Gender and Body Size Affect the Response of Erythrocyte Folate to Folic Acid Treatment

Renate M. Winkels, Ingeborg A. Brouwer, Petra Verhoef, Floor V. A. van Oort, Jane Durga, and Martijn B. Katan

The recommended dietary allowance (RDA) differs between men and women for some vitamins, but not for folate. The RDA for folate is derived mainly from metabolic studies in women. We assessed if men differ from women in their response of erythrocyte folate to folic acid supplementation. We used data from 2 randomized placebo-controlled trials with folic acid: a 3-y trial in which subjects ingested 800 μg/d of folic acid (294 men and 112 women) and a 12-wk trial in which 187 men and 129 women ingested 0, 50, 100, 200, 400, 600, or 800 μg/d of folic acid in a parallel design (n = 38–42 per treatment group). In the 3-y trial, the erythrocyte folate concentration increased 10% (143 nmol/L, [95% CI 46, 241]) less in men than in women. In the 12-wk trial, regression analysis showed that the response of erythrocyte folate upon folic acid intake for men was 47 nmol/L lower than for women (P for \( \beta_{\text{gender}} = 0.022 \)); for an intake of 800 μg/d folic acid, this resulted in a 5% lower response in men than in women. Differences in lean body size explained 56% of the difference in response of erythrocyte folate between men and women in the 3-y trial and 70% in the 12-wk trial. Men need more folic acid than women to achieve the same erythrocyte folate concentration, mainly because men have a larger lean body mass. This could be an indication that the RDA for folate should be higher for men than for women, or that the RDA should be expressed per kilogram of lean body mass. * J. Nutr. 138: 1456–1461, 2008.

Introduction

Folate intake in men is generally higher than in women (1,2). However, folate status is the same or slightly lower in men than in women (3–5).

In most countries, the recommended dietary allowance (RDA) for folate is the same for men and women, but it is derived mainly from metabolic studies in women (6–8). The RDA is 200 μg/d in the United Kingdom (9), 300 μg/d of folate from food in the Netherlands (10), and 400 μg/d of dietary folate equivalents in the US (6). In an earlier version, the RDA for folate in the US was higher for men (240 μg/d) than for women (190 μg/d) (11).

For several other vitamins, namely vitamin A, C, K, thiamin, riboflavin, and niacin, and for choline, RDAs are higher for men than for women. For vitamin A, body weight partly determines the RDA (12); thus, the RDA is higher for men than for women, because the reference body weight for an average man is higher than for an average woman. For vitamin C, the blood response to vitamin C treatment differs between men and women, partly due to gender differences in body size (13–15). For choline, animal studies suggest that males have a higher choline requirement (16) than females (17). For vitamin K, the RDA is derived from intake data and intake is usually higher in men than in women (18). For thiamin, riboflavin, and niacin, the gender differences in RDA are not based on data but on the assumption that men have higher nutritional needs because they have a larger body size (17).

In this study, we assessed whether men and women differ in their change in the concentration of folate in erythrocytes in response to a fixed dose of folic acid and, if so, if differences in body size between men and women explain this difference. The concentration of folate in erythrocytes reflects tissue folate stores and provides the best information about the long-term folate status of humans; it was used as the primary indicator to set the RDA for folate by the US Institute of Medicine in 2000 (6). Our prior hypothesis was that the change in concentration of folate in erythrocytes to folic acid treatment would be inversely associated with lean body mass, because folate is a watersoluble vitamin. In pharmacology, lean body mass is often used to predict the loading dosage of water-soluble drugs (19).

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Methods

Study population

We analyzed data from 2 folic acid supplementation trials: a 3- y trial (20,21) performed between September 2000 and December 2004 and a 12-wk trial (22) performed in 2001.

The 3- y trial. The effect of folic acid on intima-media thickening, cognitive function, and hearing was evaluated in this double-blind, placebo-controlled, randomized trial. A total of 819 men and postmenopausal women ingested placebo or folic acid capsules (800 μg/d) for 3 y. Important inclusion criteria were that subjects had to be between 50 and 70 y old and have a plasma homocysteine concentration (≥13 μmol/L but <26 μmol/L), renal or thyroid diseases, use of B-vitamin supplements, and use of medications that influence folate metabolism or atherosclerotic progression (e.g., lipid-lowering and hormone replacement therapies). A total of 819 of 4200 subjects met all inclusion criteria and were randomized. For full details of enrollment, see Durga et al. (20). Subjects were asked to refrain from the use of B vitamins during the study. Subjects provided blood samples after fasting at baseline and after 3 y of intervention. Erythrocyte folate was analyzed with an immunoassay (Immulite 2000, Diagnostic Products); intra and interassay variation coefficients were <15% (20). Compliance of the subjects to folic acid treatment was judged by capsule-return counts and by a diary that registered missed capsules. The Medical Ethics Committee of Wageningen University approved the study and subjects gave written informed consent.

The 12-wk trial. The effect of various doses of folic acid on homocysteine was studied in this double-blind, placebo-controlled, randomized, parallel trial. Subjects in the folic acid groups daily ingested a capsule with 50, 100, 200, 400, 600, or 800 μg of folic acid; subjects in the placebo group ingested a capsule daily without folic acid. The subjects had to be between 50 and 70 y old, have a plasma homocysteine concentration <26 μmol/L, and all women had to be postmenopausal. Major exclusion criteria were renal or thyroid diseases, use of medication that influences folate metabolism, and use of dietary supplements containing B vitamins. Out of 353 applicants, 316 met the inclusion criteria and were included in the trial [for full details of enrollment, see van Oort et al. (22)]. Subjects were asked to refrain from the use of B vitamins during the study. Subjects provided a fasting blood sample at the start of the intervention and after 12 wk of treatment. Erythrocyte folate was analyzed in the same laboratory and according to the same method as described above for the 3-y trial. Compliance of the subjects was judged by capsule-return counts. All participants gave written informed consent to the protocol that was approved by the Medical Ethical Committee of Wageningen University.

Calculation of body size

Weight and height were measured at baseline in both studies. We derived lean body mass and body surface area with the following formulas (19,23–25). For men:

\[
\text{Lean body mass in kg} = 1.1013 \times \text{weight in kg} - 0.0128 \times (\text{weight in kg/height in m})^2.
\]

For women:

\[
\text{Lean body mass in kg} = 1.07 \times \text{weight in kg} - 0.0148 \times (\text{weight in kg/height in m})^2.
\]

For both men and women:

\[
\text{Body surface area in m}^2 = \text{weight in kg}^{0.425} \times (100 \times \text{height in m})^{0.725} \times 0.007184.
\]

Statistical analysis

Effect of gender. We assessed if men and women differ in their response in the concentration of folate in erythrocytes to a fixed dose of folic acid and, if so, if differences in body size between men and women explained this difference. The response in erythrocyte folate is the absolute change in the concentration of folate in erythrocytes from baseline to the end of the study period. For the 3-y trial, we calculated the mean and the 95% CI of the difference between men and women in the response of erythrocyte folate to folic acid. We did not correct for the small changes seen in the placebo group. For the 12-wk trial, we constructed the following linear regression model to describe our data:

\[
\text{Response of erythrocyte folate (nmol/L)} = \text{intercept} + \beta_{\text{dose of folic acid}} \times \text{dose of folic acid in μg/d} + \beta_{\text{gender}} \times \text{gender} + \beta_{\text{gender} \times \text{dose}} \times (\text{gender} \times \text{dose}).
\]

We included gender as an interaction term in the model (gender × dose), because we considered it biologically plausible that any difference in response between men and women would increase with increasing doses of folic acid.

We checked if potentially confounding factors (alcohol intake, smoking, and change in dietary folate intake during the intervention) changed the βgender >10%. These factors did not change the association and, therefore, we did not include them in the final models.

Effect of body size. We used linear regression to assess if body size influenced the response of erythrocyte folate to folic acid. For the 3-y trial, the response of erythrocyte folate was the dependent variable and body size (lean body mass, height, weight, or body surface area) was the independent variable:

\[
\text{Response of erythrocyte folate (nmol/L)} = \text{intercept} + \beta_{\text{body size}} \times \text{body size}.
\]

For the 12-wk trial, response of erythrocyte folate was the dependent variable and dose of folic acid (in μg/d), body size, and the interaction term (body size × dose) were the independent variables:

\[
\text{Response of erythrocyte folate (nmol/L)} = \text{intercept} + \beta_{\text{dose of folic acid}} \times \text{dose of folic acid in μg/d} + \beta_{\text{body size}} \times \text{body size} + \beta_{\text{body size} \times \text{dose}} \times (\text{body size} \times \text{dose}).
\]

We included body size as an interaction term, because we reasoned that any difference in response between subjects with a large body size and a small body size would increase with increasing intakes of folic acid.

We checked if potentially confounding factors (alcohol intake, smoking, and change in dietary folate intake during the intervention) changed the β for body size >10%. These factors did not change the association and, therefore, we did not include them in the final models.

Combined effect of gender and body size. We used the following method to assess how much of the difference between men and women in response of erythrocyte folate concentrations to folic acid intake was explained by differences in body size (26). We first calculated (βgender)gender which is the βgender in a linear regression model in which gender (women = 0, men = 1) was the main independent variable that explained differences in response in erythrocyte folate. Next, we included 1 of the indicators of body size in the model and again assessed the β for gender: (βgender)gender and body size. Finally, we calculated the percentage of the gender difference in erythrocyte folate response that was explained by body size:

\[
\text{Percentage} = 100 \times \frac{[(\beta_{\text{gender}})_{\text{gender}} - (\beta_{\text{gender}})_{\text{gender and body size}}]/(\beta_{\text{gender}})_{\text{gender}}}{(\beta_{\text{gender}})_{\text{gender}}}
\]

For a detailed description of the models, see the Appendix. Baseline characteristics of men and women were compared using Student’s t test for normally distributed variables and Mann-Whitney U tests for nonnormally distributed variables. Values in the text are means ± SD or means (95% CI).

Gender difference in folate requirements 1457
Results

Both trials included more men than women. Compliance with folic acid treatment was high: 99% as assessed from capsule returns (20,22) and did not differ between men and women. All participants were white Caucasians. As expected, men were significantly taller and heavier than women and had a larger lean body mass and body surface area. Erythrocyte folate status did not differ between men and women at baseline and habitual intake of dietary folate was higher in men than in women (Table 1).

During the 3-y trial, the concentration of folate in erythrocytes increased by 1391 ± 512 nmol/L in men and by 1534 ± 77 nmol/L in women; the mean difference of 143 nmol/L (95% CI 46, 241) was significant (Table 2 and Table 3). Thus, erythrocyte folate increased ~10% less in men than in women.

### TABLE 1 Baseline characteristics of men and women who participated in 3-y and 12-wk folic acid trials

<table>
<thead>
<tr>
<th></th>
<th>3-y Trial</th>
<th>12-wk Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Men/women, n</strong></td>
<td>294 / 112</td>
<td>187 / 129</td>
</tr>
<tr>
<td><strong>Age, y</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>60 ± 6</td>
<td>60 ± 6</td>
</tr>
<tr>
<td>Women</td>
<td>61 ± 5</td>
<td>60 ± 6</td>
</tr>
<tr>
<td><strong>Height, m</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.79 ± 0.06</td>
<td>1.76 ± 0.07</td>
</tr>
<tr>
<td>Women</td>
<td>1.66 ± 0.06*</td>
<td>1.68 ± 0.08*</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>86 ± 11</td>
<td>84 ± 14</td>
</tr>
<tr>
<td>Women</td>
<td>73 ± 13*</td>
<td>75 ± 12*</td>
</tr>
<tr>
<td><strong>Lean body mass, kg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>65 ± 6</td>
<td>63 ± 7</td>
</tr>
<tr>
<td>Women</td>
<td>49 ± 4*</td>
<td>50 ± 5*</td>
</tr>
<tr>
<td><strong>Body surface area, m²</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>2.05 ± 0.15</td>
<td>2.00 ± 0.18</td>
</tr>
<tr>
<td>Women</td>
<td>1.80 ± 0.15*</td>
<td>1.84 ± 0.17*</td>
</tr>
<tr>
<td><strong>Dietary folate intake, µg/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>201 (161, 245)</td>
<td>174 (145, 207)</td>
</tr>
<tr>
<td>Women</td>
<td>180 (150, 216)*</td>
<td>160 (136, 197)*</td>
</tr>
<tr>
<td><strong>Change in dietary folate intake during intervention, µg/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>15 ± 65</td>
<td>—</td>
</tr>
<tr>
<td>Women</td>
<td>17 ± 80</td>
<td>—</td>
</tr>
<tr>
<td><strong>Current smokers, %</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Women</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td><strong>Alcohol intake, g/d</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>15 (6.25)</td>
<td>12 (3.23)</td>
</tr>
<tr>
<td>Women</td>
<td>7 (1.17)</td>
<td>6 (1.18)</td>
</tr>
<tr>
<td><strong>Serum folate, nmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>12.1 ± 4.0</td>
<td>12.2 ± 4.1</td>
</tr>
<tr>
<td>Women</td>
<td>12.9 ± 5.0</td>
<td>13.6 ± 4.2*</td>
</tr>
<tr>
<td><strong>Erythrocyte folate, nmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>699 ± 270</td>
<td>727 ± 258</td>
</tr>
<tr>
<td>Women</td>
<td>659 ± 298</td>
<td>726 ± 249</td>
</tr>
<tr>
<td><strong>Plasma total homocysteine, µmol/L</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>13.4 ± 2.6</td>
<td>12.1 ± 2.9</td>
</tr>
<tr>
<td>Women</td>
<td>13.0 ± 2.8</td>
<td>10.9 ± 3.0*</td>
</tr>
</tbody>
</table>

1 Values are means ± SD or median (interquartile range). *Different from men, P < 0.05.

In the placebo group, the increases in erythrocyte folate in men (50 ± 327 nmol/L) and women (61 ± 253 nmol/L) did not differ.

In the 12-wk trial, men generally had a lower erythrocyte folate response than women (Fig. 1) and the change was significantly less in men than in women for 2 of the doses administered (Table 2). Linear regression analysis using the pooled data from all groups showed that the dose-response curve for men was lower than that for women (P = 0.022 for β_{gender}) (Table 4), although the slopes of the dose-response curves did not differ between men and women (P = 0.674 for the interaction term gender × dose).

Response of erythrocyte folate (nmol/L) = 62 + 1.054 × dose of folic acid in µg/d + 47 × gender. At an intake of 800 µg/d of folic acid, which was the dose used in the 3-y trial, the response in erythrocyte folate in men would be 5% lower than in women: 858 nmol/L for men and 905 nmol/L for women.

Table 2

<table>
<thead>
<tr>
<th>Dose of folic acid, µg/d</th>
<th>Men</th>
<th>Women</th>
<th>Men</th>
<th>Women</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-y trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>286</td>
<td>120</td>
<td>50 ± 327</td>
<td>61 ± 255</td>
<td>−11 (−77, 55)</td>
</tr>
<tr>
<td>800</td>
<td>285</td>
<td>110</td>
<td>1391 ± 413</td>
<td>1534 ± 512</td>
<td>−143 (−241, −46)*</td>
</tr>
<tr>
<td>12-wk trial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>28</td>
<td>22</td>
<td>3 ± 126</td>
<td>29 ± 93</td>
<td>−26 (−91, 38)</td>
</tr>
<tr>
<td>50</td>
<td>24</td>
<td>18</td>
<td>22 ± 108</td>
<td>96 ± 83</td>
<td>−74 (−136, −12)*</td>
</tr>
<tr>
<td>100</td>
<td>20</td>
<td>20</td>
<td>130 ± 114</td>
<td>129 ± 137</td>
<td>4 (−77, 85)</td>
</tr>
<tr>
<td>200</td>
<td>24</td>
<td>19</td>
<td>252 ± 115</td>
<td>323 ± 129</td>
<td>−71 (−146, 4)</td>
</tr>
<tr>
<td>400</td>
<td>29</td>
<td>29</td>
<td>511 ± 186</td>
<td>574 ± 251</td>
<td>−63 (−203, 77)</td>
</tr>
<tr>
<td>600</td>
<td>21</td>
<td>23</td>
<td>616 ± 201</td>
<td>751 ± 208</td>
<td>−134 (−269, 0)*</td>
</tr>
<tr>
<td>800</td>
<td>27</td>
<td>16</td>
<td>825 ± 248</td>
<td>833 ± 294</td>
<td>−8 (−177, 161)</td>
</tr>
</tbody>
</table>

1 Values are means ± SD or mean difference (95%CI). *Difference between men and women, P < 0.05.

FIGURE 1 Responses of erythrocyte folate to various doses of folic acid in men and women in the 3-y and 12-wk trials. Values are means and 95% CI.
We calculated how the RDA for men and women should differ if we assume that men need more folic acid than women to achieve the same increase in erythrocyte folate. The current RDA for folate is 400 μg/d of dietary folate equivalents or 200 μg of folic acid. At an intake of 200 nmol/L, the response would be 47 nmol/L lower in men than in women; men would have to ingest an additional (47/1.054) = 45 μg/d of folic acid to achieve the same response. In the calculation of the RDA, folic acid is assumed to be twice as bioavailable as than food folate. Based on this assumption, the RDA for men should be 400 dietary folate equivalents per day.

In both trials, greater body size (lean body mass, height, weight, or body surface area) was associated with a lower response of erythrocyte folate when gender was not included in the linear regression models (Tables 3 and 4). There was no interaction between body size and dose in the 12-wk trial (data not shown).

Differences in lean body size explained 56% of the difference in response of erythrocyte folate between men and women in the 3-y trial and 70% in the 12-wk trial. Height, weight, or body surface area explained less of the difference than lean body mass (Table 5).

Lean body mass was correlated with gender; the correlation coefficient was 0.81 for the 3-y trial and 0.72 in the 12-wk trial (Table 5). The other measures of body size were also associated with gender.

### Discussion

The response in erythrocyte folate to folic acid treatment in men was lower than in women in a large 3-y trial with folic acid supplements. In a 2nd folic acid intervention that lasted 12 wk, we showed that the response in erythrocyte folate was lower in men than in women, although we could not show that this difference increased with increasing intakes of folic acid. It is

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**TABLE 3** Linear regression coefficients for the association between the response of erythrocyte (nmol/L) folate to folic acid treatment and gender and body size in the 3-y trial

| Predictors                        | β Estimate | 95%CI    | P-value *
|-----------------------------------|------------|---------|----------------
| Gender                            | 1534       | (1451, 1617) | 0.004
| Gender (women = 0, men = 1)       | -143       | (-241, -46)  | 0.004
| Body size                         | 1882       | (1597, 2177) | 0.002
| Lean body mass, kg                | -7.49      | (-12.32, -2.65) | 0.002
| Intercept                         | 2828       | (1947, 3710) | 0.002
| Height, m                         | -796       | (-1297, -295) | 0.002
| Intercept                         | 1773       | (1490, 2055) | 0.002
| Weight, kg                        | -4.16      | (-7.56, -0.76) | 0.017
| Intercept                         | 2138       | (1959, 2916) | 0.004
| Body surface area, m²             | -357       | (-598, -116)  | 0.004

**TABLE 4** Linear regression coefficients for the association between the response of erythrocyte folate to folic acid treatment and gender and body size in the 12-wk trial

| Predictors                        | β Estimate | 95%CI    | P-value *
|-----------------------------------|------------|---------|----------------
| Gender                            | 62         | (25, 98) | 0.022
| Dose, μg/d folic acid             | -1.054     | (0.984, 1.125) | 0.022
| Gender (women = 0, men = 1)       | -47        | (-88, -7)  | 0.022
| Body size                         | 237        | (104, 371) | 0.002
| Lean body mass, kg                | 1.053      | (0.984, 1.123) | 0.002
| Dose, μg/d folic acid             | -3.5       | (-5.8, -1.26) | 0.002
| Intercept                         | 539        | (128, 951) | 0.002
| Height, m                         | 1.053      | (0.982, 1.123) | 0.002
| Dose, μg/d folic acid             | -292       | (-530, -54)  | 0.016
| Intercept                         | 177        | (53, 302)  | 0.016
| Weight, kg                        | 1.048      | (0.978, 1.118) | 0.016
| Lean body mass, kg                | -1.77      | (-3.285, -0.257) | 0.016
| Dose, μg/d folic acid             | 308        | (97, 520)  | 0.016
| Body surface area, m²             | -1.050     | (-980, 1.120) | 0.016
| Dose, μg/d folic acid             | 227        | (59, 395)  | 0.016
| Lean body mass, kg                | 1.051      | (0.984, 1.124) | 0.016
| Dose, μg/d folic acid             | -6         | (-63, 52)  | 0.048
| Lean body mass, kg                | -3.3       | (-6.6, -0.034) | 0.048
| Dose, μg/d folic acid             | 407        | (-49, 863) | 0.048
| Lean body mass, kg                | 1.055      | (0.985, 1.125) | 0.048
| Dose, μg/d folic acid             | -31        | (-77, 15)  | 0.088
| Height, m                         | -206       | (-476, 65)  | 0.136
| Dose, μg/d folic acid             | 161        | (36, 287)  | 0.136
| Lean body mass, kg                | 1.052      | (0.982, 1.122) | 0.136
| Dose, μg/d folic acid             | -36        | (-78, -7)  | 0.101
| Weight, kg                        | -1.33      | (-2.93, 0.27) | 0.103
| Dose, μg/d folic acid             | 258        | (34, 481)  | 0.103
| Lean body mass, kg                | 1.053      | (0.983, 1.123) | 0.103
| Dose, μg/d folic acid             | -31        | (-75, 14)  | 0.174
| Body surface area, m²             | -107       | (-226, 13) | 0.082

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1 P-value for F-test of the total model was < 0.01 for all models; n = 396 (285 men, 110 women).

2 P-value for the β estimate.

3 The model is: Response of erythrocyte folate (nmol/L) = intercept + (βgender × gender).

4 The model is: Response of erythrocyte folate (nmol/L) = intercept + βbody size × body size.

5 The model is: Response of erythrocyte folate (nmol/L) = intercept + (βgender × gender and bodysize × gender + βbody size × body size).

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biologically plausible that men would have a lower response to folic acid; in general, men have a larger body size and therefore the dose of folic acid distributes over a larger volume. Our data show that gender differences in body size explain a large part of the gender difference in the response of erythrocyte folate.

Erythrocyte folate concentrations formed the primary indicator to estimate the RDA for folate, sometimes used in conjunction with plasma homocysteine and serum or plasma folate concentrations (6). The Institute of Medicine considered erythrocyte folate status the best indicator of long-term status and the primary indicator for folate adequacy (6). This means that changes in erythrocyte folate status are informative about changes in folate adequacy. Concurrently, if there is a gender difference in the amount of folic acid that is needed to achieve a certain change in erythrocyte status, this means there is a gender difference in the amount of folic acid required to achieve folate adequacy. Our data suggest that men need more folic acid to reach folate adequacy, which could suggest that the RDA for folate for men should be higher than for women. However, additional research should first confirm that gender and/or body size differences in folate responses are truly present. A study that further elucidate if any gender difference in response to folic acid treatment results from differences in lean body mass.

The men in our studies had higher dietary intake of folate than the women, which has also been shown by others (1,2). However, erythrocyte folate status did not differ between men and women in our studies and those of others (3–5). This supports our findings that men need more folate to achieve the same erythrocyte folate concentration.

We showed that the gender difference in the response of erythrocyte folate to folic acid treatment is largely explained by gender differences in body size. If future studies indeed confirm these findings, this could suggest that the RDA for folate should either be gender specific or that it should be set on body weight basis (per kilogram lean body weight). In this article, we focused on the possibility of a gender-specific RDA, to be consistent with recommendations for other vitamins. The RDA for many other vitamins is higher for men than for women, mainly because men have a larger body size (17).

We assumed linearity in the change in concentration of folates in erythrocytes in response to various doses of folic acid treatment in the 12-wk trial. The response of folates in serum to various doses of folic acid is linear (27); however, there are, to our knowledge, no trials that studied the dose response in folate in erythrocytes. We decided to use the simplest, and thus linear model, to describe our data, because the data from Figure 1 did not point to clear deviations from linearity.

Two other studies reported the response of folate concentrations in blood to folic acid separately for men and women. Both studies found a lower response in men than in women (28,29) and are therefore consistent with our findings. Coppen et al. (28) found that the plasma folate response to 500 μg of folic acid for 10 wk was 9.4 nmol/L in men and 19.7 nmol/L in women, but did not statistically test this difference. Van der Griend et al. (29) reported that post-treatment plasma folate concentrations were significantly lower in men than in women (median 38 nmol/L in men compared with 52 nmol/L in women) after a daily dose of 500 μg of folic acid for 8 wk, but did not report baseline values separately for men and women.

Gender differences in lean body mass explained at least 50% of the gender difference in response of erythrocyte folate to folic acid, but gender correlated strongly with lean body mass as well. Therefore, we could not completely separate the association with gender from the association with body size. Gender or body size were no longer always significantly associated with the response of erythrocyte folate when gender and body size were simultaneously included in the models (Tables 3 and 4). However, the F-test for the total model including gender and body size simultaneously was significant; the model with gender and body size predicted response of erythrocyte folate better than the model without these variables. We therefore assume that the separate variables no longer significantly contributed to the response because of colinearity. Our data clearly suggest that the response in erythrocyte folate differs between men and women, but our estimate of the contribution of lean body mass to the gender effect may not be entirely correct.

Several factors may limit the generalizability of our findings. First, all participants were aged between 50 and 70 y and all women were postmenopausal. Yet folic acid absorption does not seem to depend on age (30,31), but premenopausal women lose folate monthly through their menstruation. However, the amount they lose is negligible: menstrual blood loss is ~25–40 mL per cycle (32). With an average erythrocyte folate concentration of 1000 nmol/L and a hematocrit of 0.45, folate loss ranges from 11–18 nmol (or 5–8 ng) per menstrual cycle and would be negligible. Another factor that could limit generalizability is that subjects in the 3-y trial had high plasma homocysteine concentrations (>13 μmol/L) and therefore this population was not representative of a normal population. Nevertheless, participants in the 12-wk trial had normal homocysteine levels and that trial also suggested gender differences in the response of erythrocyte folate. Moreover, we are not aware of data that suggest that the absorption or bioavailability of folic acid is influenced by homocysteine or folate status (33). Nevertheless, future studies should confirm our findings in a general population.

We calculated that men might need an additional 45 μg of folic acid equivalent to ~90 μg of dietary folate equivalents. This probably slightly overestimates the additional dietary folate that men need, because we recently showed that bioavailability of folic acid relative to dietary folate was 100/80 = 1.25 (34) instead of the factor 2 used in the calculation of the U.S. dietary folate equivalents. However, we used the factor 2 in our calculations, because it was also used to derive the 400 μg of dietary folate equivalents of the current U.S. RDA.
Do men reach an intake of 490 μg of dietary folate equivalents per day in real life? Although men have a higher dietary folate intake than women, data from the NHANES showed that a large proportion of men do not ingest 490 μg/d of dietary folate equivalents; the median dietary folate intake of men varied with age and ethnicity and ranged from 312 to 552 μg/d (35). Men would have to increase their intake of fortified foods or of supplements to reach this recommendation.

In conclusion, our study suggests that men need more folic acid than women to increase their folate status by the same extent, probably because they have a larger body size. The higher need for folate of larger persons will only partly be met by a higher intake of food and thus of folate. Our findings could indicate that the RDA for folate should be higher for men than for women.

**Literature Cited**


