Lifestyle Factors and Plasma Homocysteine Concentrations in a General Population Sample

Angelika de Bree,1,2 W. M. Monique Verschuren,1 Henk J. Blom,2 and Daan Kromhout1

The authors cross-sectionally investigated the extent to which coffee, tea, and alcohol consumption, physical activity, and smoking were associated with nonfasting total plasma homocysteine concentrations in a random sample of 3,025 Dutch adults aged 20–65 years from a population-based cohort examined in 1993–1996 (n = 19,066). The lifestyle factors most strongly associated with plasma total homocysteine level were smoking (positive), alcohol drinking (negative), and coffee consumption (positive). The smoking effect was most prominent in women, and the alcohol effect was most pronounced in men. Data indicated that independently of other lifestyle factors, age, and intake of folate and B vitamin supplements, a change in lifestyle could result in a 0.1- to 1.7-µmol/liter change in plasma total homocysteine level. The authors conclude that lifestyle changes could result in a public-health-relevant change in plasma total homocysteine concentrations. Am J Epidemiol 2001;154:150–4.

From a public health viewpoint, it is important to identify modifiable factors that influence plasma total homocysteine levels, because elevated concentrations are associated with an increased risk of cardiovascular disease (1, 2). Folate intake is identified as the most important dietary determinant of plasma total homocysteine concentration (3, 4). Besides diet, other lifestyle factors such as smoking (5), physical activity (6), and consumption of alcohol (7–9), coffee (5, 10–13), and tea (10) may have an effect on plasma total homocysteine levels in the general population. Inconclusive results for alcohol and coffee consumption and a lack of representative populations with a broad age range create the need to investigate further. In addition, only two studies have corrected for the confounding effect of folate intake (7, 11). Using a representative sample of the general Dutch population aged 20–65 years, we studied the association of these lifestyle factors with plasma total homocysteine concentrations, taking folate intake into account.

MATERIALS AND METHODS

The subjects and methods of this study have been described extensively elsewhere (14). Briefly, we drew an age- and sex-stratified random sample (n = 3,025) of subjects in a population-based cohort that was examined during 1993–1996 (n = 19,066) (15).

Data were collected with self-administered questionnaires and a physical examination. On the basis of responses to questions about smoking, we categorized subjects as either nonsmokers or light (<10 cigarettes/day), moderate (≥10–<20 cigarettes/day), or heavy (≥20 cigarettes/day) smokers. Physical activity was reported as the average amount of time (minutes/week) spent in various activities during leisure time, work time, household activity, and commuting (e.g., cycling to work) over the previous year. For the statistical analyses, we defined four levels of weekly activity: “sedentary,” <0.5 hours; “moderately active,” ≥0.5–<3.5 hours; “active,” ≥3.5 hours, of which ≤2 hours was heavy activity; and “very active,” ≥3.5 hours, of which >2 hours was heavy activity (16). Information from subjects who participated in 1993 (n = 408) was not included, since the questions on activity in 1993 were different from those posed in 1994–1996.

A semiquantitative food frequency questionnaire (17, 18) provided information on the consumption of alcohol, coffee, and tea, which was recoded into units per day. Energy-adjusted folate intake was estimated as described elsewhere (4).

Plasma total homocysteine concentrations from nonfasting venous blood samples were determined as described by Fiskerstrand et al. (19), with some modifications (20). We used SAS software (version 6.12) for all statistical analyses (SAS Institute, Inc., Cary, North Carolina). Comparisons between men and women were conducted using Wilcoxon’s two-sample tests for continuous variables and chi-squared tests for proportions. The distribution of plasma total homocysteine values was normalized by logarithmic transformation. Thus, geometric means are presented.
unless stated otherwise. Each lifestyle–plasma total homocysteine association was evaluated by univariate and multivariate linear regression. Differences in adjusted mean plasma total homocysteine concentrations as compared with a reference category were tested using analyses of covariance. In the multivariate models, we adjusted for age (years), folate intake (µg/day), intake of B vitamin supplements (no/yes), and the other studied lifestyle factors. When considered as confounders, the lifestyle factors were included continuously, except for smoking, which was included as three indicator variables (light, moderate, and heavy smoking) with nonsmokers used as the reference group.

RESULTS

Age and the prevalences of inactivity and smoking were equal for men and women (table 1). Women had a lower plasma total homocysteine concentration, folate intake, and alcohol and coffee consumption than men but higher tea consumption than men.

Univariate linear regression (table 2), in which the beta coefficients express a proportional change in plasma total homocysteine level because of the logarithmic transformation, showed that in both men and women coffee consumption was positively associated, tea consumption was negatively associated, and physical activity was not associated with plasma total homocysteine concentration. Alcohol consumption showed a negative association with plasma total homocysteine level in men only, and smoking showed a positive association in women only.

The multivariate results showed essentially the same associations, except that the inverse relation with tea disappeared and activity level became positively associated in women. In table 2, results for alcohol consumption in men are also presented stratified for folate intake (below and above the median intake level of 204 µg/day), because of the interaction between alcohol and folate intake (4). Smoking also showed an interaction with folate intake in men (4), demonstrating a significant positive association among men with folate intakes below the first quintile (i.e., <176 µg/day). We did not stratify the results for folate intake, since this would only have affected the power of the analyses, whereas the direction of the association between smoking and plasma total homocysteine remained positive.

### TABLE 1. Selected characteristics of Dutch men and women aged 20–65 years who were examined in 1993–1996

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men (n = 1,493)†</th>
<th>Women (n = 1,532)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean or %</td>
<td>Mean or %</td>
</tr>
<tr>
<td></td>
<td>SD†</td>
<td>SD§</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.5</td>
<td>41.0</td>
</tr>
<tr>
<td>Plasma total homocysteine§ (µmol/liter)</td>
<td>14.6</td>
<td>13.1*</td>
</tr>
<tr>
<td>Folate intake§ (µg/day)</td>
<td>240</td>
<td>194*</td>
</tr>
<tr>
<td>Coffee consumption (cups/day)</td>
<td>4.9</td>
<td>4.1*</td>
</tr>
<tr>
<td>Tea consumption (cups/day)</td>
<td>1.5</td>
<td>2.1*</td>
</tr>
<tr>
<td>Alcohol consumption (glasses/day)</td>
<td>1.9</td>
<td>0.7*</td>
</tr>
<tr>
<td>Less than 30 minutes of moderate or heavy physical activity per week (%)</td>
<td>21.7</td>
<td>20.9</td>
</tr>
<tr>
<td>Current smoker (%)</td>
<td>35.4</td>
<td>37.3</td>
</tr>
</tbody>
</table>

* p < 0.001 (significantly different from males).
† The minimum number of valid observations was 1,488 for men and 1,529 for women, except for physical activity, where the percentages were based on 1,297 men and 1,319 women.
‡ SD, standard deviation.
§ Arithmetic value.

### TABLE 2. Proportional change in plasma total homocysteine concentration associated with a one-unit change in various lifestyle factors among Dutch men and women aged 20–65 years who were examined in 1993–1996

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Men Univariate analysis (n = 1,493)*</th>
<th>Men Adjusted† analysis (n = 1,287)*</th>
<th>Women Univariate analysis (n = 1,532)*</th>
<th>Women Adjusted† analysis (n = 1,314)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% 95% CI*</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
<td>% 95% CI</td>
</tr>
<tr>
<td>Coffee consumption (cups/day)</td>
<td>0.77 0.32, 1.22</td>
<td>0.48 −0.02, 0.99</td>
<td>1.67 1.20, 2.14</td>
<td>1.03 0.46, 1.60</td>
</tr>
<tr>
<td>Tea consumption (cups/day)</td>
<td>−1.03 −1.77, −0.29</td>
<td>−0.43 −1.25, 0.38</td>
<td>−1.00 −1.56, −0.44</td>
<td>−0.18 −0.80, 0.44</td>
</tr>
<tr>
<td>Alcohol consumption (glasses/day)</td>
<td>−1.15 −1.77, −0.54</td>
<td>−1.06 −1.71, −0.41</td>
<td>−0.16 −1.40, 1.08</td>
<td>−0.47 −1.77, 0.82</td>
</tr>
<tr>
<td>Low folate intake§</td>
<td>−1.52 −2.73, −0.31</td>
<td>−0.79 −1.52, −0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High folate intake†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity#</td>
<td>0.39 −1.08, 1.86</td>
<td>1.05 −0.44, 2.54</td>
<td>0.34 −1.16, 1.84</td>
<td>1.71 0.25, 3.16</td>
</tr>
<tr>
<td>Nonsmoking vs. smoking</td>
<td>1.79 −1.19, 4.77</td>
<td>0.84 −2.38, 4.07</td>
<td>6.21 3.45, 8.97</td>
<td>3.56 0.52, 6.59</td>
</tr>
</tbody>
</table>

* The minimum number of valid observations was 1,488 for men and 1,529 for women, except for physical activity, where the data were based on 1,297 men and 1,319 women. Multivariate results were based on a minimum of 1,287 men and 1,314 women.
† Adjustments were made for age, intake of folate and vitamin B supplements, and the other lifestyle factors in the table; in the analyses in which smoking was considered as a confounder, the data were adjusted with indicator variables, using nonsmokers as the reference group.
‡ CI, confidence interval.
§ Below the median value of 204 µg/day.
# Refer to Materials and Methods section in text for definition of categories.
The adjusted results for coffee, alcohol, and smoking are displayed in figures 1–3, respectively. Drinking >6 cups of coffee per day was associated with a 1.4-µmol/liter higher plasma total homocysteine concentration in men and a 1.1-µmol/liter higher concentration in women, as compared with coffee abstainers (figure 1). Drinking >2 glasses of alcoholic beverages per day was associated with a 1.7-µmol/liter lower plasma total homocysteine level in men with a low folate intake (<200 µg/day) and a 0.8-µmol/liter lower level in men with a high folate intake (>204 µg/day), as compared with alcohol abstainers (figure 2). Women who smoked heavily had a 0.8-µmol/liter higher plasma total homocysteine concentration than women who did not smoke (figure 3).

**FIGURE 1.** Mean adjusted plasma total homocysteine (P-tHcy) concentrations according to coffee consumption among Dutch men (⧫) and women (▴) aged 20–65 years examined in 1993–1996. Mean values were adjusted for age, tea consumption, alcohol consumption, smoking, physical activity, and intake of folate and B vitamin supplements. Asterisks indicate a significantly different plasma total homocysteine concentration in comparison with the first category (**p < 0.01; ***p < 0.001). Bars, 95% confidence interval.

**FIGURE 2.** Mean adjusted plasma total homocysteine (P-tHcy) concentrations according to alcohol consumption among Dutch men (⧫) and women (▴) aged 20–65 years examined in 1993–1996. Results for men are stratified by folate intake (low intake, <204 µg/day; high intake, >204 µg/day). Mean values were adjusted for age, tea consumption, coffee consumption, smoking, physical activity, and intake of folate and B vitamin supplements. Asterisks indicate a significantly different plasma total homocysteine concentration in comparison with the first category (**p < 0.01). Bars, 95% confidence interval.

**FIGURE 3.** Mean adjusted plasma total homocysteine (P-tHcy) concentrations according to cigarette smoking among Dutch men (⧫) and women (▴) aged 20–65 years examined in 1993–1996. Mean values were adjusted for age, consumption of tea, coffee, and alcohol, physical activity, and intake of folate and B vitamin supplements. Asterisks indicate a significantly different plasma total homocysteine concentration in comparison with the first category (**p < 0.05; ***p < 0.001). Bars, 95% confidence interval.

**DISCUSSION**

In this study, smoking and coffee and alcohol consumption were associated with plasma total homocysteine concentrations independently of other lifestyle factors, age, use of B vitamin supplements, and folate intake, the latter being the major dietary determinant of plasma total homocysteine concentration. The effect of smoking was most pronounced in women, and the alcohol effect was most pronounced in men.

The positive dose-response relation that we found between coffee consumption and plasma total homocysteine concentration is consistent with most (10–12) but not all (13) observational studies. Reservations about the effect of coffee on plasma total homocysteine level have been refuted by recent intervention studies (21, 22). The randomized crossover designs of the latter studies increase the likelihood of there being a causal relation between coffee consumption and plasma total homocysteine concentration.

The intervention studies showed similar effects for filtered coffee (1.5 µmol/liter) (21) and unfiltered coffee (1.2 µmol/liter) (22), indicating that the factor which raises plasma total homocysteine is not removed by the use of paper filters. Caffeine is a proposed factor (10, 22), as it
might obstruct the conversion from homocysteine to cysteine by acting as a vitamin B6 antagonist (22). Therefore, the null finding for tea in the present study was not unexpected, since the amount of caffeine in tea is small (23). Intervention studies with assessment of both decaffeinated coffee and regular coffee would provide more insight on this topic.

High levels of alcohol consumption increase plasma total homocysteine concentration (24, 25); however, we studied moderate alcohol consumption (only 16 percent of our male drinkers consumed ≥4 glasses/day). An inverse relation between moderate alcohol consumption and plasma total homocysteine levels has been observed previously in men from Caerphilly (7) and in Norwegian men and women (8). However, as in the present study, no statistically significant association was found among US women in another study (9); this could be due to the small range of alcohol intakes in women.

The fact that we observed no association in women could indicate a dose effect of ethanol. On the other hand, the type of alcoholic beverage consumed could be important (25). Beer was the most frequently consumed alcoholic beverage among men, and it contains folate, vitamin B3, and vitamin B6, all vitamins that serve as cofactors in homocysteine metabolism. Although residual confounding cannot be excluded, beer consumption was significantly inversely associated with plasma total homocysteine concentration independently of these vitamins. This might point to ethanol or another substance in beer (other than B vitamins) being the plasma total homocysteine-lowering factor. Betaine, which is present in wine (26), could also have contributed to the overall inverse relation between alcohol and plasma total homocysteine level. Betaine is used in a metabolic route independent of folate to methylate homocysteine to methionine (27). Because of the lack of dietary data on betaine, we were unable to adjust for it.

In the multivariate analyses, we observed a weak positive relation between physical activity and plasma total homocysteine level in women. This contrasts with the protective effect observed in the Hordaland Homocysteine Study (6). Since activity is generally associated with a healthier lifestyle (28), our finding is the opposite of what would be expected. Intervention studies might be able to elucidate the effect of activity on plasma total homocysteine concentration.

In accordance with other population-based studies (6, 9), smoking was positively associated with plasma total homocysteine level, the effect being clearest in women. The mechanism behind the increase is unidentified. Smokers often have a lower plasma folate status than nonsmokers (29–31); however, in the present study, this was explained by a lower folate intake. Thus, smoking does not seem to reduce the availability of folate for the remethylation of homocysteine to methionine. This suggests that smoking either induces local effects in cells exposed to cigarette smoke (30), changes plasma thiol redox status (homocysteine is an aminothiol) (32, 33) by a higher formation of reactive oxygen species (34), or inhibits the action of enzymes such as methionine synthase (35).

Subjects participating in surveys like ours might be more health-conscious than nonparticipants. All subjects invited to participate in the MORGEN Study (15) received a response card; approximately 70 percent returned the response card, while approximately 50 percent completed the full assessment. A nonresponse study indicated that nonresponders were more likely to be men, smokers, nondrinkers of alcohol, and physically inactive. However, a comparison of the distributions of data on gender, lifestyle, and demographic factors in our random sample with nationwide data (36) showed similar percentages (14). Therefore, our results may be applicable to a wider population of Dutch men and women.

In summary, smoking and alcohol and coffee consumption were major lifestyle correlates of plasma total homocysteine concentrations in this study. It is important to increase the public’s awareness that feasible changes in lifestyle may favorably alter plasma total homocysteine levels, which in turn might reduce the risk of cardiovascular disease.

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REFERENCES