican American |
| Participants, n      | 1227              | 1139                | 1197             | 1731              | 1543                | 1249              |
| Age, y               | 40.8 (39.0–42.6)   | 35.9 (34.5–37.2)    | 33.2 (32.3–34.2) | 43.4 (41.7–45.0)  | 37.4 (36.5–38.4)    | 33.6 (32.4–34.9)  |
| Total homocysteine, μmol/L† | 9.6 (9.3–9.9)     | 9.5 (9.3–9.8)       | 9.0 (8.7–9.4)    | 8.1 (7.9–8.3)     | 8.0 (7.8–8.2)       | 7.0 (6.8–7.3)     |
| Folate concentration, nmol/L† | 12.8 (11.6–14.1)  | 10.2 (9.7–10.7)     | 10.9 (10.1–11.7) | 14.6 (13.5–15.8)  | 10.7 (10.2–11.3)    | 11.7 (10.7–12.9)  |
| Vitamin B12 concentration, pmol/L† | 313 (305–321)     | 385 (374–396)       | 336 (320–352)    | 315 (307–313)     | 391 (384–399)       | 351 (336–366)     |
| Creatinine concentration, μmol/L† | 100.8 (99.2–102.3) | 107.6 (105.9–109.4) | 95.1 (94.0–96.2) | 85.0 (84.1–85.9)  | 86.4 (84.9–87.9)    | 75.4 (74.1–76.7)  |

* Unless otherwise indicated, values are given as the mean (95% CI).
† Geometric mean.
between total homocysteine and vitamin B₁₂ was adjusted for folate concentrations. We tested the associations between homocysteine and vitamins for interactions with age, sex, and ethnicity. We tested for trend of total homocysteine concentration across vitamin concentrations by using linear regression with the logarithm of the continuous vitamin concentration as the independent variable, adjusting as described above. We showed the trend by using the SYSTAT LOWESS procedure to fit smoothed curves (24) to the geometric mean total homocysteine concentrations in the vitamin decile categories (25).

It has been suggested that population reference ranges for the total homocysteine concentration be established in samples of persons without established risk factors for a high homocysteine concentration (10, 15–17). For our reference sample, we included persons whom we assumed to be folate- and vitamin B₁₂-replete (that is, their serum concentrations of both vitamins were above the 50th percentile) and had normal serum creatinine concentrations (<90 μmol/L for women and <110 μmol/L for men). Pregnant women were excluded. We used the 5th and the 95th percentiles from the reference sample to estimate population reference ranges.

To identify the potential impact of low vitamin concentrations on high total homocysteine concentration, we needed to establish values for high total homocysteine and low vitamin concentrations. We used the sex-specific 95th percentiles in the participants 20 to 39 years of age (the reference sample) to define high total homocysteine concentrations for all age groups. We defined low vitamin concentrations as a folate concentration less than 11 nmol/L (26, 27) and a vitamin B₁₂ concentration less than 185 pmol/L (28, 29). We calculated the prevalence of high total homocysteine concentration; the prevalence ratio for high total homocysteine concentration; the attributable risk percentage; and the population attributable risk percentage for persons with low concentrations of folate, vitamin B₁₂, or both compared with persons who had adequate concentrations of both of these vitamins. The attributable risk percentage estimates the excess cases of high homocysteine concentrations among persons with low vitamin concentrations, whereas the population attributable risk percentage takes into account the prevalence of low vitamin concentrations in the population and estimates the excess of high homocysteine concentrations associated with low vitamin concentrations in the entire population.

We used the design effect for total homocysteine concentration, which is the ratio of the complex sampling design variance derived by using SUDAAN software (23) to the simple random sample variance calculated by using SAS software (30), to determine the recommended minimum sample size needed to achieve stable estimates of means, proportions, and percentiles according to the National Center for Health Statistics analytic guidelines (19). On the basis of an average design effect of approximately 1.4 for our sample, means and medians derived from fewer than 42 participants, 10th and 90th percentiles derived from fewer than 112 participants, and 5th and 95th percentiles derived from fewer

| Variable | Percentile | 10th | 20th | 30th | 40th | 50th | 60th | 70th | 80th | 90th |
|----------|------------|------|------|------|------|------|------|------|------|------|
| Folate concentration, nmol/L | Male participants | 12–19 y | 5.7 | 7.3 | 8.4 | 9.5 | 11.1 | 12.9 | 15.4 | 17.9 | 22.9 |
| | | 20–39 y | 5.2 | 6.3 | 7.5 | 8.4 | 9.5 | 10.9 | 12.5 | 15.0 | 19.5 |
| | | 40–59 y | 5.7 | 6.8 | 7.9 | 9.1 | 10.7 | 12.5 | 14.7 | 17.9 | 23.8 |
| | | ≥60 y | 6.8 | 8.6 | 10.4 | 12.2 | 14.3 | 17.0 | 20.8 | 26.1 | 37.2 |
| | Female participants | 12–19 y | 5.9 | 7.5 | 8.6 | 9.7 | 11.1 | 12.9 | 15.4 | 18.4 | 24.0 |
| | | 20–39 y | 5.4 | 6.6 | 7.9 | 9.1 | 10.7 | 12.5 | 15.0 | 18.4 | 25.2 |
| | | 40–59 y | 6.1 | 7.5 | 8.6 | 10.0 | 11.3 | 13.6 | 16.8 | 22.0 | 29.5 |
| | | ≥60 y | 7.5 | 9.1 | 11.1 | 13.1 | 15.9 | 19.5 | 24.7 | 33.8 | 46.9 |
| Vitamin B₁₂ concentration, pmol/L | Male participants | 12–19 y | 246 | 271 | 307 | 342 | 374 | 409 | 454 | 509 | 591 |
| | | 20–39 y | 224 | 257 | 291 | 318 | 350 | 385 | 427 | 477 | 556 |
| | | 40–59 y | 204 | 246 | 282 | 305 | 331 | 358 | 394 | 460 | 556 |
| | | ≥60 y | 177 | 213 | 244 | 274 | 309 | 347 | 395 | 457 | 557 |
| | Female participants | 12–19 y | 235 | 277 | 309 | 352 | 396 | 436 | 490 | 549 | 639 |
| | | 20–39 y | 201 | 240 | 268 | 303 | 337 | 372 | 415 | 468 | 562 |
| | | 40–59 y | 212 | 257 | 291 | 324 | 357 | 398 | 451 | 502 | 596 |
| | | ≥60 y | 186 | 225 | 258 | 297 | 339 | 382 | 444 | 506 | 623 |

Table 2. Percentile Values Used To Define Decile Categories of Serum Folate and Vitamin B₁₂ Concentrations
than 224 participants were deemed unstable. Sample size for stable estimates of the proportions varied by the magnitude of the proportion, ranging from 42 for proportions of 0.5 to 224 for proportions of 0.05 or 0.95. We indicate in the text and tables statistics that did not meet the appropriate sample size.

We categorized participants into three ethnic groups: non-Hispanic white, non-Hispanic black, and Mexican American. We excluded persons from other ethnic groups (n = 436) because their inclusion produced unstable estimates of mean total homocysteine concentration after adjustment for ethnicity. Our analyses are based on 8086 participants with complete data on serum total homocysteine, folate, vitamin B12, and creatinine concentrations.

**Results**

Table 1 shows selected characteristics of the sample by sex and ethnic group. On average, non-Hispanic white participants were the oldest and Mexican American participants were the youngest. Except among Mexican Americans, female participants were older than male participants. Serum homocysteine concentrations were higher in male participants than in female participants in all ethnic groups. They were highest for non-Hispanic black male participants and lowest for Mexican American male participants and were lower for Mexican American female participants than for non-Hispanic female participants.

**Geometric Mean of the Serum Total Homocysteine Level by Vitamin Decile Category**

Table 2 shows the age- and sex-specific vitamin deciles used to define the categories shown in Figures 1 and 2.

**Folate**

Figure 1 shows the relation between serum total homocysteine and folate concentrations. A strong inverse association was seen at all ages in male participants and female participants (P for trend < 0.001). The magnitude of the difference in homocysteine concentrations from the lowest to the highest folate decile varied slightly by age and sex. These differences ranged from 6.2 μmol/L in male participants 20 to 39 years of age to 3.5 μmol/L in female participants 12 to 19 years of age. However, no statistically significant interactions were seen, which we interpret to mean that the strength of the relation between total homocysteine and folate concentrations did not vary by age, sex, or ethnic group.

**Vitamin B12**

The relation between total homocysteine and vitamin B12 concentrations, shown in Figure 2, was weaker than the relation between folate and total homocysteine concentrations. Higher geometric mean
homocysteine concentrations were confined to vitamin B₁₂ concentrations less than approximately 250 pmol/L. The inverse association between homocysteine and vitamin B₁₂ concentrations was statistically significant in each age and sex category, but the magnitude of the difference in homocysteine concentrations across the vitamin B₁₂ decile categories was generally small. The difference between homocysteine concentrations in the lowest and highest vitamin B₁₂ categories ranged from 0.7 to 3.7 µmol/L in all age and sex groups, except for male participants 60 years of age or older, in whom the difference was 5.3 µmol/L. Despite the range of observed differences, no statistically significant interactions with age, sex, or ethnicity were observed, indicating that the relation between serum total homocysteine and vitamin B₁₂ concentrations was similar in these population subgroups.

Population Reference Ranges for Serum Total Homocysteine Concentration

The 5th and 95th percentiles for the total homocysteine concentration in the reference sample are presented as reference ranges (Table 3). We also included the 10th and 90th percentiles because the numbers of reference patients in some age and sex groups were not large enough to meet the criterion for stability of the 5th and 95th percentiles. The percentile values were consistently greater in male participants, although the range between the upper and lower percentiles was generally similar in participants of either sex. The ranges also tended to increase slightly with age regardless of sex.

Prevalence of High Homocysteine Concentration and Relation to Vitamin Concentrations

The prevalence of high total homocysteine concentrations, shown in Table 4, was based on the sex-specific 95th percentile values for men and women in the reference sample (participants 20 to 39 years of age) (Table 3). The prevalence of high total homocysteine concentration increased with age, but it was greater than 10% in all age categories except female participants 12 to 19 years of age. Even among male participants 12 to 19 years of age, the prevalence was twofold greater than that in the reference sample.

Table 5 shows the mean total homocysteine concentration, the prevalence and prevalence odds ratio for high total homocysteine concentration, attributable risk percentage, and population attributable risk percentage for persons with low concentrations of folate and vitamin B₁₂ compared with persons who have adequate concentrations of both vitamins. These measures were adjusted for ethnic group and serum creatinine concentration, as well as for age and sex when appropriate. The mean total homocysteine concentration and prevalence of high total homocysteine concentration was greatest for both men and women who had low concentrations of folate and vitamin B₁₂ (P < 0.001 compared with persons who had adequate concentrations of both vitamins). The total population attributable risk for high homocysteine concentration associated with low concentrations of either vitamin was 62.9% in male participants and 66.4% in female participants, indicating that almost two thirds of cases of high total homocysteine concentrations in the United States were due to low concentrations of folate and vitamin B₁₂.
States are associated with low concentrations of folate, vitamin B₁₂, or both. However, a low vitamin B₁₂ concentration alone contributed little to the prevalence of high total homocysteine concentrations in the entire population, as evidenced by its small contribution to the population attributable risk. The total population attributable risk associated with low concentrations of either vitamin decreased from approximately 75% in persons younger than 40 years of age to about 31% for persons 60 years of age or older.

**Discussion**

Our study describes the quantitative importance of folate and vitamin B₁₂ concentrations as determinants of total homocysteine concentration in a nationally representative sample of healthy adolescents and adults in the United States. The analyses demonstrate the importance of folate and vitamin B₁₂ as determinants of total homocysteine concentrations at the population level. More than 60% of the high total homocysteine concentrations in this sample were linked to low folate concentrations (with or without low vitamin B₁₂ concentrations). The important role of vitamin B₁₂ in homocysteine metabolism is indicated by the high attributable risk percentage among persons with low vitamin B₁₂ concentrations. However, vitamin B₁₂ concentration is much less important than folate concentration in the general population as a cause of high total homocysteine concentration because low vitamin B₁₂ concentrations are less prevalent.

Age seems to influence the association between high homocysteine and vitamin concentrations. Among persons 12 to 39 years of age, approximately 75% of the cases of high homocysteine concentrations were associated with low folate or vitamin B₁₂ concentrations. This decreased to about 30% of cases among persons 60 years of age or older. This finding occurred because of a decreased contribution of low folate concentration, but not low vitamin B₁₂ concentration, to high homocysteine concentrations in older persons. The age difference is probably explained by increased prevalence of low vitamin B₁₂ concentrations with age along with the accrual with age of other risk factors for high total homocysteine, such as declining renal function, a critical factor in homocysteine metabolism (1, 31, 32) and, possibly, estrogen status in women (1, 33). Little is known about other factors that affect homocysteine metabolism at older ages.

Our results confirm the well-established role of folate and vitamin B₁₂ in homocysteine metabolism (6, 8–14). Homocysteine is formed during metabolism of methionine at the intersection of two metabolic pathways (3). In a reaction catalyzed by a vitamin B₁₂-dependent methyltransferase, homocysteine can acquire a methyl group from methyltetrahydrofolate to reconstitute methionine. Excess homocysteine that is not required for the regeneration of methionine is catabolized to cysteine by a pathway containing two vitamin B₆-dependent enzymes.

Our findings are consistent with those of three earlier studies that considered these relations in representative population samples from Europe and the United States with more limited age ranges. Bates and colleagues (8) reported strong inverse associations among plasma homocysteine concentrations, serum folate and vitamin B₁₂ concentrations, and folate intake in a representative sample of 972 British men and women 65 years of age or older from the National Diet and Nutrition Survey. In the Hordaland Homocysteine Study (10), which was

| Participants | Sample | Total Homocysteine Concentration | 5th Percentile | 10th Percentile | 90th Percentile | 95th Percentile |
|--------------|--------|---------------------------------|---------------|----------------|----------------|----------------|
| Male participants | 12–19 y | 167 | 4.3† | 4.7 | 8.9 | 9.9† |
| 20–39 y | 248 | 5.2 | 5.5 | 10.2 | 11.4† |
| 40–59 y | 158 | 5.7† | 6.1 | 11.9 | 12.9† |
| ≥60 y | 156 | 5.9† | 6.3 | 12.3 | 15.3† |
| Female participants | 12–19 y | 183 | 3.3† | 3.4 | 6.9 | 7.2† |
| 20–39 y | 358 | 4.1 | 4.5 | 8.5 | 10.4† |
| 40–59 y | 229 | 4.1 | 4.4 | 9.1 | 10.2 |
| ≥60 y | 185 | 4.9† | 5.3 | 10.3 | 11.6† |

* The reference sample consisted of participants with apparently replete folate and vitamin B₁₂ concentrations (≥50th percentile) and with serum creatinine concentrations < 110 μmol/L for male participants and < 90 μmol/L for female participants. Pregnant women were excluded from the reference sample.
† Did not meet sample size criteria (n = 224) for stability of estimated 5th and 95th percentiles.
‡ The 95th percentile values from men and women 20 to 39 years of age were used to define high total serum homocysteine concentrations for all age categories.

**Table 4. Prevalence of High Serum Total Homocysteine Concentration by Sex and Age**

| Participants | Sample | Participants with High Total Homocysteine Concentration | n | %* |
|--------------|--------|--------------------------------------------------------|---|----|
| Male participants | 12–19 y | 603 | 10.5 |
| 20–39 y | 1206 | 21.0 |
| 40–59 y | 768 | 28.6 |
| ≥60 y | 986 | 43.2 |
| Female participants | 12–19 y | 706 | 7.9 |
| 20–39 y | 1660 | 16.4 |
| 40–59 y | 1021 | 21.1 |
| ≥60 y | 1136 | 46.5 |

* Defined as > 11.4 μmol/L for male participants and > 10.4 μmol/L for female participants.
based on a representative sample of residents of Hordaland County, Norway, selected as part of a national cardiovascular disease risk survey, investigators observed a strong inverse association between folate intake scores and plasma total homocysteine concentrations in 11,941 men and women 40 to 67 years of age with no history of vascular disease, diabetes, or hypertension. In 1,160 members of the original Framingham Heart Study cohort (67 to 96 years of age), which was originally designed as a representative sample of the adult population of Framingham, Massachusetts, total plasma homocysteine concentrations were inversely correlated with plasma folate and vitamin B12 concentrations and folate intake (9). This latter study also reported that approximately two thirds of patients with moderately elevated total homocysteine concentrations (≥14 μmol/L) were associated with low plasma concentrations of folate, vitamin B12, or vitamin B6.

The homocysteine data from NHANES III present a unique opportunity to establish population reference ranges in a nationally representative sample of Americans. Investigators have recommended that reference ranges for total homocysteine be established in populations with apparently adequate vitamin status (10, 15–17). Previously published reference ranges were based on various samples. Upper reference limits based on the 97.5th percentile from a population-based sample of Norwegians were 12.6 μmol/L in women and 14.5 μmol/L in men 40 to 42 years of age, and 16.7 μmol/L in women and 17.8 μmol/L in men 65 to 67 years of age (10). Other investigators calculated 95% reference intervals as the mean total homocysteine ± 2 SDs from smaller convenience samples (15–17). The upper limit of the 95% reference interval calculated in this manner is an estimate of the 97.5th percentile if the data are normally distributed. Ubbink and colleagues (15) suggested a reference range of 4.9 to 11.7 μmol/L, which was calculated from the

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### Table 5. Mean and Prevalence of High Serum Total Homocysteine Concentrations Associated with Low Serum Vitamin Status

| Participants | Low Vitamin Concentration* | Sample n | Total Homocysteine Concentration μmol/L | Participants with High Total Homocysteine Concentration† | Prevalence Odds Ratio | Attributable Risk % | Population Attributable Risk % |
|--------------|----------------------------|----------|----------------------------------------|----------------------------------------------------------|----------------------|----------------|--------------------------|
| Men          | Yes Yes                    | 136      | 13.5†                                  | 50.2†                                                   | 5.9                  | 82.9          | 6.9                      |
|              | Yes No                     | 1,617    | 10.7†                                  | 35.9†                                                   | 4.2                  | 76.1          | 5.3                      |
|              | No Yes                     | 89       | 11.0†                                  | 28.8†                                                   | 3.4                  | 70.2          | 2.2                      |
|              | No No                      | 1,721    | 8.1                                    | 8.6                                                     | 1                    | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |
| Female       | Yes Yes                    | 154      | 12.2†                                  | 68.6†                                                   | 10.7                 | 90.6          | 11.1                     |
|              | Yes No                     | 1,877    | 9.0†                                   | 30.9†                                                   | 4.8                  | 79.2          | 5.3                      |
|              | No Yes                     | 142      | 8.1†                                   | 19.2†                                                   | 3.0                  | 66.6          | 2.1                      |
|              | No No                      | 2,350    | 6.7                                    | 6.4                                                     | 1                    | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |
| 12–19 y      | Yes Yes                    | 21       | 16.0†                                  | 86.3†                                                   | 46.5                 | 97.9          | 18.1                     |
|              | Yes No                     | 624      | 8.1†                                   | 10.9†                                                   | 5.9                  | 83.0          | 5.3                      |
|              | No Yes                     | 7        | 7.3                                    | 0.1†                                                   | –                    | –             | –                        |
|              | No No                      | 657      | 6.2                                    | 2.0                                                     | 1                    | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |
| 20–39 y      | Yes Yes                    | 104      | 11.6†                                  | 50.5†                                                   | 10.2                 | 90.2          | 8.1                      |
|              | Yes No                     | 1,493    | 9.3†                                   | 31.5†                                                   | 6.4                  | 84.3          | 67.6                     |
|              | No Yes                     | 54       | 7.0                                    | 5.9                                                     | 1.2                  | 16.5          | 0.1                      |
|              | No No                      | 1,215    | 6.8                                    | 5.0                                                     | 1.0                  | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |
| 40–59 y      | Yes Yes                    | 59       | 13.4†                                  | 68.4†                                                   | 6.5                  | 84.7          | 7.4                      |
|              | Yes No                     | 824      | 9.7†                                   | 38.8†                                                   | 3.7                  | 73.0          | 50.2                     |
|              | No Yes                     | 42       | 9.3†                                   | 33.1                                                   | 3.2                  | 68.3          | 2.0                      |
|              | No No                      | 864      | 7.4                                    | 10.5                                                   | 1.0                  | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |
| 60–69 y      | Yes Yes                    | 106      | 14.9†                                  | 80.4†                                                   | 2.5                  | 59.7          | 5.1                      |
|              | Yes No                     | 553      | 12.3†                                  | 71.5†                                                   | 2.2                  | 54.7          | 21.6                     |
|              | No Yes                     | 128      | 13.5†                                  | 67.4†                                                   | 2.1                  | 52.0          | 4.5                      |
|              | No No                      | 1,335    | 9.5                                    | 32.4                                                   | 1.0                  | –             | –                        |
| Total‡       |                            |          |                                        |                                                         |                      |               |                          |

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* Defined as ≤ 11 nmol/L for folate and ≤ 185 pmol/L for vitamin B12.
† Defined as > 11.4 μmol/L for male participants and > 10.4 μmol/L for female participants. Means and prevalences were adjusted for sex or age, ethnic group, and creatinine concentration.
‡ P < 0.001 for the difference between participants with adequate folate and vitamin B12 concentrations and those with other vitamin status.
§ Population attributable risk percentage for any low vitamin concentration.
| P < 0.01 for the difference between participants with adequate folate and vitamin B12 concentrations and those with other vitamin status.
expected effects of vitamin supplementation in South African men 18 to 65 years of age. Rasmussen and coworkers (16) provide age-specific reference ranges based on vitamin-replete Danish men and women: 4.6 to 8.1 μmol/L for men and women 20 to 29 years of age, 4.5 to 7.9 μmol/L for women and 6.3 to 11.2 μmol/L for men 30 to 59 years of age, and 5.8 to 11.9 μmol/L for men and women 60 years of age or older. Joosten and colleagues (17) defined a reference range of 5.3 to 12.7 μmol/L for vitamin-supplemented Belgian, German, and Dutch persons 65 to 96 years of age.

Our study has some limitations. First, despite the size of the study, we did not have sufficient numbers of patients in the reference sample to calculate stable estimates of the 2.5th and 97.5th percentiles. Instead, we used the 5th and 95th percentiles for establishing reference ranges, although some of our age- and sex-specific reference groups were not even large enough to meet the recommended criteria for stability for these less extreme percentiles.

Second, the fact that the NHANES homocysteine data are derived from a representative U.S. sample allowed us to estimate the contribution of low vitamin status to high homocysteine concentrations in the United States. However, our ability to estimate this contribution by using population attributable risk is limited by the dependence of this statistic on the definition of high total homocysteine concentration, for which no generally accepted definition is available. Ideally, the definition of a high circulating homocysteine concentration should be based on some health outcome, such as risk for vascular disease. However, a threshold total homocysteine concentration associated with increased risk for vascular disease has not been clearly established. Most studies suggest that homocysteine concentrations of 10 to 12.5 μmol/L or higher are associated with significantly increased risk for vascular disease (34–36), and Malinow and colleagues (37) reported an elevated risk for thickening of the carotid artery intima media starting at total homocysteine concentrations as low as 8.3 μmol/L. Lacking any standard definition based on disease risk, we used the upper reference limit from vitamin-replete young adults to define high total homocysteine concentration. Even though the strategy that we used to define high homocysteine was not based on vascular disease risk, it is unlikely that our estimates of population attributable risk for high homocysteine associated with low folate and vitamin B_{12} would be altered substantially if a strategy based on risk was used to define high homocysteine concentration; our cut-off values (11.4 μmol/L for men and 10.4 μmol/L for women) fall within the range of values associated with increased risk for vascular disease (34–37).

Third, our estimates of population attributable risk are dependent on our definitions of low vitamin concentrations. The values we used to define “low”—less than 11 nmol/L for folate and less than 185 pmol/L for vitamin B_{12}—are not those commonly used to define deficiency. We used the designation of “low” to include not only vitamin-deficient persons but also those whose vitamin concentrations fall in the range between apparently deficient and apparently adequate (26–29). There is metabolic evidence of insufficient vitamin status (9, 29), as well as evidence of clinical abnormalities (28), within this range. Our cut-off value identified almost half of the U.S. population as having low circulating folate concentrations. The data are consistent with the information from the NHANES III sample on folate intake, which indicated that 50% of the U.S. population had folate intakes well below the recommended dietary allowance (38).

Finally, the use of nonfasting serum samples may have affected the observed relation between homocysteine and its vitamin determinants (15% of the samples were from persons who fasted <6 hours), although homocysteine and vitamin concentrations in NHANES III were not significantly affected by length of fast (18, 39).

In conclusion, these data from NHANES III show that folate and, to a lesser extent, vitamin B_{12} concentrations in a large segment of adolescents and adults in the United States from 1991 to 1994 were not sufficient to prevent high total homocysteine concentrations. However, the role of folate as the principal determinant of high homocysteine concentrations in this population may be changing. Since the completion of this survey, the U.S. Food and Drug Administration published a regulation that all enriched grain products be fortified with folic acid by January 1998 (40). The first report on the effect of folic acid fortification indicated a 92% reduction in the prevalence of circulating folate concentrations less than 7 nmol/L and a 48% reduction in prevalence of homocysteine concentrations greater than 13 μmol/L (41). Comparison of the NHANES III data with those from similar surveys conducted after full implementation of folic acid fortification of grain products will be required to assess the impact of fortification on total homocysteine concentrations.

From Tufts University, Boston, Massachusetts; and Centers for Disease Control and Prevention, Hyattsville, Maryland, and Atlanta, Georgia.

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Requests for Reprints: Jacob Selhub, PhD, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111.

Current Author Addresses: Drs. Selhub, Jacques, and Rosenberg and Ms. Rogers: Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111.

Dr. Bowman: Division of Nutrition and Physical Activity, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, 4770 Buford Highway, MS K261, Atlanta, GA 30341.

Ms. Gunter: Division of Environmental Heart Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Highway, Atlanta, GA 30341.

Ms. Wright and Mr. Johnson: Division of Health Examination Statistics, National Center for Health Statistics, Centers for Disease Control and Prevention, 6525 Belcrest Road, Hyattsville, MD 20782.

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