Homocysteine, folate, vitamin B-12, and 10-y incidence of age-related macular degeneration

Bamini Gopinath, Victoria M Flood, Elena Rochtchina, Jie Jin Wang, and Paul Mitchell

ABSTRACT

Background: Epidemiologic evidence of a relation between serum total homocysteine (tHcy), vitamin B-12, and folate and age-related macular degeneration (AMD) is inconsistent and unresolved.

Objective: In this cohort study, we aimed to investigate associations between intakes and serum concentrations of folate and vitamin B-12 or serum tHcy and 10-y AMD incidence.

Design: Serum folate, vitamin B-12, and tHcy were determined from blood samples drawn in 1997–1999 from cohort members aged ≥55 y. AMD was assessed in 1760 survivors from retinal photographs taken in 2002–2004 and 2007–2009. Total intakes of folate and vitamin B-12 were assessed by using a food-frequency questionnaire.

Results: After adjustment for age, sex, current smoking, white blood cell count, and fish consumption, each 1-SD increase in serum tHcy was associated with increased risk of incident early and any AMD [ORs (95% CIs): 1.33 (1.09, 1.63) and 1.33 (1.11, 1.60), respectively]. Participants with a serum vitamin B-12 deficiency (<185 pmol/L) had higher risk of incident early and late AMD [ORs (95% CIs): 1.58 (1.06, 2.36) and 2.36 (1.38, 4.73), respectively]. Folate deficiency (<11 nmol/L) was associated with 75% and 89% increased risk of incident early and any AMD, respectively, 10 y later. Participants who reported supplementary vitamin B-12 intake had 47% reduced risk of incident any AMD (OR: 0.53; 95% CI: 0.33, 0.85).

Conclusion: Elevated serum tHcy and folate and vitamin B-12 deficiencies predicted increased risk of incident AMD, which suggests a potential role for vitamin B-12 and folate in reducing AMD risk. Am J Clin Nutr 2013;98:129–35.

INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blindness and low vision in older adults (1). Although there have been recent significant advances in the understanding of AMD, knowledge of the specific underlying mechanisms responsible for the development and progression of AMD remains unclear. Current treatment options are limited to patients with late-stage neovascular or intermediate AMD (2, 3). For the majority of the population with early or no AMD, there is no strategy for disease prevention except the avoidance of cigarette smoking (4).

Homocysteine is an independent risk factor for ischemic heart disease and peripheral vascular disease (5, 6). Major determinants of homocysteine are age, dietary intakes, circulating concentrations of B vitamins (ie, folate, vitamin B-12, and vitamin B-6), kidney dysfunction, and traditional cardiovascular disease risk factors such as blood pressure, BMI, and serum lipids (6, 7). Damaging sequelae of elevated homocysteine concentrations thought to underlie increased risk of cardiovascular disease could also contribute to the development of AMD (4). Cross-sectional data from the Blue Mountains Eye Study (BMES) of 2335 participants (mean age: 66.8 y) showed that increased serum total homocysteine (tHcy) and low vitamin B-12 were independently associated with increased odds of AMD (8). However, folate deficiency was not associated with the prevalence of AMD (8). In a clinic-based sample of 60 patients, elevated plasma homocysteine and lower vitamin B-12 concentrations were associated with the prevalence of AMD (9). In contrast, in a cross-sectional US study of 4145 participants aged ≥40 y, nonsignificant associations were observed between serum tHcy, red blood cell folate, and serum vitamin B-12 and the prevalence of AMD (10). A recent randomized trial of 5442 women aged ≥40 y examined the effect of therapy to lower homocysteine concentrations in AMD (4). This study showed that daily supplementation with a combination of folic acid, pyridoxine, and cyanocobalamin (vitamin B-12) was associated with 35–40% decreased risk of AMD (4).

Epidemiologic data available on the relation between serum tHcy, vitamin B-12, and folate and AMD are largely cross-sectional and equivocal. Moreover, to our knowledge, there have been no population-based studies that have assessed whether dietary intakes of vitamin B-12 and folate are associated with incident AMD. Hence, in this relatively large cohort of older

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2 The Blue Mountains Eye Study was supported by the Australian National Health and Medical Research Council (grants 974159, 991407, 211069, and 262120), Kellogg’s Pty Ltd, and Westmead Millennium Institute. BG is supported by a Macular Degeneration Foundation and Blackmores Dr Paul Beaumont Fellowship.

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4 Abbreviations used: AMD, age-related macular degeneration; BMES, Blue Mountains Eye Study; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FFQ, food-frequency questionnaire; tHcy, total homocysteine.

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adults, we aimed to establish the 1) temporal association between serum tHcy, folate, and vitamin B-12 and the 10-y incidence of AMD, independent of potential confounders and 2) prospective relation between dietary intakes of folate and vitamin B-12 and incident AMD.

SUBJECTS AND METHODS

Study population

The BMES is a population-based cohort study of common eye diseases and other health outcomes in a suburban Australian population located west of Sydney. Study methods and procedures have been described elsewhere (11). Participants were noninstitutionalized residents aged ≥49 y who were invited to attend a detailed baseline eye examination after a door-to-door census of the study area. The selection bias at baseline was minimized after multiple call-back visits, including door knocking, telephone reminders, and letters at recruitment. The participation rate was 82.4% at baseline. Baseline examinations of 3654 residents aged ≥49 y were conducted during 1992–1994 (BMES-1: 82.4% participation rate). Surviving baseline participants were invited to attend examinations after 5 y (1997–1999; BMES-2), 10 y (2002–2004; BMES-3), and 15 y (2007–2009; BMES-4). At BMES-2, 3- and 4-year follow-ups, 2334 participants (75.1% of survivors), 1952 participants (75.6% of survivors), and 1149 participants (55.4% of survivors) with complete data were reexamined, respectively. Serum tHcy, folate, and vitamin B-12 (exposures of interest) were only obtained once at BMES-2. Therefore, BMES-2 was considered the baseline for the present analyses, and our study estimated the 10-y risk (ie, from BMES-2 to BMES-4) of developing early, late, and any AMD associated with these exposures. The University of Sydney and the Western Sydney Area Health Service Human Research Ethics Committees approved the study, and written informed consent was obtained from all participants at each examination.

Assessment of AMD

The incidence of AMD was the main outcome 5 or 10 y later. We took two 30° stereoscopic color retinal photographs of the macula of both eyes, which were graded for the presence of early and late AMD by using the Wisconsin AMD Grading System (12, 13). Intergrader and intragrader reliability showed good agreement in the identification of individual lesions (14). The detailed methodology of AMD ascertainment in this population has been reported extensively elsewhere (12, 13). Incident early AMD was defined as the absence of late AMD and presence of either 1) large (≥125-μm diameter) indistinct soft or reticular drusen or 2) both large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation) at BMES-3 or -4 in either eye of persons free of early AMD in both eyes at BMES-2 (13). Similarly, incident late AMD was defined as the appearance of neovascular AMD or geographic atrophy at BMES-3 or -4 in either eye of persons without late-AMD lesions in both eyes at BMES-2 (13). Incident any AMD was defined as having early or late AMD at BMES-3 or -4. A retinal specialist (PM) adjudicated all uncertain retinal pathology and confirmed all late-AMD cases.

Assessment of serum tHcy, folate, and vitamin B-12

Fasting blood samples were drawn within 4 wk after the day of examination, and blood testing was performed on the same day as blood collection at the Institute of Clinical Pathology and Medical Research, Westmead Hospital, Sydney, Australia. Total serum homocysteine was determined from blood samples by using the fluorescent polarization immunoassay method on an Abbott IMx Analyzer. An elevated homocysteine concentration or hyperhomocysteinemia was defined as >15 μmol/L, which is a laboratory recommended cutoff for older adults (15). Serum vitamin B-12, folate, and red blood cell folate assays were performed by using the competitive-binding assay method on a Beckman Access analyzer (Beckman Coulter Inc). Individuals were classified as having low serum vitamin B-12 if serum concentrations were <185 pmol/L (16). Individuals were classified as having low serum folate if serum concentrations were <11 nmol/L (17).

Estimation of total intakes of folate and vitamin B-12

For the current study, we assessed associations between intake data collected at BMES-2 (during 1997–1999) and the 10-y incidence of AMD. Information on intakes were collected by using a 145-item self-administered food-frequency questionnaire (FFQ) that was modified for the Australian diet and vernacular from an early Willett FFQ (18) and included reference portion sizes. Participants used a 9-category frequency scale to indicate the usual frequency of consumption of individual food items during the past year. The FFQ included details about frequency estimates and details about vitamin B-12 and folic acid supplements. The FFQ was validated by comparing nutrients from the FFQ to 3 × 4-d weighed food records collected over 1 y (n = 79) to assess the seasonal variation. Most nutrient correlations were between 0.50 and 0.60 for energy-adjusted intakes, similar to other validated FFQ studies (19, 20). A dietitian coded data from the FFQ into a customized database that incorporated the Australian Tables of Food Composition 1990 (NUTTAB 90) and follow-up data used NUTTAB95 (21, 22). Folate and vitamin B-12 nutrient-composition values were obtained from the Australian Food and Nutrient Database (23) and UK tables of food composition (24), respectively. In addition, folate-fortified foods were also included in the nutrient database (25, 26). Participants were asked to complete questions on supplement use, including details on the frequency, strength, and length of time used for supplement brand and type. The total intake of vitamin B-12 was calculated by adding the crude dietary intake and supplement intake. Total dietary folate equivalents were calculated as follows (27):

\[
\text{One microgram of dietary folate equivalent} = \left[ \text{micrograms of food folate} + \left(1.7 \times \text{micrograms of synthetic folic acid}\right) \right] (I)
\]

where the additional bioavailability of synthetic folic acid was taken into account for synthetic folic acid in fortified food products and supplements.

Assessment of covariates

Covariates that were included in the final, multivariable model were those that were previously identified as risk factors for AMD...
in the BMES cohort, including smoking (29), white blood cell count (29), and fish consumption (30). Smoking status was determined from the patient history as never smoked, past smoker, and current smoker (which included subjects who had ceased smoking within the past 12 mo). Fasting blood samples were also processed for white blood cell counts. We extracted separate data on the frequency of consuming fish, including salmon, tuna, and sardines from the FFQ. Supplementary analyses also involved adjustment for the presence of chronic kidney disease (CKD) because it has been shown to be associated with both serum tHcy (31) and risk of AMD (32). We calculated the estimated glomerular filtration rate (eGFR) by using the 4-variable Modification of Diet in Renal Disease Study equation (33), and moderate CKD was defined as an eGFR <60 mL·min⁻¹·1.73 m⁻². Dietary intakes of antioxidants (ie vitamin C, vitamin E, β carotene, zinc, and combined lutein and zeaxanthin) have also been shown to influence the incidence of AMD (34); hence, supplementary analyses also involved adjustment for these potential confounders in multivariable analyses.

Statistical analyses

SAS statistical software (version 9.2; SAS Institute) was used for analyses including t tests, chi-square tests, and logistic regression. Associations between serum tHcy, folate, and vitamin B-12 (both serum and total dietary intakes) and incident AMD (study outcome) were examined in discrete linear logistic regression models adjusted for age, sex, current smoking, white blood cell count, and fish consumption. Supplementary analyses involved adjustment for CKD and antioxidant intake. Findings from these analyses are expressed as adjusted ORs with 95% CIs. We analyzed tHcy, folate, and vitamin B-12 both as categorized (tertiles and diagnostic and clinical cutoffs) and as continuous (per-SD) variables. Statistical significance was defined as P < 0.05.

RESULTS

Of the 2334 participants examined at BMES-2, 1760 subjects had participated in at least one follow-up examination (ie, at BMES-3 and/or BMES-4). Of these participants, 1390 subjects had fasting blood measures at BMES-2 and incident AMD data at either BMES-3 or -4. As shown in Table 1, participants with AMD compared with participants without incident AMD were more likely to be women, older, and have higher serum tHcy but lower serum vitamin B-12 and total dietary and supplementary vitamin B-12 intake.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>With incident any AMD (n = 219)</th>
<th>Without incident AMD (n = 1711)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>71.6 ± 6.7</td>
<td>66.7 ± 7.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex (M) [n (%)]</td>
<td>69 (31.5)</td>
<td>509 (43.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Current smoking [n (%)]</td>
<td>18 (8.2)</td>
<td>90 (7.7)</td>
<td>0.79</td>
</tr>
<tr>
<td>White blood cell count (×10⁹/L)</td>
<td>6.30 ± 1.6</td>
<td>6.29 ± 1.7</td>
<td>0.90</td>
</tr>
<tr>
<td>Serum tHcy (μmol/L)</td>
<td>13.0 ± 4.6</td>
<td>12.0 ± 4.2</td>
<td>0.002</td>
</tr>
<tr>
<td>Serum folate (nmol/L)</td>
<td>18.0 ± 9.6</td>
<td>18.0 ± 8.5</td>
<td>0.96</td>
</tr>
<tr>
<td>Serum vitamin B-12 (pmol/L)</td>
<td>263.4 ± 116.6</td>
<td>284.3 ± 138.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Fish consumption (≥1 serving/wk) [n (%)]</td>
<td>130 (66.7)</td>
<td>716 (67.3)</td>
<td>0.86</td>
</tr>
<tr>
<td>Consumed folate supplement [n (%)]</td>
<td>23 (11.8)</td>
<td>138 (13.0)</td>
<td>0.65</td>
</tr>
<tr>
<td>Consumed vitamin B-12 supplement [n (%)]</td>
<td>23 (11.8)</td>
<td>195 (18.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Total intake of folate equivalents (μg/d)²</td>
<td>440.8 ± 228.4</td>
<td>462.5 ± 257.0</td>
<td>0.23</td>
</tr>
<tr>
<td>Total intake of vitamin B-12 (μg/d)²</td>
<td>7.9 ± 9.9</td>
<td>11.2 ± 20.0</td>
<td>0.0004</td>
</tr>
</tbody>
</table>

1 AMD, age-related macular degeneration.
2 P values were obtained by using t tests for continuous variables and chi-square analyses for categorical data.
3 Mean ± SD (all such values).
4 Total intakes were calculated by adding crude dietary and supplement intakes.

Associations between serum tHcy, folate, and vitamin B-12 and incident AMD

After adjustment for age, sex, current smoking, white blood cell count, and fish consumption, each 1-SD increase in serum vitamin B-12 was associated with 27%, 23%, and 34% reduced risk of incident any, early, and late AMD, respectively (Table 2). Each 1-SD increase in serum tHcy was associated with 33% increased risk of either incident early and any AMD (Table 2). As shown in Table 3, having a folate deficiency (<11 nmol/L) at baseline was associated with 75% and 89% increased risk of developing incident early and any AMD 10 y later. Older adults with a serum vitamin B-12 deficiency (<185 pmol/L) were also at higher risk of developing incident early, late, and any AMD [ORs (95% CIs): 1.58 (1.06, 2.36), 2.56 (1.38, 4.73), and 1.82 (1.28, 2.58), respectively] (Table 3).

Supplementary analyses involved adjustment for an eGFR <60 mL·min⁻¹·1.73 m⁻² in the multivariable model. However, CKD was not a significant covariate in the model and did not appreciably change the strengths of the association between serum tHcy and the incidence of AMD. For example, after additional adjustment for CKD, risk [OR (95% CI) of incident early and any AMD was 1.37 (1.11, 1.69) and 1.38 (1.15, 1.67), respectively]. Hence, we chose not to adjust for CKD in the final, parsimonious model.

1 AMD, age-related macular degeneration.
2 P values were obtained by using t tests for continuous variables and chi-square analyses for categorical data.
3 Mean ± SD (all such values).
4 Total intakes were calculated by adding crude dietary and supplement intakes.
Associations between total intakes of vitamin B-12 and folate and incident AMD

For analyses of associations with total B-12 and folate intakes, 131 participants who did not have complete FFQ data at BMES-2 were excluded, which left 1259 participants. Each 1-SD (1 SD = 246.3 μg/d) increase in total vitamin B-12 intake was associated with 24% reduced risk of the 10-y incidence of any AMD (multivariable-adjusted OR: 0.76; 95% CI: 0.60, 0.96). There was no significant association between each 1-SD (1 SD = 246.3 μg/d) increase in the total intake of dietary folate equivalents and the 10-y incidence of AMD (data not shown). Although, as shown in Table 4, participants in the highest compared with lowest tertiles of the total intake of dietary folate equivalents had 55% reduced risk of developing incident late AMD. Tertiles of total vitamin B-12 intake were not associated with incident AMD (Table 4).

We also assessed associations between supplementary vitamin B-12 intake only and risk of developing AMD. In participants with incident early, late, and any AMD at BMES-3, 19 subjects (9.8%), 5 subjects (2.3%), and 24 subjects (12.1%) reported taking vitamin B-12 supplements at BMES-2, respectively. Participants who self-reported supplementary vitamin B-12 intake had reduced risk of the 10-y incidence of early, late, and any AMD (multivariable-adjusted ORs (95% CIs): 0.58 (0.35, 0.98), 0.37 (0.14, 0.99), and 0.53 (0.33, 0.85), respectively). Moreover, we included vitamin B-12 intake from the diet only and supplementary vitamin B-12 intake only in the multivariable model, and only associations with supplementary vitamin B-12 intake persisted for incident early and any AMD [ORs (95% CIs): 0.58 (0.35, 0.98) and 0.53 (0.33, 0.85), respectively]. Associations with vitamin B-12 intake from the diet only became non-significant (data not shown).

Supplementary analyses involved additional adjustment for antioxidant intake in the multivariable model. These dietary factors were not significant covariates in the final model (ie antioxidant intake did not exert an independent influence on developing incident AMD, and their inclusion in the multivariable model did not greatly change observed estimates. For instance, after additional adjustment for antioxidant intake, each 1-SD increase in total vitamin B-12 intake at baseline was associated with 26% reduced risk of any AMD 10 y later (OR: 0.74; 95% CI: 0.57, 0.95). Hence, we chose not to adjust for antioxidant intake in the final, parsimonious model.

**DISCUSSION**

To our knowledge, this study adds new understanding by documenting a prospective association between elevated serum tHcy concentrations and increased risk of incident early and late AMD. Risk of developing incident early and late AMD over a 10-y period was also significantly associated with having either a vitamin B-12 or folate deficiency at baseline. An inverse

**TABLE 2**

Associations between serum homocysteine, vitamin B-12, and folate and the 10-y incidence of AMD in the Blue Mountains Eye Study from 1997–1999 to 2007–2009 (n = 1390)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Incident any AMD (n = 219)</th>
<th>Incident early AMD (n = 162)</th>
<th>Incident late AMD (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine (1-SD increase)</td>
<td>1.33 (1.11, 1.60)</td>
<td>1.33 (1.09, 1.63)</td>
<td>1.25 (0.93, 1.69)</td>
</tr>
<tr>
<td>Vitamin B-12 (1-SD increase)</td>
<td>0.73 (0.60, 0.89)</td>
<td>0.77 (0.62, 0.96)</td>
<td>0.66 (0.45, 0.96)</td>
</tr>
<tr>
<td>Folate (1-SD increase)</td>
<td>0.91 (0.77, 1.07)</td>
<td>0.93 (0.77, 1.13)</td>
<td>0.89 (0.66, 1.20)</td>
</tr>
</tbody>
</table>

1 All values are adjusted ORs; 95% CIs in parentheses. Values were calculated by using logistic regression analyses and were adjusted for age, sex, current smoking, white blood cell count, and fish consumption. AMD, age-related macular degeneration.

2 One SD = 5.09 μmol/L.

3 One SD = 144.9 pmol/L.

4 One SD = 9.1 nmol/L.

**TABLE 3**

Associations between serum total homocysteine, folate, and vitamin B-12 (by diagnostic cutoffs) and the 10-y incidence of AMD in the Blue Mountains Eye Study from 1997–1999 to 2007–2009 (n = 1390)

<table>
<thead>
<tr>
<th>Variables</th>
<th>n (%)</th>
<th>Any AMD (n = 219)</th>
<th>Early AMD (n = 162)</th>
<th>Late AMD (n = 57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homocysteine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 15 μmol/L</td>
<td>1157 (85.2)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>&gt; 15 μmol/L</td>
<td>201 (14.8)</td>
<td>1.53 (1.00, 2.34)</td>
<td>1.56 (0.97, 2.51)</td>
<td>1.33 (0.63, 2.78)</td>
</tr>
<tr>
<td>Folate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 11 nmol/L</td>
<td>1094 (80.9)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>&lt; 11 nmol/L</td>
<td>259 (19.1)</td>
<td>1.89 (1.30, 2.74)</td>
<td>1.75 (1.15, 2.66)</td>
<td>1.94 (0.97, 3.88)</td>
</tr>
<tr>
<td>Vitamin B-12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 185 pmol/L</td>
<td>914 (78.7)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>&lt; 185 pmol/L</td>
<td>248 (21.3)</td>
<td>1.82 (1.28, 2.58)</td>
<td>1.58 (1.06, 2.36)</td>
<td>2.56 (1.38, 4.73)</td>
</tr>
</tbody>
</table>

1 AMD, age-related macular degeneration.

2 All values are ORs; 95% CIs in parentheses. Values were calculated by using logistic regression analyses and were adjusted for age, sex, current smoking, white blood cell count, and fish consumption.
relation was also observed between total vitamin B-12 and folate equivalent intakes and the 10-y incidence of AMD. Moreover, supplemental vitamin B-12 intake was associated with reduced risk of incident AMD. These associations were independent of potential confounders such as smoking, white blood cell count, fish consumption, renal function, and antioxidant intake.

The longitudinal association between elevated serum tHcy and risk of developing incident AMD supports previous studies that have reported a cross-sectional link between increased serum tHcy concentrations and higher prevalence of AMD (8, 9, 35). Several underlying pathways could explain the association between elevated serum tHcy and AMD risk, including: endothelial dysfunction (36–38), impaired vascular reactivity (39), and augmented inflammatory processes (4, 38, 40). Moreover, AMD is a disease that likely involves damage to small vessels of the choroid (41, 42), and some evidence has suggested that homocysteine could be a more important risk factor for small-vessel disease than for large-vessel disease (43).

Because homocysteine metabolism requires vitamin B-12 and folate (10), the observed inverse association between serum vitamin B-12 and folate and the incidence of early and late AMD was not surprising. Previous cross-sectional studies have reported similar associations with the prevalence of AMD in older adults (8, 9). Beneficial effects of vitamin B-12 and folate on AMD risk could potentially be, at least partly, mediated via lowering serum tHcy concentrations (8). In addition, high folate and vitamin B-12 concentrations could also reverse endothelial dysfunction independent of the effect of lowering tHcy concentrations (44, 45). Because endothelial dysfunction is implicated in the pathogenesis of AMD (46), this could be a potential pathway by which optimal folate and vitamin B-12 status is protective against the development of AMD.

Folate and vitamin B-12 intakes are both important contributors to serum tHcy concentrations (47). In the BMES, increasing total intakes of folate were associated with reduced risk of incident AMD. Although vitamin B-12 intake from the diet only was not associated with the incidence of AMD, participants who self-reported supplementary vitamin B-12 intake at baseline had reduced risk of developing AMD 10 y later. Christen et al (4) hypothesized that a direct antioxidant effect of vitamin B-12 and folate and enhancement of endothelial nitric oxide concentrations in the choroidal vasculature with an associated increase in vascular reactivity (48) could underlie the beneficial effects of vitamin B-12 and folic acid supplements on AMD risk (4). In the case of vitamin B-12, the achievement of an optimal status may present a particular challenge for many older adults because of the frequent problem of age-related food-bound B-12 malabsorption (49). Because vitamin B-12 deficiency was associated with higher risk of incident AMD, the high prevalence of low vitamin B-12 status in older adults is a potentially important public health issue that needs to be addressed (49, 50). Our findings suggested that older adults could also benefit from taking vitamin B-12 supplements, which are more bioavailable than the naturally occurring vitamin when taken in larger doses of the crystalline form, in cases where the gut is not functioning well (51).

Associations between elevated serum tHcy, folate, and vitamin B-12 deficiencies and greater risk of incident early AMD are important because individuals with early AMD are at increased risk of developing advanced AMD, which is the leading cause of severe, irreversible vision loss in older adults (4). Moreover, there are few effective preventive or therapeutic strategies available that target early AMD signs (52). Hence, additional studies are warranted to investigate whether a high consumption of folate-rich foods (eg, vegetables and cereals) and vitamin B-12 supplements could play a role in the primary prevention of AMD.

Strengths of this study included its prospective design and long-term follow-up of a stable population-based sample; hence, our findings are applicable to general older Australians and could also be applicable to older persons in Western countries. In addition, this study used high-quality stereoscopic retinal photography with validated grading to assess macular conditions and a detailed side-by-side comparison of baseline and follow-up photographs to ensure negligible misclassification of incident AMD (30, 53). Study limitations also deserve discussion. First, we could not exclude the possibility of residual confounding; eg, the apparent beneficial effect of dietary vitamin B-12 intake on AMD risk could be explained by other health-related factors, because older adults with higher vitamin B-12 intake are also likely to lead a healthier lifestyle than those with poorer dietary

### Table 4

<table>
<thead>
<tr>
<th>Dietary variables</th>
<th>n</th>
<th>Incident any AMD</th>
<th>Incident early AMD</th>
<th>Incident late AMD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total dietary folate equivalents</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First tertile (≤44.4 µg/d)</td>
<td>416</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Second tertile (44.5–475.2 µg/d)</td>
<td>436</td>
<td>1.07 (0.73, 1.56)</td>
<td>1.15 (0.75, 1.77)</td>
<td>0.76 (0.38, 1.50)</td>
</tr>
<tr>
<td>Third tertile (≥475.3 µg/d)</td>
<td>408</td>
<td>0.92 (0.62, 1.37)</td>
<td>1.08 (0.69, 1.69)</td>
<td>0.45 (0.21, 0.98)</td>
</tr>
<tr>
<td><strong>Total vitamin B-12</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First tertile (≤4.14 µg/d)</td>
<td>407</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Second tertile (4.15–6.66 µg/d)</td>
<td>415</td>
<td>0.99 (0.68, 1.45)</td>
<td>1.01 (0.66, 1.55)</td>
<td>0.96 (0.47, 1.95)</td>
</tr>
<tr>
<td>Third tertile (≥6.67 µg/d)</td>
<td>437</td>
<td>0.81 (0.54, 1.20)</td>
<td>0.84 (0.54, 1.31)</td>
<td>0.69 (0.33, 1.47)</td>
</tr>
</tbody>
</table>

1Total intakes were calculated by adding the crude dietary intake and supplement intake. AMD, age-related macular degeneration.

2All values are adjusted ORs; 95% CIs in parentheses. Values were calculated by using logistic regression analyses and were adjusted for age, sex, current smoking, white blood cell count, and fish consumption.
vitamin B-12 intake. Second, the number of participants who developed incident late AMD were small; hence, we may have underestimated some of the associations. Third, analyses only examined associations between baseline intakes of dietary folate equivalents and the incidence of AMD; hence, there is a possibility that synthetic folic acid in foods could have increased because of fortification over the 10 y. However, voluntary fortification of selected foods (primarily breakfast cereals) with folic acid was introduced in Australia in 1995 (ie, before the BMES-2 examination). Since September 2009, Australian millers have been required to add folic acid to wheat flour. Because BMES-4 finished at the end of 2009, only voluntary fortification was in effect for the majority of the 10-y follow-up period. Indeed, there was only a small increment in dietary folate equivalents intake ($\sim 20 \mu g/d$) in the overall study population during the 10 y. Hence, because dietary folate equivalents did not change appreciably during the follow-up, it would be acceptable to present the associations between baseline folate intake and the 10-y incidence of AMD. Finally, because we have examined several associations, the possibility of chance findings could not be excluded.

In conclusion, this study showed that elevated serum tHcy could signal increased risk of developing AMD in the long-term. In addition, older adults with vitamin B-12 and folate deficiencies could be at increased risk of incident early AMD. Additional research and replication in other large cohort studies are clearly needed. However, if our findings are confirmed, efforts at the prevention of elevated serum tHcy either by treatment with folic acid and vitamin B-12 supplements or a simple strategy of eating a healthful diet by incorporating a range of foods that contain folate such as leafy green vegetables and fortified foods could potentially contribute to reducing the burden of blindness from AMD.

The authors’ responsibilities were as follows—BG and PM: study concept and design; ER: analysis of data; BG, VMF, JJW, and PM: interpretation of data; BG: drafting of the manuscript; and all authors: critical revision of the manuscript. None of the authors had a conflict of interest.

REFERENCES