Reduced plasma homocysteine levels in elderly Australians following mandatory folic acid fortification — A comparison of two cross-sectional cohorts

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ABSTRACT

Objective: In 2009, Australia implemented mandatory folic acid fortification in wheat flour for bread-making. The primary aim was to improve folate status in reproductive-aged women to reduce neural tube defect incidence. However, folic acid consumption has consequently increased in all demographics. Blood folate is inversely associated with homocysteine levels, a risk factor for multiple diseases. Therefore, we assessed the impact of mandatory folic acid fortification on homocysteine levels in elderly Australians.
Methods: Homocysteine and blood folate levels were compared between two elderly cross-sectional cohorts (pre-versus post-mandatory folic acid fortification). Importantly, dietary habits were assessed to evaluate the confounding influence of altered dietary patterns not related to fortification.
Results: Post-fortification, plasma homocysteine levels (10.6 vs. 14.5 μmol/L) and hyperhomocysteinemia incidence (27.2% vs 56.3%) were significantly reduced, relative to the pre-fortification subjects. This was associated with increased blood folate (red cell: 1243 vs 1066 nmol/L, serum 28.0 vs 23.9 nmol/L), and increased intake of synthetic folic acid (366.8 vs 231.0 DFE/day) but not natural folate (332.7 vs 323.6 DFE/day). Limited other differences were detected in dietary intake patterns between groups. The positive relationship between homocysteine levels and age was abrogated post-fortification (p = 0.3 vs p = 0.0003).
Conclusions: A potential off-target benefit of mandatory folic acid fortification in Australia was demonstrated. With many countries still considering the merits and consequences of mandatory fortification policies, it is important to unravel the off-target effects including dietary context.

1. Introduction

Mandatory fortification of wheat flour used for bread-making with folic acid commenced in Australia in September 2009. The Australian New Zealand Food Standards require fortification at 2—3 mg of folic acid per kilogram of wheat flour, resulting in approximately 0.13 mg of folic acid per 100 g of bread [1]. Prior to mandatory fortification, some foods, primarily breakfast cereals, were voluntarily fortified by manufacturers. The primary aim of mandatory fortification was to improve the folate status of women of reproductive-age to reduce the incidence of neural tube defects [2]. However, consumption of fortified products is not limited to this target group and consequently, fortification has led to increased exposure to folic acid in other demographics, including the elderly. While off-target exposures has been the source of some controversy [3], there may also be benefits.

Low folate levels are also associated with elevated levels of blood homocysteine (Hcy) [4]. Ultimately, Hcy levels are a function of the equilibrium between the methylation and transsulfuration pathways and dietary intake of folate, vitamin B_{12}, and to a lesser

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extent vitamin B_6 [5]. As such, elevated Hcy can be caused by a deficiency of intake of one or more of these vitamins. Lifestyle exposures such as excessive alcohol consumption, cigarette smoking, and physical inactivity may also play a role in modulating Hcy levels [6]. Hcy levels also increase with aging [7] and are higher in males [8].

Hyperhomocysteinemia increases risk of cardiovascular diseases, including atherosclerosis, myocardial infarction and stroke [9–13] and neurodegenerative diseases such as dementia [14,15], Alzheimer's disease [16,17], Parkinson's disease [18,19] and cognitive decline [20]. Mechanisms may be direct or indirect and include promotion of low-density lipoprotein oxidation [21,22] and inflammation [23], competition with inhibitory neurotransmitters [24], and damage to endothelial and neuronal cells [20,22,25–28].

While folic acid and vitamin B_{12} supplementation can reduce Hcy, a consensus has yet to be reached as to whether this reduces risk or improves symptoms of either cardiovascular or neurodegenerative diseases, as results have been inconsistent [13,14,29–41]. These inconsistencies may be largely attributed to limitations of study design. Considerable differences exist in the dose and duration of supplementation across trials [13,14,29–41]. Other studies are based on reported supplement use, rather than a standardised intervention. For multi-factorial diseases the benefits of lowering Hcy may be too small for trials to detect. Additionally, supplementation trials conducted in countries with mandatory or voluntary fortification might lead to a masking of the experimental effects, due to higher baseline levels [42].

Moreover, as neurodegenerative and cardiovascular diseases commonly present in later life [43–45], trials of supplementation in elderly cohorts [13,14,31,36–39], particularly following the onset of hyperhomocysteinemia or symptomatic disease, may not be sufficient to reverse the damage already sustained by sub-clinical deficiencies in the preceding decades. Therefore, reducing Hcy levels via systematic fortification over the long term may be more practical for reducing risk of primary disease than later life supplementation. Indeed, this may be an off-target benefit of mandatory folic acid fortification in Australia. However, the influence of Australia's mandatory folic acid fortification policy on homocysteine levels, particularly in the at risk elderly demographic, remains to be investigated. It is also important to remember that any dietary intervention must be considered in the context of other background dietary exposures.

In this study, the blood levels of folate (serum and red cell) and Hcy were assessed in two elderly cross-sectional cohorts from the Central Coast of NSW, Australia. The samples from one cross-sectional cohort were collected shortly prior to the introduction of mandatory folic acid fortification in Australia, and the other was collected shortly after implementation. The dietary habits of participants were also assessed to evaluate the potential confounding influence of altered dietary intake of folate, from sources not related to mandatory fortification.

2. Methods

2.1. Subjects and sample collection

The pre-fortification subjects was obtained from a larger study of patients undergoing routine colonoscopy at Gosford Hospital (Central Coast, NSW, Australia) [46]. Two-hundred and seven participants who gave blood and completed food frequency questionnaires were recruited prior to the introduction of mandatory folic acid fortification in Australia in September 2009. The post-fortification subjects (sample collection commencing mid-2010) was obtained from a cross-sectional cohort of elderly individuals (≥65 years) living on the Central Coast of NSW (The Retirement

Health and Lifestyle Study [47]). A total of 649 participants in this group gave blood and completed food frequency questionnaires.

Only participants ≥65 years of age were included in this analysis. Participants were excluded if blood biochemistry measures were missing or food frequency questionnaire were deemed invalid. Criteria for deeming a questionnaire invalid were missed pages, extreme excess (>30,000 kj/day) or deficient (<3500 kj/day) energy consumption [48], or reported excessive consumption of a single food group (≥11 serves/day). After exclusions, 115 participants (50% female; mean age 72.7 year, standard error ± 0.5 years) were included in the pre-fortification cohort and 475 participants (54% female: mean age 77.2 years, standard error ± 0.3 years) were included in the post-fortification cohort.

Fasting blood samples were collected in EDTA lined tubes and stored at −20 °C as whole blood, or collected in Lithium Heparin tubes (plasma) or with a tubes containing clot activator (serum). Samples for serum and plasma were centrifuged (1500 g, 10 min) to obtained serum or plasma (serum left for 30 min for clotting), and stored at −80 °C. Written informed consent was obtained from all participants. Approval was obtained from the University of Newcastle Human Research Ethics Committee (approval numbers H-429-0407 and H-2008-0431 for the pre- and post-fortification cohorts, respectively).

2.2. Blood biochemistry

Serum and red cell folate, and serum vitamin B_{12} levels were assessed by the Institute for Clinical Pathology and Medical Research (pre-fortification subjects) and the Hunter Area Pathology Service (post-fortification subjects) using standardised chemiluminescent assays. Total plasma Hcy was measured by selective fluorescence assay (JD Biotech Corp, Taipei, Taiwan) [46].

2.3. Food frequency questionnaires

The pre-fortification food frequency questionnaires were analysed using Foodworks™ 2.10.146 (Xyris Software, Brisbane, QLD, Australia), which includes the AusFoods (brands), Aus Nut (base foods) and the New Zealand Vitamin and Mineral Supplements 1999 databases. Data for foods commonly voluntary fortified with folic acid were entered in a brand specific manner [49]. The post-fortification food frequency questionnaires were analysed using Foodworks Professional 7 (Xyris Software, Brisbane, QLD, Australia), which reflects folic acid values post fortification using 2010 databases. Food frequency questionnaires were as published in previous studies [46,50] and were adapted from the CSIRO version [51]. Lists of supplements and frequency of intake were provided by participants. Serves per day were calculated for the major food groups based on the standard serving sizes in the Australian dietary guidelines [52]. Food groups were further stratified where the strata may significantly contribute to folate intake (for example bread and cereal as strata to the food group grains). Alcohol intake was included in the food frequency questionnaires. Smoking habits were also collected in a lifestyle survey (grouped as current, never or ex-smokers).

2.4. Statistics

Nominal logistic and linear regression were used for multivariable models. Least-squares means and 95% confidence intervals are reported throughout, with adjustment for age and sex age excluded where it is a variable of interest. Least-squares means were compared using t-tests (or non-parametric equivalents) or χ^2 tests, with effect likelihood ratio tests. Outcomes were considered to be statistically significant at p ≤ 0.05. Where homocysteine was the

outcome variable, additional adjustments were applied for smoking history, reported alcohol consumption and serum vitamin B_{12} levels.

3. Results

3.1. Comparison of pre- and post-fortification folate and Hcy levels

In the pre-fortification subjects, only one participant had red cell folate levels indicative of deficiency (<340 nmol/L [53]). No participants were deficient in the post-fortification cohort. In the pre-fortification subjects, the mean red cell folate level was 1066 nmol/L (95% CI: 977–1156). As anticipated, the mean red cell folate level was significantly higher in the post fortification subjects, at 1243 nmol/L (95% CI: 1203–1283; p = 0.0005; Fig. 1A). These results were not significantly altered when adjustments were applied for smoking history and reported alcohol intake.

The percentage of participants with serum folate levels indicative of deficiency (<10 nmol/L [53]) was significantly reduced post-fortification, from 6.3% in the pre-fortification subjects, to 2.9% post-fortification (χ^2 = 9.4, p = 0.002). The mean serum folate level in the pre-fortification subjects was 23.9 nmol/L (95% CI: 21.1–26.6), which increased to 28.0 nmol/L (95% CI: 26.8–29.3; p = 0.008) post-fortification (Fig. 1B). These results were not significantly altered when adjustments were applied for smoking history and reported alcohol intake.

Mean plasma Hcy levels were 14.5 μmol/L (95% CI: 13.3–15.7) pre-fortification, which was significantly higher than the post-fortification mean of 10.6 μmol/L (95% CI: 10.0–11.1; p < 0.0001; Fig. 1C). Pre-fortification, 56.3% of participants had elevated plasma Hcy (>13 μmol/L [54]) which was significantly lowered to 27.2% post-fortification (χ^2 = 25.7, p < 0.0001). This result remained significant when additional adjustment was applied for serum vitamin B_{12} levels (χ^2 = 41.1, p < 0.0001), and with additional adjustments for smoking history and reported alcohol intake.

3.2. Comparison of reported intakes of natural and synthetic folate containing foods & supplements

Analysis of food frequency questionnaires and reported supplement intake revealed that there was no significant difference in the intake of natural folate (naturally found in foods) between the pre- and post-fortification cross-sectional cohorts. Average estimated intake of natural folate was 323.6 DFE/day (95% CI: 299.8–347.5) in the pre-fortification subjects, and 332.7 DFE/day (95% CI: 319.5–340.2) in the post-fortification subjects (p = 0.6;

Fig. 2A). However, the estimated total intake of synthetic folate (folic acid) was significantly increased from 231.0 DFE/day (95% CI: 137.0–324.7) pre-fortification to 366.8 DFE/day (95% CI: 328.1–412.5) in the post-fortification subjects (p = 0.01; Fig. 2B).

Pre-fortification, 22.5% of participants reported consuming a supplement containing folic acid. A significantly lower percentage (13.5%) of the post-fortification subjects reported taking supplements (χ^2 = 6.3, p = 0.01). However, the average intake of folic acid from supplements was not significantly different with a mean intake of 62.0 μg/day (95% CI: 17.2–107.0) pre-fortification, and 52 μg/day (95% CI: 28.5-75-4) post-fortification.

3.3. Interactions with sex and age

Sex was an independent predictor of plasma Hcy (p = 0.008), with males having higher levels. However, there was no interaction between sex and cohort in predicting Hcy level (p-interaction = 0.3), indicating that the relationship between cohort (fortification) and Hcy levels was not biased by sex. Red cell and serum folate levels did not vary by sex (p = 0.3 and 0.2, respectively).

There was a significant interaction between age and cohort in predicting Hcy level (p-interaction = 0.05), with a greater effect of fortification seen with increasing age. Interestingly, the correlation between age and Hcy was limited to the pre-fortification cohort (β = 0.12 r^2 = 0.330, p = 0.0003; Fig. 3A), with no significant correlation in the post-fortification cohort (β = 0.04, r^2 = 0.010, p = 0.3; Fig. 3B). Additional adjustment for sex, smoking history, alcohol intake and vitamin B_{12} levels did not significantly alter these results. No significant relationships between age and red cell or serum folate levels were observed.

4. Discussion

While it is well established that folate status and Hcy levels are inversely related, the specific impact of mandatory folic acid fortification on circulating Hcy has not been well established. This study demonstrates a reduction in Hcy levels in post-fortification subjects' relative to a pre-fortification cohort recruited from the same geographical region of Australia. Importantly, the results presented here demonstrate an increase intake of synthetic folic acid but not natural folate following fortification, despite a reduction in the reported consumption of brassica vegetables. Assessment of background diet as a potential confounder is often ignored in studies comparing pre- and post-fortification cohorts. Including this analysis is important if changes in Hcy levels are to be attributed to
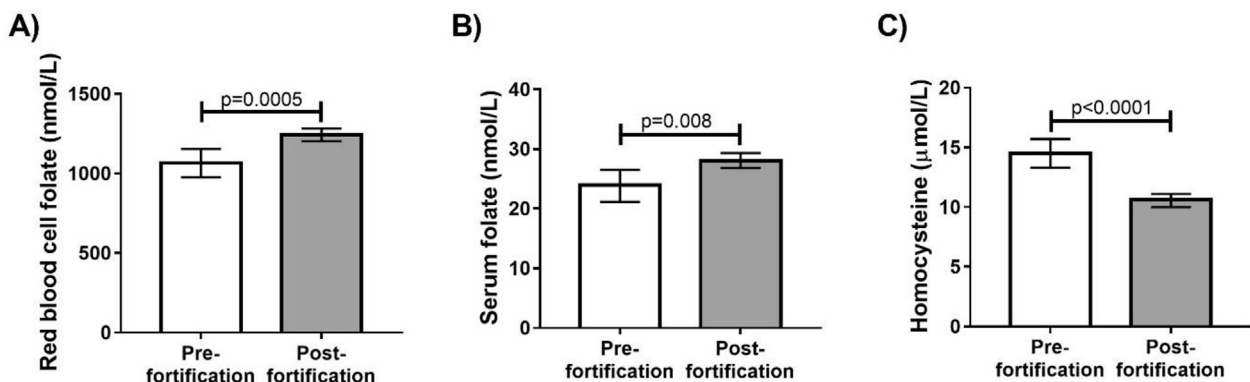

Fig. 1. Comparison of A) red cell folate, B) serum folate and C) plasma homocysteine levels in two cohorts, prior to (pre-fortification) and following (post-fortification) mandatory folic acid fortification of bread-making wheat flour in Australia. Least-squares means and confidence intervals (adjusted for age and sex) are presented. Homocysteine results are further adjusted for smoking history, alcohol intake and vitamin B_{12} levels. n = 115 (pre-fortification) and 475 (post-fortification).
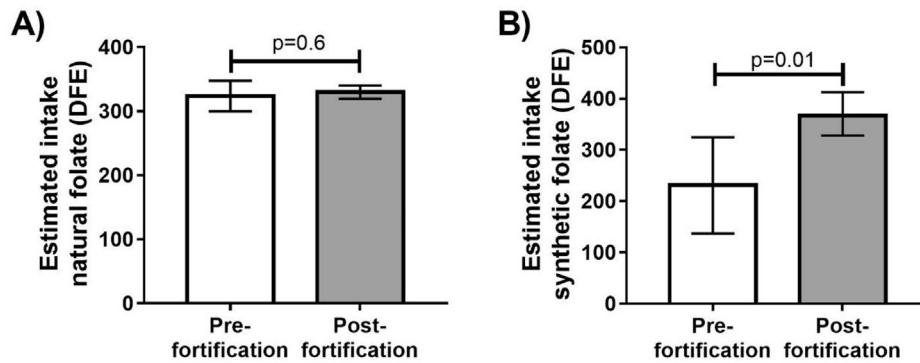
Fig. 2. Comparison of estimated intake of A) natural folate and B) synthetic folate, prior to (pre-fortification) and following (post-fortification) mandatory folic acid fortification of bread-making wheat flour in Australia. Results are reported as dietary folate equivalents (DFE) due to the differences in bioavailability between natural and synthetic folates. Least-squares means and confidence intervals (adjusted for age and sex) are presented. n = 115 (pre-fortification) and 475 (post-fortification), To assess if these differences in synthetic folate (folic acid) intake levels could be attributed to mandatory fortification, patterns of dietary intakes were compared between the pre- and post-fortification cohorts, using standard daily serves data for a range of food groups that may influence folate intake. The only significant difference detected in dietary intakes was a relative reduction in the reported consumption of brassica vegetables in the post-fortification subjects (Table 1).

Table 1
Estimated reported dietary intakes from food frequency questionnaires.

Food Group	Pre-fortification		Post-fortification		p-value
	Mean	95% CI	Mean	95% CI	
Grains total serves[a]	4.55	4.03–5.06	4.66	4.44–4.88	0.7
Bread products[b]	2.57	2.17–2.96	2.70	2.52–2.89	0.6
Breakfast cereals[c]	1.35	1.06–1.65	1.45	1.32–1.58	0.6
Pasta[d]	0.22	0.17–0.27	0.17	0.15–0.19	0.06
Rice[d]	0.26	0.19–0.34	0.28	0.25–0.31	0.6
Noodles[d]	0.13	0.07–0.11	0.07	0.04–0.09	0.06
Vegetables total serves[e]	5.26	4.74–5.78	5.56	5.34–5.78	0.3
Brassica vegetables[f]	0.74	0.61–0.85	0.54	0.49–0.59	**0.003**
Fruit total serves[g]	3.11	2.68–3.53	3.09	2.90–3.27	0.9
Meats & protein alternatives total serves[h]	3.12	2.78–3.46	3.14	2.99–3.29	0.9
Red Meat[i]	1.20	1.03–1.37	1.32	1.25–1.40	0.2
Poultry[j]	0.38	0.31–0.44	0.34	0.32–0.37	0.3
Seafood[k]	0.29	0.23–0.35	0.33	0.30–0.35	0.2
Legumes/beans/tofu[l]	0.15	0.10–0.20	0.14	0.12–0.16	0.7
Eggs[m]	0.27	0.21–0.33	0.31	0.28–0.33	0.2
Nuts[n]	0.35	0.21–0.49	0.31	0.25–0.37	0.9
Processed meats[o]	0.32	0.23–0.40	0.38	0.35–0.43	0.2
Dairy[p]	1.73	1.50–1.96	1.65	1.55–1.75	0.5
Alcohol[q]	1.22	0.93–1.52	1.04	0.91–1.16	0.3
Smoking history % (current/previously/never)	50/47/3		42/34/24		**0.01**

Outcomes were considered to be statistically significant at p ≤ 0.05 (Bold).

[a] Includes bread, breakfast cereals, pasta, rice, noodles & other grains. ~500 kJ per standard serve.

[b] Includes bread (1 slice, ½ medium bread roll or 40 g per serve), crisp-bread (3 or 45 g per serve), english muffins (1 small or 35 g per serve), crumpets (1 or 60 g per serve), flatbread & tortillas (½ each or 40 g per serve).

[c] Breakfast cereals (2/3 cup, 30 g or 2 biscuits per serve), including porridge (1/2 cup or 120 g cooked porridge per serve).

[d] ½ cup cooked per serve.

[e] Does not include beans or legumes (included in protein alternatives), except green peas. Includes potatoes & other starchy vegetables (1/2 medium per serve) & tomatoes (1 medium per serve). For all other vegetables ½ cup cooked or 1 cup raw per standard serve. ~100–350 kJ per serve.

[f] Includes broccoli, cauliflower, cabbage, & brussel sprouts. Reported consumption of other brassica vegetables were negligible.

[g] 1 medium or two small fruits, a "handful" of dried fruit, 1 cup of fruit juice, or 1 cup of diced or canned fruit, ~350 kJ per standard serve.

[h] Includes red meat, poultry, fish, seafood, legumes, beans, tofu, eggs nuts & processed meats. ~500–600 kJ per standard serve.

[i] 65 g cooked weight per serve.

[j] 80 g cooked weight per serve.

[k] 1 fillet, 100 g cooked fish, 115 g raw fish, 1 small can of fish or ½ cup other seafood (crab, prawn, lobster oyster or salted fish) per serve.

[l] Includes legumes & beans (1 cup cooked per serve) & tofu (~170 g per serve).

[m] 2 large eggs per serve.

[n] Includes nuts, seeds, & nut/seed pastes (30 g per serve).

[o] Includes bacon & deli meats.

[p] Includes milk & milk alternatives (1 cup fresh, UHT, long-life or reconstituted from powder or ½ cup evaporated per serve), cheese (40 g or 2 slices per serve) & yoghurt (3/4 cup or 200 g).

[q] ~10 g alcohol per standard drink. *Least-squares means and confidence intervals (adjusted for age and sex) are presented. n = 115 (pre-fortification) and 475 (post-fortification).*
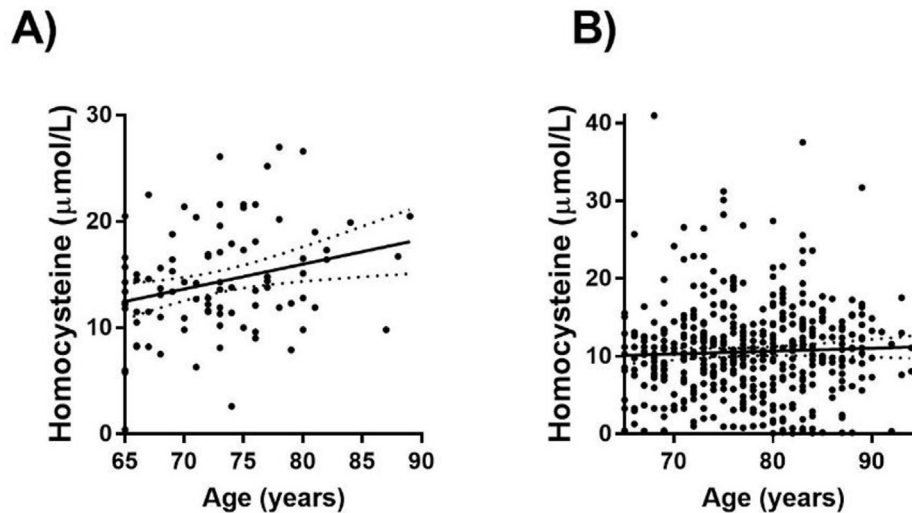
Fig. 3. A) Relationship between age and Hcy in the pre-fortification cohort ($r^2 = 0.330$, $p = 0.0003$). B) Relationship between age and Hcy in the post-fortification cohort ($r^2 = 0.010$, $p = 0.3$). p-interaction (age x cohort) = 0.05. Unadjusted data are presented, however, additional adjustment for sex, smoking history, alcohol intake and vitamin B_{12} levels did not significantly alter these results. n = 115 (pre-fortification) and 475 (post-fortification).

fortification and not to other dietary changes over time. Additionally, the results presented here indicate that mandatory fortification may mitigate the effect of age on Hcy levels. This is important, as the elderly are at increased risk of morbidities associated with hyperhomocysteinemia.

These findings are largely consistent with those reported for different regimens of mandatory fortification in other countries [55,56], however, magnitude of effect size varies. The results reported here reflect changes in plasma folate and Hcy levels previously identified in the Framingham Offspring Study cohort following the introduction of mandatory folic acid fortification in the USA [55]. However, in the Framingham Offspring Study, fortification appeared to have a larger impact on folate status, with a 12 nmol/L increase in the mean plasma folate levels post-fortification, compared to a 4.1 nmol/L increase in serum folate in the present study.

The discrepancy in findings may be explained by differences in: the period of time elapsed between the commencement of fortification and sample collection; sample collection duration; patterns of use of voluntary fortification prior to mandatory fortification; country-specific phase-in policies; dietary habits; assay methods; and assessment of a broader age cohort in the former study relative to the present study. In the USA all enriched flour, rice, pasta, cornmeal, and other grain products must contain 140 μg of folic acid per 100 g [55], whereas the Australian policy is limited to bread-making wheat flours only at a level of 120—135 μg of folic acid per 100 g [1].

However, it is interesting that the relative magnitude of change in folate status was not reflected in the magnitude of change seen in Hcy levels. A reduction in mean Hcy levels of approximately 0.7 μmol/L was detected in the Framingham Offspring Study [55], compared to 3.9 μmol/L in the present cohort. As stated above, this may reflect different assay methods, or it may demonstrate an increased benefit in the elderly cohort. However, a similar magnitude of Hcy reduction (5.5 μmol/L) was identified in women of reproductive age when mandatory fortification was introduced in Iran (150 μg/100 g flour) [56]. Varying time-frames of the commencement and duration of sample collection relative to the implementation of fortification, and different phase-in policies between countries, may also explain these differences.

Another intervention study in an elderly cohort (50—75 years;

n = 143), using folate-fortified bread, reported a mean reduction in Hcy of 1.6 μmol/L after 12 weeks [57]. This small reduction was in spite of the participants increasing folic acid intake by almost 600 μg per day [57], considerably more than the estimated increased intake following mandatory fortification. This may indicate that a longer period of increased consumption is more important than a high dose in the management of Hcy levels. This hypothesis is supported by another study of 18—80 year olds using fortified breakfast cereals (200 μg/day) which found an incremental decrease in mean Hcy levels over time, with a 0.6 μmol/L reduction after 4 weeks, and a reduction of 1.1 μmol/L after 24 weeks [58]. The influence of time-frame of supplementation on Hcy levels requires further investigation.

The results reported here also support the findings of Hickling et al. [54] who found a reduction in Hcy levels and rates of hyperhomocysteinemia in an Australian longitudinal cohort assessed in 1995 and in 2001. In 1995 few foods were fortified with folic acid, and in 2001 approximately 50% of breakfast cereals available in Australia were voluntarily fortified [54]. The serum folate values obtained in 2001 (mean 23.1 nmol/L) were similar to those found in the pre-fortification cohort presented here (mean 23.9 nmol/L), suggesting that the current pre-fortification cohort was representative of a voluntary fortification cohort. The combined findings of the two studies clearly shows the incremental improvement of folate status via voluntary and then mandatory fortification in Australia. Notably, the Hcy levels in the 2001 cohort (2.4 μmol/L) were considerably lower than in the present pre-fortification cohort, although this is likely due to the inclusion of younger participants (27—77 years) in the earlier cohort [54].

Analysis of the food frequency and supplement questionnaire data demonstrated that the observed increase in serum and red cell folate levels was not related to changes in the consumption of foods not subject to mandatory fortification, or supplement usage. In fact, in the present study, blood folate levels were higher post-fortification, despite reduced reported consumption of brassica vegetables, which are high in folate, and decreased reported usage of folate containing supplements. This suggests mandatory fortification is the principal factor associated with the increased blood folate levels and associated decrease in circulating Hcy. This is further demonstrated by the change in estimated intake of synthetic, and not natural forms of folate. While there are notable

limitations of dietary intake assessment via food frequency questionnaires, including potential over reporting of healthful foods and under-reporting of discretionary foods they are useful for comparisons of habitual intakes, and useful for comparisons between groups.

Although the use of two independent cohorts, rather than a longitudinal follow-up of the same subjects may be seen as a limitation of this study, it reduces the influence of an aging cohort on the outcome. Hcy levels are known to increase with age, and dietary habits may also vary with age and deterioration of taste sensation. Assessing two independent cohorts, sourced from the same geographical area, allows us to specifically assess the influence of mandatory folate fortification in the elderly demographic with minimal impact from these potentially confounding factors, and reduces the potential impact of participant loss to follow-up. Adjustments were applied where variables were known to influence outcomes of interest, or means varied between groups. However, the potential for undocumented differences in the two convenience samples used cannot be discounted.

Adjustment of the analyses here for factors known to influence Hcy levels (vitamin B_{12} levels, alcohol consumption, smoking, age, and sex) strengthens the results presented here. However, it is a limitation of the study design that other factors known to influence Hcy, such as physical activity [6], were not able to be controlled for.

The mitigation of the relationships between age and plasma Hcy in the post-fortification cohort generates optimism that reduced disease risk in the elderly, a particularly at-risk cohort, may be achievable with continued fortification. It is possible that population-wide fortification over an extended time-frame may be more successful in reducing symptoms and risk of cardiovascular and neurodegenerative diseases than previous intervention studies. Additional studies are needed to determine if the lowering of Hcy levels in post-fortification cohorts results in a reduction of risk of cardiovascular and neurodegenerative diseases. Although the post-fortification group was older on average, this reflects the higher maximum age, as such, the abrogation of the relationship between age and homocysteine in this larger study group is even more striking.

While the introduction of a mandatory folate fortification program that impacts the broader population to benefit a small percentage of the population may be seen as controversial, the results presented here demonstrate a potential benefit of folate fortification in the elderly. Decreased mean plasma Hcy and decreased rates of hyperhomocysteinemia across all age groups, may translate into a decreased disease burden in the Australian population in future generations, particularly if coupled with additional risk reduction strategies. The results presented here constitute important information to be considered by policy makers in jurisdictions that are still considering the potential risks and benefits of fortification.

Acknowledgements and declarations

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