Background: Lowering serum homocysteine levels with folic acid is expected to reduce mortality from ischemic heart disease. Homocysteine reduction is known to be maximal at a folic acid dosage of 1 mg/d, but the effect of lower doses (relevant to food fortification) is unclear.

Methods: We randomized 151 patients with ischemic heart disease to 1 of 5 dosages of folic acid (0.2, 0.4, 0.6, 0.8, and 1.0 mg/d) or placebo. Fasting blood samples for serum homocysteine and serum folate analysis were taken initially, after 3 months of supplementation, and 3 months after folic acid use was discontinued.

Results: Median serum homocysteine level decreased with increasing folic acid dosage, to a maximum at 0.8 mg of folic acid per day, when the homocysteine reduction (placebo adjusted) was 2.7 µmol/L (23%), similar to the known effect of folic acid dosages of 1 mg/d and above. The higher a person’s initial serum homocysteine level, the greater was the response to folic acid, but there were statistically significant reductions regardless of the initial level. Serum folate level increased approximately linearly (5.5 nmol/L for every 0.1 mg of folic acid). Within-person fluctuations over time in serum homocysteine levels, measured in the placebo group, were large compared with the effect of folic acid, indicating that monitoring of the reduction in an individual is impractical.

Conclusions: A dosage of folic acid of 0.8 mg/d appears necessary to achieve the maximum reduction in serum homocysteine level across the range of homocysteine levels in the population. Current US food fortification levels will achieve only a small proportion of the achievable homocysteine reduction.
PATIENTS AND METHODS

Patients at St Richard’s Hospital, Chichester, England, who were known to have ischemic heart disease (previous myocardial infarction or angina) were invited to participate in the study. The trial was approved by the local research ethics committee. Patients already taking vitamin supplements or taking anticonvulsant therapy were excluded from participating in the study. Patients with a myocardial infarction in the previous 3 months were also excluded, as serum homocysteine level increases in the acute phase after a myocardial infarction and then decreases. 1

A total of 151 patients were randomized to 5 folic acid supplementation groups and a placebo group. Identical tablets containing no folic acid (placebo) and doses of 0.2, 0.4, 0.6, 0.8, and 1.0 mg of folic acid (Cantassium Vitamins, London, England) were used. The trial was double-blind. Bottles of tablets were numbered in random order and dispensed in numerical order by the pharmacy. The tablets were taken for 3 months.

Fasting blood samples for serum folate and serum homocysteine measurement were taken from each patient on 3 occasions: before commencing the tablets, after taking them for 3 months, and after stopping them for 3 months. The blood samples were placed on ice immediately after collection and centrifuged at 4°C within 2 hours, and the serum was stored at −20°C. Serum homocysteine assays were performed at Trinity College, Dublin, Ireland, by isocratic high-performance liquid chromatography; 2 with the use of a fluorescent conjugate (SBD-1). 3 A 1:10 dilution of 0.5 mL of whole blood in 1-g/L sodium ascorbate was also stored at −20°C, until analyzed for serum folate by microbiologic assay. 4 Laboratory staff were unaware of the randomization group to which patients were assigned.

Of the 151 patients (mean age, 65 years), 125 were male; 84 had had a myocardial infarction and the other 67 had angina. There were 26 patients each in the placebo, 0.4-mg, 0.6-mg, and 0.8-mg groups; 27 patients in the 0.2-mg group; and 24 patients in the 1.0-mg group. Three patients were unavailable for follow-up at 3 months (1 in each of the placebo, 0.2-mg, and 0.4-mg groups), and an additional 3 patients were unavailable for follow-up at 6 months (2 in both the placebo and 0.4-mg groups and 1 in the 0.2-mg group). In the other groups, no patients were unavailable for follow-up. The effect of folic acid after 3 months was determined from the 148 patients who attended the first and second visits, and the effect of stopping folic acid supplementation was determined from the 143 patients who attended all 3 visits.

At the clinic visit after 3 months of supplementation, all patients said they had taken their tablets regularly. Counts of unused tablets in the containers showed a maximum of 5 (of 91 initially), and more than half of the patients had taken all their tablets.

about 2% to 3% of individuals without ischemic heart disease of the same age. 5 These values remained high in the placebo group at the 3- and 6-month visits, indicating that they were genuinely high and not simply the result of individual fluctuations or measurement error. Because of the skewed distribution and the presence of outlying values, the median was used instead of the mean as a measure of central tendency.

**Figure 1.** Distribution of serum homocysteine concentrations at initial visit.

**Table 1. Median, Mean, and 10th and 90th Percentiles of Serum Homocysteine and Serum Folate Levels at Initial Visit (n = 151)**

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Mean</th>
<th>10th Percentile</th>
<th>90th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum homocysteine, µmol/L</td>
<td>13.4</td>
<td>14.6</td>
<td>9.4</td>
<td>20.8</td>
</tr>
<tr>
<td>Serum folate, nmol/L</td>
<td>15.4</td>
<td>18.4</td>
<td>9.1</td>
<td>31.2</td>
</tr>
</tbody>
</table>

*To convert nanomoles per liter to micrograms per liter, divide by 2.266.*

**Figure 2A** shows the median increase in serum folate level between the initial and the 3-month visit according to supplementation group (148 patients). Results were placebo-adjusted (subtracting the median change in the placebo group from the median change in each supplementation group). Serum folate level increased with increasing folic acid dose; for each 0.1 mg of folic acid, serum folate level increased by about 5.5 nmol/L. **Figure 2B** shows the placebo-adjusted median reduction in serum homocysteine levels between the initial and 3-month visit according to supplementation group. Serum homocysteine levels showed a continuous decline with increasing folic acid dose up to a dosage of 0.8 mg/d, where the median serum homocysteine reduction was 2.7 µmol/L (23% of the median starting homocysteine level in that supplementation group). The SEs of the changes in serum homocysteine level from a linear regression analysis of the change in serum homocysteine level on folic acid dose were about 0.7 µmol/L, and while the trial did not have the statistical power to show statistically significant differences between adjacent folic acid dosage groups, the trend in increasing homocysteine reduction in relation to increasing folic acid dosages up to 0.8 mg/d was significant. The high serum homocysteine response in the 0.8-mg and 1.0-mg dosage groups was not due to a chance preponderance of subjects with very high (>20 µmol/L) initial serum homocysteine levels in these 2 dosage groups (the 0.8-mg group had none of these). The homocysteine reduction with the 1-mg folic acid dose was slightly less than that with 0.8
mg; this is probably due to chance, but it does suggest that the effect of 1 mg is unlikely to be greater than that of 0.8 mg. The serum homocysteine–lowering effect of folic acid was similar in older and younger patients. The mean reduction in each supplementation group was a little greater than the median, and at higher doses it was about 3 µmol/L (21% of the mean starting homocysteine level), similar to the effect of folic acid dosages between 1 and 5 mg/d, shown in the meta-analysis of the Homocysteine Lowering Trialists’ Collaboration.¹

Table 2 shows the placebo-adjusted reduction in serum homocysteine concentrations according to tertile levels for all supplementation groups, suggesting that the variation across a single tertile level is mainly due to true differences between individuals. Even in the lowest dosage group, with a mean reduction in each supplementation group was a little greater than the median, and at higher doses it was about 3 µmol/L (21% of the mean starting homocysteine level). The effect of folic acid in lowering serum homocysteine level was statistically significant in all 3 tertile groups (.001,  .001, and = .04 from a linear regression analysis in the highest, middle, and lowest tertile groups, respectively). A folic acid dosage of 0.8 mg/d achieved the maximum median reduction in serum homocysteine level in all 3 tertile groups, but in the highest group a dosage of 0.4 mg/d appeared sufficient to achieve the maximum effect. The results suggest that the higher the initial homocysteine level, the lower the folic acid dose needed to attain the maximum reduction. This trend was confirmed in the 10% of patients with very high initial homocysteine levels (≥20 µmol/L). This group showed a dramatic response to folic acid supplementation, even in the lowest dosage group, with a median serum homocysteine reduction of 7.1 µmol/L (mean reduction, 9 µmol/L).

Table 3 shows the extent to which the effect of folic acid on serum folate and serum homocysteine levels was sustained 3 months after folic acid supplementation was stopped (143 patients in total). Serum folate concentration decreased after cessation but (apart from the 0.2-mg dosage group) did not return to initial levels; it was 9.3 nmol/L higher in the 0.8-mg/d supplementation group and 12.4 nmol/L higher in the 1.0-mg/d supplementation group (the latter representing a 75% return to the initial level). Serum homocysteine concentrations returned to near initial levels for all supplementation groups, suggesting that a sustained serum homocysteine reduction relies on continued folic acid supplementation.

The 3 sets of measurements in 22 placebo-treated subjects allowed calculation (from an analysis of variance) of the within- and between-person SDs for serum homocysteine and serum folate levels (Table 4). For serum homocysteine, the between-person SD (6.8 µmol/L) is large in relation to the within-person SD (2.3 µmol/L), indicating that the variation across a single set of measurements is mainly due to true differences between in-
individuals rather than to random variation in the same individual. Applying the data in Table 4 to prospective (cohort) studies of homocysteine and the incidence of heart disease and stroke, the “regression dilution correction factor” (the ratio of total to between-person variance) for serum homocysteine is 1.12 ([2.33^2+6.76^2]/6.76^2), a low value, indicating that the observed regression coefficient (slope) of cardiovascular disease on homocysteine needs to be increased by only 12% to allow for the diluting effect of random fluctuations in homocysteine level.7,8

The data in Table 4 can also be used to assess the value of using homocysteine measurements before and after the start of folic acid treatment to monitor the homocysteine reduction. This might be worthwhile if the effect of treatment were large in relation to the within-person fluctuation in homocysteine level. In fact it is relatively small, as shown by the “monitoring factor” for homocysteine of 0.45, so monitoring in this way is not useful. This monitoring factor is the median reduction from 0.8 mg of folic acid per day, divided by 2.56 times the within-person SDs (which estimates the 10th to 90th percentile range of values in an individual over time). For serum folate level, on the other hand, the monitoring factor is large (3.5).

### Table 4. Within- and Between-Person SDs for Serum Homocysteine and Serum Folate Levels

<table>
<thead>
<tr>
<th></th>
<th>Serum Homocysteine</th>
<th>Serum Folate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within-person SD (W)</strong></td>
<td>2.3 µmol/L</td>
<td>5.3 nmol/L*</td>
</tr>
<tr>
<td><strong>Between-person SD (B)</strong></td>
<td>6.8 µmol/L</td>
<td>8.7 nmol/L*</td>
</tr>
<tr>
<td><strong>Regression dilution correction factor</strong></td>
<td>1.12</td>
<td>1.37</td>
</tr>
<tr>
<td><strong>[(B^2+W^2)/B^2]</strong></td>
<td>0.45</td>
<td>3.4</td>
</tr>
</tbody>
</table>

*To convert nanomoles per liter to micrograms per liter, divide by 2.266.

### Table 5. Serum Homocysteine Reduction in Trials Using Folic Acid at Dosages Below 1 mg/d, in Which Mean Age of Subjects Was More Than 40 Years

<table>
<thead>
<tr>
<th>Source*</th>
<th>Folic Acid, mg</th>
<th>Mean Age, y</th>
<th>Initial</th>
<th>Change†</th>
<th>Ratio, Final to Initial</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ward et al⁹</td>
<td>0.1</td>
<td>45</td>
<td>9.0</td>
<td>-0.9</td>
<td>0.91</td>
<td>9</td>
</tr>
<tr>
<td>Malinow et al⁹‡</td>
<td>0.13</td>
<td>64</td>
<td>10.0</td>
<td>-0.7</td>
<td>0.93</td>
<td>7</td>
</tr>
<tr>
<td>Schorah et al¹¹‡</td>
<td>0.2</td>
<td>45</td>
<td>10.2</td>
<td>-1.3</td>
<td>0.87</td>
<td>13</td>
</tr>
<tr>
<td>Ward et al⁸</td>
<td>0.2</td>
<td>45</td>
<td>9.0</td>
<td>-1.4</td>
<td>0.84</td>
<td>16</td>
</tr>
<tr>
<td>Ward et al⁸</td>
<td>0.4</td>
<td>45</td>
<td>9.0</td>
<td>-1.9</td>
<td>0.79</td>
<td>21</td>
</tr>
<tr>
<td>Lobo et al¹⁰</td>
<td>0.4</td>
<td>61</td>
<td>14.0</td>
<td>-4.0</td>
<td>0.71</td>
<td>29</td>
</tr>
<tr>
<td>Riddell et al¹³</td>
<td>0.45</td>
<td>53</td>
<td>11.7</td>
<td>2.1</td>
<td>0.82</td>
<td>18</td>
</tr>
<tr>
<td>Malinow et al¹⁰‡</td>
<td>0.5</td>
<td>66</td>
<td>11.4</td>
<td>-2.0</td>
<td>0.82</td>
<td>18</td>
</tr>
<tr>
<td>Den Heijer et al¹⁴</td>
<td>0.5</td>
<td>53</td>
<td>12.2</td>
<td>-2.1</td>
<td>0.83</td>
<td>17</td>
</tr>
<tr>
<td>Coppen and Bailey¹⁵</td>
<td>0.5</td>
<td>42</td>
<td>9.5</td>
<td>-2.1</td>
<td>0.78</td>
<td>22</td>
</tr>
<tr>
<td>Malinow et al¹¹‡</td>
<td>0.67</td>
<td>63</td>
<td>14.6</td>
<td>4.2</td>
<td>0.71</td>
<td>29</td>
</tr>
</tbody>
</table>

*All studies were randomized except those of Ward et al⁹ and Lobo et al¹⁰.
†All values are placebo-adjusted except for those from Ward et al⁹.
‡Cereal fortification.

Our results show a clear effect of increasing folic acid dose on serum homocysteine reduction. The maximum effect was attained at 0.8 mg/d, where the serum homocysteine reduction was 2.7 µmol/L, a 23% reduction, similar to the effect shown for folic acid dosages between 1 and 5 mg/d.¹

The results confirm that the higher the initial serum homocysteine level, the more sensitive is the response to folic acid. The study had the statistical power to show that there was a significant serum homocysteine-lowering effect of folic acid in people with relatively low initial serum homocysteine levels, indicating some benefit in folic acid supplementation regardless of serum homocysteine level. Although a dosage of 0.4 mg of folic acid per day is sufficient to attain close to the maximum serum homocysteine reductions in those with higher initial serum homocysteine concentrations, it will achieve only about half of the smaller reductions in those with lower serum homocysteine levels—overall, about 75% of the total effect. The higher dosage appears necessary for the full benefit. The consistent pattern of our results, both unstratified by initial serum homocysteine level (Figure 2B) and stratified according to initial serum homocysteine level (Table 2), suggests that we can be reasonably confident of the pattern of the dose-response relationship observed between serum homocysteine level and supplemental folic acid despite the fairly wide confidence intervals that apply to any one dosage group or subgroup within a dosage group.

Table 5 summarizes the results of published studies that have investigated the effect on serum homocysteine level of folic acid supplements (in pills or fortified breakfast cereal) in dosages less than 1.0 mg/d.⁹⁻¹⁵ Two studies in selected populations with very high serum homocysteine concentrations (>40 µmol/L) were excluded.¹⁶,¹⁷ We also restricted our analysis to studies in which the mean age was more than 40 years, because analysis of the published trials shows that the increase in serum folate for a specified increase in folic acid in

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COMMENT
take is greater in older than in younger people. It is likely, therefore, that for a given intake of folic acid, homocysteine levels will decrease more in older than in younger people. The data support our own results in suggesting that folic acid dosages of 0.1 and 0.2 mg/d do not attain the full effect and that dosages of 0.4 and 0.5 mg/d reduce serum homocysteine levels by about 20%—achieving most but not all of the known maximum effect of about 25%. There is little previously published information on dosages between 0.6 and 1.0 mg/d.

The data in Table 4 show that the within-person fluctuation in serum homocysteine level over time is large in comparison with the maximum average decrease in serum homocysteine level produced by folic acid. It is therefore impractical to monitor the change in serum homocysteine level produced by folic acid in an individual patient (as opposed to a group); the relatively modest true reduction would be obscured.

On the other hand, the within-person SD of homocysteine is small in relation to the between-person SD (2.3 vs 6.8 µmol/L), indicating that, in cohort studies of serum homocysteine and cardiovascular disease, the “diluting” effect of fluctuations in homocysteine will be small. The reduction in ischemic heart disease mortality that can be expected through serum homocysteine reduction should be close to the observed reduction in ischemic heart disease mortality in cohort studies (systematic underestimation of the true effect will be only about 10%). This compares with the larger effect of within-person fluctuation in serum cholesterol level, where the effect of serum cholesterol reduction on ischemic heart disease is about 50% greater than that estimated in cohort studies.6

In an analysis of variance, such as that used to produce the results in Table 4, it may be better to adopt 2 modifications: to use logarithms (because the variation is likely to be proportional to the mean) and to minimize the effect of outliers by estimating the SD from the 10th to the 90th percentiles in the placebo group to calculate the between-person SD. Analyzing the data in this way yields estimates of 0.31 and 0.13 for the between- and within-person SDs of homocysteine (units are loge), and 0.38 and 0.26, respectively, for serum folate. These numbers can be made interpretable in the original units of measurement by taking the antilogarithm and using this together with a mean value to calculate the range defined by the mean±1 SD. For example, the range of mean±1 within-person SD for homocysteine with a mean of 10 µmol/L would be 7.3 µmol/L (10 divided by antilog, 0.31) to 13.6 µmol/L (10 times antilog, 0.31). This compares with a range of 7.7 to 12.3 µmol/L using the estimate in Table 4.

Our estimates for the homocysteine regression dilution correction factor (1.12 from Table 4 or 1.18 from the above estimates) is close to the estimate of 1.14 from Clarke and colleagues,18 but their estimates of both within- and between-person SD were lower. This may reflect the lower homocysteine values in that study (because the subjects did not have ischemic heart disease). An analysis of variance using logarithms largely reconciles the differences between the 2 studies. In another study, the within-person SD of homocysteine was small (0.6 µmol/L), but the duration of the study was short (4 weeks); the between-person SD was 2.8 µmol/L.19

Public health initiatives that fortify food with folic acid are likely to be the most effective means of increasing folic acid consumption in the population. This is an inexpensive and simple strategy. Fortification has already been introduced in the United States to prevent neural tube defects. The level of fortification mandated by the US government (0.14 mg of folic acid per 100 g of cereal grain, with the intention of supplementing a person’s diet by about 0.1 mg of folic acid per day)20 will achieve only a small proportion of the maximum serum homocysteine–lowering effect. In the United Kingdom, the relevant government advisory committee has recommended the universal fortification of flour at a level of 0.24 mg per 100 g of flour.21 Even this will have only a partial effect in lowering serum homocysteine level.

The advantages in selecting a study population with ischemic heart disease were that they represent a significant proportion of people who stand to benefit most from a reduction in serum homocysteine level through food fortification. As expected, they had higher serum homocysteine concentrations than unaffected persons of the same age (median, 13.4 µmol/L compared with about 12 µmol/L) and included a higher proportion of people with particularly high serum homocysteine levels (likely to be caused by genetic variants, such as homozygotes for the 677 methylenetetrahydofolate reductase variant). This important high-risk group stands to benefit considerably from serum homocysteine reduction; their median serum homocysteine reduction of 7 µmol/L would be expected to reduce risk by about 25%.2 Randomized trials of the efficacy of folic acid supplements in the prevention of ischemic heart disease are ongoing, but the existing evidence of an effect is persuasive.1 It would be reasonable for clinicians to consider advising patients with ischemic heart disease to take 0.8 mg of folic acid each day.

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REFERENCES