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Effects of Folic Acid Supplementation on Hearing in Older Adults

A Randomized, Controlled Trial

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Background: Age-related hearing loss is a common chronic condition of elderly persons. Low folate status has been associated with poor hearing.

Objective: To determine whether folic acid supplementation slows age-related hearing loss.

Design: Double-blind, randomized, placebo-controlled trial conducted from September 2000 to December 2004.

Setting: The Netherlands.

Participants: 728 older men and women recruited from municipal and blood bank registries with plasma total homocysteine concentrations 13 μmol/L or greater serum and vitamin B_{12} concentrations 200 pmol/L or greater at screening, and no middle ear dysfunction, unilateral hearing loss, or pathologic ear conditions unrelated to aging.

Intervention: Daily oral folic acid (800 μg) or placebo supplementation for 3 years.

Measurements: 3-year change in hearing thresholds, assessed as the average of the pure-tone air conduction thresholds of both ears of the low (0.5-kHz, 1-kHz, and 2-kHz) and high (4-kHz, 6-kHz, and 8-kHz) frequencies.

Results: Initial median hearing thresholds were 11.7 dB (interquartile range, 7.5 to 17.5 dB) for low frequencies and 34.2 dB (interquartile range, 22.5 to 50.0 dB) for high frequencies. Sixteen participants (2%) were lost to follow-up. After 3 years, thresholds of the low frequencies increased by 1.0 dB (95% CI, 0.6 to 1.4 dB) in the folic acid group and by 1.7 dB (CI, 1.3 to 2.1 dB) in the placebo group (difference, −0.7 dB [CI, −1.2 to −0.1 dB]; $P = 0.020$). Folic acid supplementation did not affect the decline in hearing high frequencies.

Limitations: The strict criterion for participation on the basis of serum homocysteine concentrations limits extrapolation to the general population. Folic acid fortification of food was prohibited in the Netherlands during the study, so baseline folate levels in participants were about half of those found in the U.S. population.

Conclusions: Folic acid supplementation slowed the decline in hearing of the speech frequencies associated with aging in a population from a country without folic acid fortification of food. The effect requires confirmation, especially in populations from countries with folic acid fortification programs.

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Hearing loss is one of the most common chronic conditions of elderly persons, and age-related hearing loss is the most common type of hearing impairment (1). Age-related hearing loss refers to a sensorineural hearing loss due to aging and other physiologic, environmental, and pathologic processes that occur throughout the lifespan (2). Factors that vary with age, such as the increase in plasma total homocysteine concentrations, may play an etiologic role in age-related hearing loss. Folate is an important dietary determinant of plasma total homocysteine concentrations (3, 4), and folic acid supplementation can lower those levels by approximately 25% (5).

Recent research has focused on the link between folate metabolism and sensorineural hearing loss (6–13). Two studies have examined the link between folate and age-related hearing loss. The first epidemiologic study found that high concentrations of erythrocyte folate ($r = -0.37$; $P = 0.01$) and serum folate ($r = -0.36$; $P = 0.01$) were associated with lower hearing thresholds and, thus, with better hearing in 55 women who had age-related hearing loss (7). The explanations given by the authors included the role of folate-related homocysteine lowering in cellular metabolism or in the nervous and vascular systems. However, the association of increased folate concentrations with better hearing was not confirmed in a second epidemiologic study of 91 audiologic patients with suspected age-related hearing loss (8).

We investigated whether daily oral folic acid supplementation (800 μg) for 3 years improved hearing thresholds compared with placebo in 728 older adults with age-related hearing loss.

METHODS

Participants

The study participants were 819 men and postmenopausal women 50 to 70 years of age from the Gelderland province in the Netherlands who were participating in the

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Context

Epidemiologic evidence suggests an association between folate and homocysteine levels and hearing status.

Contributions

The investigators randomly assigned 728 older adults with high homocysteine levels to receive daily folic acid or placebo for 3 years. They found that hearing thresholds for low frequencies increased more slowly in participants taking folic acid, suggesting a protective effect.

Cautions

The trial was conducted in the Netherlands at a time when folate fortification of food was prohibited.

Implications

Folic acid supplementation seemed to slow the decline of low-frequency hearing in folate-deficient, older adults.

—The Editors

Folic Acid and Carotid Intima-media Thickness trial, a study investigating whether folic acid supplementation retards atherosclerotic progression. Additional outcomes were age-related decline in cognitive function and hearing. We recruited participants by using municipal and local blood bank registries.

Assuming that high concentrations of plasma total homocysteine were risk factors, we selected participants who were expected to benefit from the homocysteine-lowering effect of folic acid. We excluded participants with plasma total homocysteine levels less than 13 μmol/L (73rd percentile of those screened) or greater than 26 μmol/L. We also excluded participants with elevated homocysteine concentrations that were possibly due to factors other than suboptimal folate concentrations, including serum vitamin B_{12} concentration less than 200 pmol/L (10th percentile of those screened [vitamin B_{12} concentrations >160 pmol/L indicated deficiency]), self-reported diagnosis of renal or thyroid disease, or self-reported use of medications that influence folate metabolism (14). In addition, we excluded participants with self-reported intestinal disease or terminal cancer and participants who reportedly used vitamin B supplements or medications that may influence atherosclerotic progression. In addition to using the eligibility criteria related to homocysteine metabolism, we excluded participants with hearing problems that were unlikely to be due to age-related hearing loss. Middle ear dysfunction and unilateral hearing loss are pathologic ear conditions that are not related to aging. We excluded 91 participants because of middle ear dysfunction (defined as air–bone gap ≥15 dB on the audiogram in either ear) or unilateral hearing loss (≥20-dB difference in average pure-tone hearing thresholds for 0.5 kHz, 1 kHz, and 2 kHz between the right and left ear) (13). Finally, we required self-reported adherence of 80% or more during a 6-week placebo run-in period (**Figure**).

The medical ethics committee of Wageningen University, Wageningen, the Netherlands, approved the study, and participants gave written informed consent.

Randomization, Blinding, and Adherence

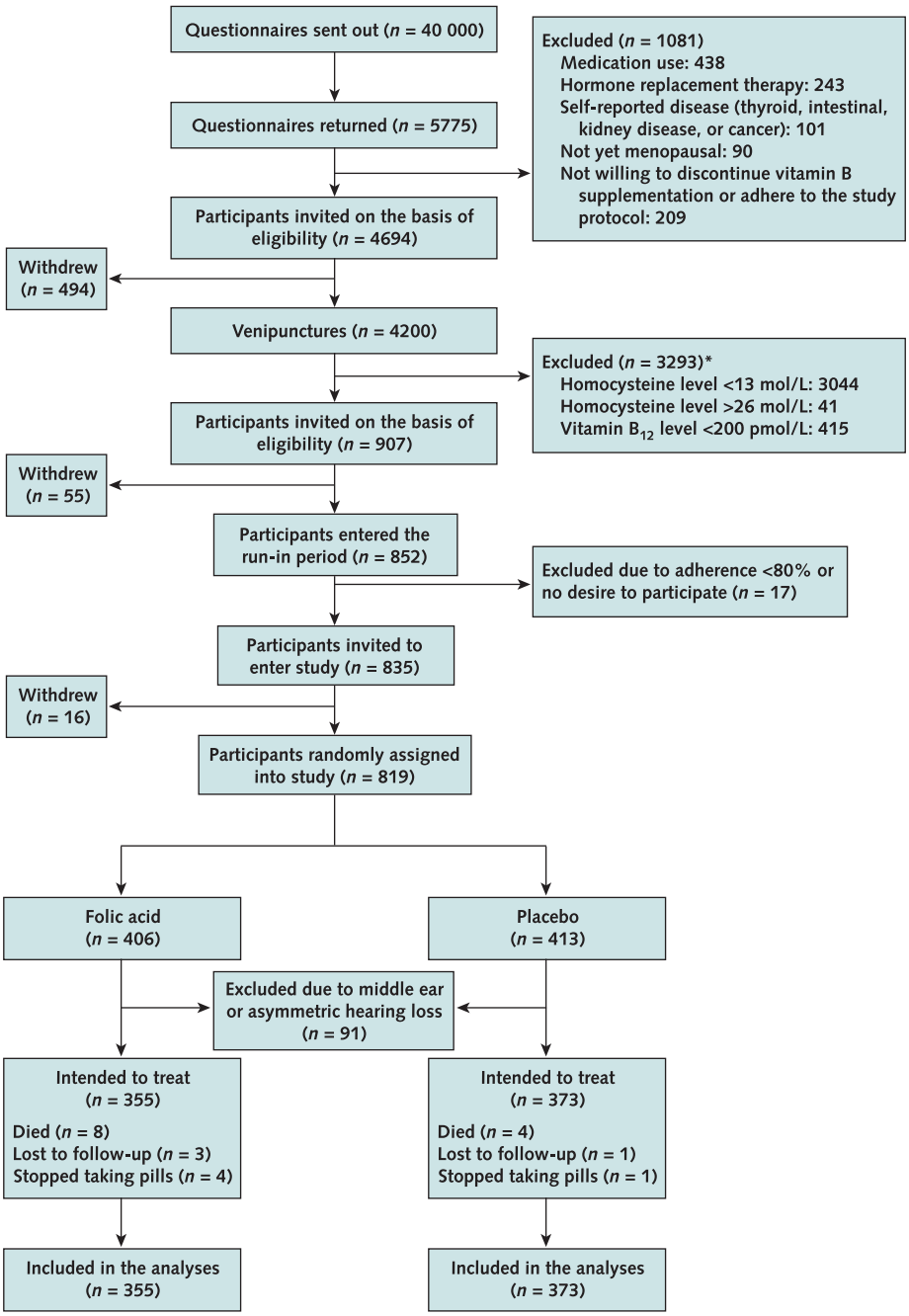
After the initial measurement sessions, we allocated participants to folic acid (800 μg/d) or placebo. When we designed the study, 800 μg/d folic acid was considered a low dosage for a clinical trial. We allocated the sequence of study entry to either treatment by using permuted blocks with randomly varied block sizes of 4 and 6. Specialized personnel who were not involved in the study allocated and labeled the capsule boxes with the participants' unique sequence numbers. Forty-eight participants had at least one other household member in the study. The second member of the same household received the same treatment as their previously randomly assigned partner. The capsules, produced by Swiss-Caps Benelux (Heerhugowaard, the Netherlands), were indistinguishable in appearance. Capsules were individually packaged in foil strips containing 28 capsules per strip, with the days of the week printed on the back. Participants received a 13-month supply of capsules each year. We judged adherence by counting returned capsules and reviewing a diary that registered missed capsules, which were both returned by participants every 12 weeks.

At the end of the study, the proportion of participants who thought that they received the folic acid or placebo capsule did not differ significantly between the 2 groups ($P = 0.26$). All personnel, including the authors, were blinded to group assignment until completion of the trial and subsequent data analyses.

Audiometry

If present, excessive cerumen was removed from participants' ears. We obtained audiometric assessments with participants seated in an acoustical booth (Audiofon G, Audiovox, Hauppauge, New York) by using an audiometer (Madsen Voyager 522, Madsen Electronics, Taastrup, Denmark) and circumaural earphones. At baseline and the final measurements, we calibrated the audiometer according to the International Organization of Standardization (ISO) standard 389 (15). The acoustical booth muted sounds up to 42 dB, but was not calibrated according to ISO standards, and was placed in a quiet room (a small, isolated, carpeted room above the college library). We performed audiometric testing by using a variation of the Hughson and Westlake (16) method: thresholds based on ascending responses using 5-dB steps up and 10-dB steps down (16). We measured pure-tone air conduction hearing thresholds at 0.5 kHz, 1 kHz, 2 kHz, 4 kHz, 6 kHz, and 8 kHz. If the difference between the right and left ear was 50 dB or more for the air conduction hearing level, then we used contralateral masking to determine the air conduction hearing thresholds. To calculate air–bone gaps for the

Figure. **Study flow diagram.**



The flowchart shows the flow of participants through the recruitment phases (November 1999 to April 2001) and the study (September 2000 to December 2004). *Participants may have been excluded for several reasons.

exclusion of participants with possible middle ear dysfunction at the start of the study, we measured bone conduction hearing thresholds at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz by using contralateral masking.

Other Measurements

Elevated concentrations of plasma total homocysteine may be a consequence of low blood levels of B vitamins involved in one-carbon metabolism, alone or in combination with genetic polymorphisms, such as methylenetetrahydrofolate reductase (MTHFR) 677C→T, or impaired renal function (14). We measured these determinants of fasting plasma total homocysteine concentrations in venous blood that was immediately processed and stored at −80 °C. We measured serum folate, erythrocyte folate, and serum vitamin B_{12} concentrations by using a chemiluminescent immunoassay (Immulite 2000, Diagnostic

Products Corp., Los Angeles, California). We determined the plasma total homocysteine level with high-performance liquid chromatography (HPLC) and fluorometric detection (17). Plasma vitamin B_6 concentration was also measured by HPLC (18). We determined serum creatinine and lipid levels by using the Hitachi 747 (Roche Diagnostics, Basel, Switzerland). We determined the MTHFR C677T genotype by polymerase chain reaction of DNA and restriction digestion with *Hin*Fl (19).

We ascertained self-reported medical history, including current drug use and smoking habits, by questionnaire, and a research assistant reviewed the information with the participants. Education was grouped according to the highest attained level (20). We measured height and weight and calculated body mass index. We measured blood pressure by using an automated meter (Dinamap Compact Pro 100, GE Healthcare, Waukesha, Wisconsin) and used the average of 8 measurements. We used a food frequency questionnaire to measure folate intake in the previous 3 months in the elderly patients. The questionnaire asked about the intake of foods that contribute at least 80% of the average folate intake as determined in an analysis of the second Dutch Food Consumption Survey for the subpopulation of men and women 50 to 70 years of age (21). The crude Spearman correlation coefficient between serum folate concentrations and folate intake estimated by the questionnaire was 0.16 ($P > 0.01$), which was within the range found by other investigators (correlation coefficient range, 0.13 to 0.34).

Genotyping and attained educational level were measured at the beginning of the study, and all other measurements were taken at the beginning and end of the study. We measured plasma total homocysteine, serum folate, and serum vitamin B_{12} concentrations and obtained information on medical condition and drug use each year.

Statistical Analyses

Descriptive statistics are given as medians (interquartile ranges). The outcome of the study was the 3-year change in the average of the pure-tone air conduction hearing thresholds of the low (0.5-kHz, 1-kHz, and 2-kHz) and high (4-kHz, 6-kHz, and 8-kHz) frequencies in the folic acid group versus the placebo group. Because we excluded participants with asymmetrical hearing loss from the study, we averaged the hearing thresholds of the low and high frequencies from both ears. We used the *t*-test to determine whether the change in hearing thresholds between treatment groups differed. We conducted all analyses without knowledge of follow-up folate and homocysteine concentrations. The treatment code was broken after an independent statistician verified data and all authors formally approved the tables that illustrated the main effects. We defined statistical significance as a *P* value less than 0.05 (2-tailed). We conducted analyses on an intention-to-treat basis by using SPSS 11.0 for Windows (SPSS Inc., Chicago, Illinois).

At baseline, 29 participants had hearing thresholds greater than 100 dB, which exceeded the limits of our audiometer (2 kHz [$n = 1$], 4 kHz [$n = 4$], 6 kHz [$n = 6$], and 8 kHz [$n = 29$]), and we assigned these participants a hearing threshold of a 105-dB hearing level. At the end of the study, we assigned 49 participants a hearing threshold of a 105-dB hearing level (2 kHz [$n = 1$], 4 kHz [$n = 4$], 6 kHz [$n = 9$], and 8 kHz [$n = 47$]). We gave 16 participants who were lost to follow-up the median hearing threshold of the low or high frequencies of the total study population at year 3.

In secondary analyses, we determined whether the effect of folic acid supplementation was dependent on the initial concentrations of folate or homocysteine or the MTHFR C677T genotype. We determined the effect of folic acid supplementation per stratum by using the *t*-test. We examined whether the difference in treatment effects among strata was statistically significant.

Role of the Funding Sources

The Netherlands Organisation for Health Research and Development, Wageningen University, and Wageningen Centre for Food Sciences funded the study. The Wageningen Centre for Food Sciences is an alliance of major Dutch food industries, research institutes, and the Dutch government and performs long-term strategic research for the development of new and innovative food with attention to health aspects. The funding sources had no role in the design, analysis, or reporting of the study or in the decision to submit the manuscript for publication.

RESULTS

The **Figure** shows the flow of the participants through the trial. Sixteen participants (2%) did not return for the audiometric assessment after 3 years, and 5 participants stopped treatment prematurely. The proportion of these participants did not differ between the groups ($P = 0.178$). Except for these participants, adherence was high (99% of the capsules were reportedly consumed). The lowest reported average adherence over a 12-week period was consumption of 87% of the capsules.

The initial median hearing threshold of the low frequencies was a 11.7-dB hearing level (interquartile range, 7.5 to 17.5 dB), which is similar to the ISO standardized average hearing threshold for participants 60 years of age (22). The median hearing threshold of the high frequencies (34.2-dB hearing level [interquartile range, 22.5 to 50.0 dB]) was higher than the ISO standardized average (29.7-dB hearing level) (**Table 1**).

Baseline plasma total homocysteine concentrations were lower than those at screening, a likely result of regression to the mean (**Table 2**). During our study, folic acid fortification of foods was prohibited in the Netherlands, so baseline folate levels were approximately half were those found in the U.S. population (23). Compared with placebo, folic acid supplementation increased serum folate

Table 1. **Baseline Characteristics***

Characteristic	Folic Acid Group (n = 355)	Placebo Group (n = 373)
Mean age (SD), *y*	60 (5)	60 (6)
Men, *n (%)*	255 (72)	267 (72)
Education level, *n (%)*		
High	135 (38)	155 (42)
Middle	126 (36)	147 (39)
Low	94 (36)	71 (19)
Median hearing threshold (IQR), *dB*†		
Low frequencies	11.7 (7.5–17.5)	11.7 (6.7–18.3)
High frequencies	33.3 (21.7–50.0)	35.0 (22.5–50.8)
Self-reported hearing problems, *n (%)*	35 (10)	39 (10)
MTHFR 677C→T genotype, *n* (%)		
677CC	128 (36)	152 (41)
677TC	158 (45)	172 (46)
677TT‡	67 (19)	47 (130
Median serum vitamin B_{12} concentration (IQR), *pmol/L*	289 (238–366)	285 (245–360)
Median plasma vitamin B_6 concentration (IQR), *nmol/L*§	33 (25–43)	31 (24–42)
Mean serum creatinine level (SD)		
μmol/L	92 (13)	92 (12)
mg/dL	1.04 (0.15)	1.04 (0.14)
Mean serum total cholesterol level (SD)		
mmol/L	5.83 (1.10)	5.81 (1.06)
mg/dL	225 (42)	224 (41)
Mean serum LDL cholesterol level (SD)‖		
mmol/L	4.02 (0.98)	3.99 (0.92)
mg/dL	155 (38)	154 (36)
Mean serum HDL cholesterol level (SD)		
mmol/L	1.24 (0.34)	1.23 (0.37)
mg/dL	48 (13)	47 (14)
Dyslipidemia, *n (%)*¶	135 (38)	123 (33)
Mean systolic blood pressure (SD), *mm Hg*‡	133 (17)	132 (16)
Mean diastolic blood pressure (SD), *mm Hg*‡	77 (8)	77 (8)
Hypertension, *n (%)*‡**	81 (23)	75 (20)
Mean BMI (SD), kg/m^2	26.6 (3.6)	26.6 (3.7)
Current smoking, *n (%)*	70 (20)	75 (20)
Diabetes mellitus, *n (%)*	12 (3)	12 (3)
Self-reported CVD, *n (%)*††	48 (14)	33 (9)

* BMI = body mass index; CVD = cardiovascular disease; HDL = high-density lipoprotein; IQR = interquartile range; LDL = low-density lipoprotein; MTHFR = methylenetetrahydrofolate reductase.
† Average pure-tone air conduction threshold of both ears at 0.5 kHz, 1 kHz, and 2 kHz for low frequencies and at 4 kHz, 6 kHz, and 8 kHz for high frequencies.
‡ Data were available for 355 participants in the folic acid group and 371 participants in the placebo group.
§ Data were available for 372 participants in the placebo group.
‖ Data were available for 371 participants in the placebo group.
¶ Total cholesterol level >6.5 mmol/L (>251 mg/dL), HDL cholesterol level <0.9 mmol/L (<35 mg/dL), or use of lipid-lowering medication.
** Systolic blood pressure ≥160 mm Hg, diastolic blood pressure ≥95 mm Hg, or use of antihypertensive medication.
†† Diagnosis of angina pectoris, myocardial infarction, arrhythmia, stroke, or peripheral arterial disease or having undergone angioplasty, coronary bypass surgery, or aortic aneurysm surgery.

concentrations by 573% (95% CI, 533% to 612%) and decreased plasma total homocysteine concentrations by 26% (CI, 23% to 28%) after 3 years.

After 3 years, hearing thresholds were statistically significantly increased in both groups. In the folic acid group, the median hearing threshold was a 12.5-dB hearing level (interquartile range, 8.3 to 18.3 dB) for the low frequencies and a 40.0-dB hearing level (interquartile range, 25.8 to 53.3 dB) for the high frequencies. In the placebo group, the median hearing threshold was a 13.3-dB hearing level (interquartile range, 8.3 to 20.0 dB) and a 40.0-dB hearing level (interquartile range, 27.5 to 55.4 dB), respectively. The annual rate of decline in hearing for high frequencies (1.6 dB) in participants in the placebo group was slightly higher than estimates from other study populations (approximately 1.1 dB) (24).

The increases in hearing threshold of the low frequencies were 1.0 dB (CI, 0.6 to 1.4 dB) in the folic acid group and 1.7 dB (CI, 1.3 to 2.1 dB) in the placebo group, for a mean difference of −0.7 dB (CI, −1.2 to −0.1 dB) (P = 0.020) (**Table 3**). Folic acid treatment did not affect the decline in hearing the high frequencies. These results were unchanged when we excluded the 16 participants who were lost to follow-up and the 54 participants with a hearing threshold above that measured by our audiometer (mean difference of the hearing thresholds in the low frequencies, −0.8 dB [CI, −1.3 to −0.3 dB]). When we restricted our analyses to participants with a hearing threshold above that measured by our audiometer at the beginning or end of the study, we did not detect an improvement in hearing levels due to folic acid supplementation (mean difference of hearing thresholds in the low fre-

Table 2. **Folate Status and Total Homocysteine Concentrations during the Study***

Variable	Folic Acid Group	Placebo Group	*P* Value†
Serum folate level, *nmol/L*‡			
Baseline	11.5 (9.4–14.6)	11.8 (9.7–14.9)	–
1 year	52.7 (43.2–85.8)	11.9 (9.4–15.4)	<0.001
2 years	49.4 (41.7–81.5)	12.4 (9.3–15.3)	<0.001
3 years	75.0 (50.0–102.8)	13.2 (10.3–16.8)	<0.001
Erythrocyte folate level, *nmol/L*§			
Baseline	630 (493–820)	672 (531–814)	–
3 years	2040 (1743–2446)	692 (537–883)	<0.001
Dietary folate intake, *μg/d*‖			
Baseline	195 (159–241)	195 (159–242)	–
3 years	181 (152–219)	179 (151–225)	0.84
Plasma total homocysteine level, *μmol/L*¶			
Baseline	13.0 (11.6–14.7)	12.8 (11.4–14.7)	–
1 year	9.4 (8.4–10.5)	12.4 (10.6–14.6)	<0.001
2 years	9.7 (8.5–10.9)	12.5 (11.0–14.8)	<0.001
3 years	10.0 (9.0–11.2)	13.4 (11.5–15.2)	<0.001

* Values are expressed as medians (interquartile ranges).
† *P* values are based on nonparametric tests.
‡ At baseline, data were available for 355 participants in the folic acid group and 373 participants in the placebo group. At year 1, data were available for 347 participants and 368 participants, respectively. At year 2, data were available for 340 participants and 363 participants, respectively. At year 3, data were available for 344 participants and 366 participants, respectively.
§ At baseline, data were available for 355 participants in the folic acid group and 373 participants in the placebo group. At year 3, data were available for 344 participants and 366 participants, respectively.
‖ At baseline, data were available for 355 participants in the folic acid group and 373 participants in the placebo group. At year 3, data were available for 344 participants and 368 participants, respectively.
¶ At baseline, data were available for 355 participants in the folic acid group and 373 participants in the placebo group. At year 1, data were available for 347 participants and 366 participants, respectively. At year 2, data were available for 341 participants and 365 participants, respectively. At year 3, data were available for 343 participants and 366 participants, respectively.

quencies, 1.5 dB [CI, −1.4 to 4.4 dB]). Finally, we did not detect a trend in threshold shift reduction across individual frequencies (**Appendix Table 1** and **Appendix Table 2**, available at www.annals.org), although this was a post hoc analysis that, unlike average values, has no physiologic parallel to cochlear function.

For the MTHFR C677T genotype, the mean difference between treatment groups in hearing thresholds of the low frequencies was −1.5 dB (CI, −2.8 to −0.2) in the MTHFR 677TT participants; −0.7 dB (CI, −1.5 to 0.1) in the MTHFR 677CT participants; and −0.3 dB (CI, −1.2 to 0.6) in the MTHFR 677CC participants ($P = 0.171$ for interaction). The effect of folic acid treatment seemed pronounced in participants with initial serum folate concentrations less than the median (median serum folate concentration, <11.8 nmol/L). In these participants, the mean difference between treatment groups in hearing thresholds of the low frequencies was −1.1 dB (CI, −1.8 to −0.3) compared with −0.2 dB (CI, −1.0 to 0.6) among participants with initial serum folate concentrations of 11.8 nmol/L or greater ($P = 0.117$ for interaction). These results were unchanged when folate was modeled as a continuous variable (data not shown).

Four participants in the folic acid group reported forgetfulness, tinnitus, weight gain, and dark urine, and 5 participants in the placebo group reported muscle aches, weight gain, queasiness, skin irritations, and a bitter taste in the mouth. No participant stopped the trial prematurely because of these side effects.

Discussion

In 728 older adults with symmetrical sensorineural hearing loss, daily oral folic acid supplementation for 3 years slowed the decline in hearing of the low frequencies by 0.7 dB. Folic acid did not affect hearing thresholds of the high frequencies.

In our study population, hearing loss was probably of cochlear origin, although we did not measure this directly. We selected participants with symmetrical sensorineural hearing dysfunction that was probably due to aging. Asymmetrical sensorineural thresholds can be a symptom of unilateral noise-induced hearing damage or neural pathology, such as acoustic neuroma. We excluded participants with conductive hearing loss, because in the presence of middle ear dysfunction, such as otitis media or otosclerosis, bone conduction thresholds can be altered, leading to pseudosensorineural hearing loss (25). Finally, few participants are likely to have had central auditory disorders because these disorders are not highly prevalent in the participants' age group (26, 27).

Following an a priori data analysis plan, we chose our outcome measure—the average of the hearing thresholds of the low and high frequencies—to reflect possible physiologic differences that may help elucidate the cause of hear-

ing loss. The apex and base of the cochlea are responsible for transducing low-frequency sounds and high-frequency sounds, respectively. It has been postulated that the cochlear sensory cells of the apex may be the most susceptible to aberrations in microcirculation in the stria vascularis because the apex is the farthest from the blood supply of the cochlea (28). An improvement in microcirculation may explain the effect of folic acid on low-frequency thresholds. Studies of cerebral tissues in animals suggest that microvascular function is responsive to dietary folate deprivation and folic acid supplementation (29, 30). In humans, however, 2-year folic acid and vitamin B_6 supplementation did not affect markers of cerebral microangiography (31). Measures, such as cochlear blood flow, could determine whether folic acid supplementation protects against hearing loss through improved microcirculation. Alternatively, a greater biological variation associated with hearing thresholds of the high frequencies compared with those of the low frequencies may explain why a potential beneficial effect of folic acid could not be detected in the high-frequency hearing range. In that case, we should have recruited more participants or extended the duration of the study to detect an effect of folic acid supplementation. Finally, age-related hearing loss initially affects high-frequency hearing and slowly extends to hearing in the lower frequencies; hence, corresponding sensory cell loss occurs earlier in the high frequencies than in the lower frequencies. Thus, our finding of an effect in low-frequency hearing may be because high-frequency hearing loss occurs earlier and may have already been established, so folic acid could only prevent or confine sensory cell damage at the low frequencies. If this is indeed the case, then similar trials should be repeated in younger persons to determine whether folic acid supplementation affects all frequencies, including those greater than 8 kHz. Tests that measure sensory cell function, such as otoacoustic emission tests, could also be included in future studies.

In the placebo group, the annual rate of decline in hearing for the low (0.6 dB) and high (1.6 dB) frequencies was approximately 0.5 dB and 1.1 dB, respectively—slightly higher than estimates from other study populations (24). These slight differences may be related to the fact that we selected participants with elevated plasma total homocysteine concentrations. We previously reported that initial plasma total homocysteine concentrations were not associated with hearing thresholds (13) and they did not modify the effect of folic acid treatment. On the other hand, 9% and 2% of the participants were deficient in serum and erythrocyte folate concentrations, respectively (32), and the median folate intake in our study population was less than the recommended dietary allowances of 300 μg/d (33). Our data suggest that participants with low baseline folate status showed a stronger treatment effect. Consequently, the effect of folic acid on hearing thresholds may be weaker in people with better folate status, for example, in countries, such as the United States, with mandated fortification of flour with folic acid and average concentrations of serum folate that are approximately twice those of the Dutch population (23).

We believe that our trial is the first to show a beneficial effect of long-term folic acid supplementation on hearing thresholds. These findings must be confirmed in other trials, which are currently under way, that examine the homocysteine-lowering effect on recurrent vascular disease (34). The link between folate and hearing has only recently gained interest among researchers. Two small studies have been conducted in people with age-related hearing loss, which have led to divergent results (7, 8). More recent research has shown an association of low folate concentrations (9) and elevated total homocysteine concentrations (9, 12) with sudden sensorineural hearing loss and noise-induced hearing loss (10), another form of sensorineural hearing loss. Progressive sensorineural loss has been reported in children with low concentrations of folate in cerebrospinal fluid (11), and folic acid treatment has been associated with lesion healing in temporal bone (35). Finally, the sensorineural ototoxic effect of cisplatin, a folate analogue commonly used in chemotherapy, has been extensively studied (36). The importance of folate metabolism in sensorineural hearing requires further study.

Delineation of folic acid as an agent that slows the decline in hearing the speech frequencies seen with aging, even in individuals without a hearing impairment, may limit the incidence of hearing loss and improve quality of life (37). While the decrease in hearing loss observed in our study was small—only 0.7 dB during 3 years of folic acid supplementation—after 30 years, folic acid supplementation could spare a 7-dB hearing level if this function is

Table 3. **Increase in Hearing Thresholds over 3 Years Using Intention-to-Treat* Analyses**

Variable	Mean Change in Hearing Thresholds (95% CI), *dB*		Mean Difference (95% CI)	*P* Value
	Folic Acid Group (n = 355)	Placebo Group (n = 373)		
Low frequencies†	1.0 (0.6 to 1.4)	1.7 (1.3 to 2.1)	−0.7 (−1.2 to −0.1)	0.020
High frequencies†	4.6 (4.0 to 5.3)	4.8 (4.1 to 5.4)	−0.2 (−1.1 to 0.8)	0.73

* Folic acid group (n = 11) and placebo group (n = 5) used group median at year 3 for imputations of the low frequencies (12.5-dB hearing level) and of the high frequencies (40.0-dB hearing level).
† Average pure-tone air conduction threshold of both ears at 0.5 kHz, 1 kHz, and 2 kHz for low frequencies and at 4 kHz, 6 kHz, and 8 kHz for high frequencies.

linear. However, hearing loss is not linear but increases over time, such that the effect of folic acid on hearing could be greater. More disabling than an increase in pure-tone thresholds is the progressive degradation of speech understanding in everyday noisy surroundings. Although this steady deterioration cannot fully be attributed to pure-tone threshold shifts, it depends clearly on auditory thresholds and age (38). Additional studies should include sensitive tests of speech perception under unfavorable conditions next to auditory thresholds. Whether the potential effect of folic acid treatment is even greater in younger persons in which cochlear degeneration is typically still limited needs to be determined. Considering that the folate status of older adults is generally low in countries without folic acid fortification programs, our findings suggest a possible way to diminish the public health burden of hearing loss in those countries.

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Appendix Table 1. **Change in Hearing Thresholds across Individual Frequencies Using All Collected Data**

Frequency, *kHz*	Mean Difference between Groups (95% CI), *dB*	*P* Value
0.5	−0.4 (−1.1 to 0.2)	0.159
1	−0.9 (−1.5 to −0.2)	0.010
2	−0.7 (−1.4 to 0.1)	0.092
4	−0.8 (−1.7 to 0.1)	0.069
6	0.7 (−0.6 to 1.9)	0.29
8	0.2 (−1.1 to 1.5)	0.75

Appendix Table 2. **Change in Hearing Thresholds across Individual Frequencies for 658 Participants***

Frequency, *kHz*	Mean Difference between Groups (95% CI) (*n* = 658), *dB*	*P* Value
0.5	–0.6 (–1.2 to 0.0)	0.069
1	–1.1 (–1.7 to –0.4)	0.002
2	–0.8 (–1.5 to –0.0)	0.040
4	–0.8 (–1.7 to 0.1)	0.097
6	0.7 (–0.5 to 2.0)	0.24
8	0.4 (–0.9 to 1.7)	0.55

* Participants who were lost to follow-up ($n = 16$) and participants with a hearing threshold above that detected by the audiometer ($n = 54$) were excluded.