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EVIDENCE BASED PUBLIC HEALTH POLICY AND PRACTICE

Impact of voluntary folate fortification on plasma homocysteine and serum folate in Australia from 1995 to 2001: a population based cohort study

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Study objective: To investigate the effect of the voluntary folate fortification policy in Australia on serum folate and total plasma homocysteine (tHcy) concentrations.

Design: Population based cohort study.

Setting: Perth, Western Australia.

Participants: Men and women aged 27 to 77 years (n = 468), who were originally randomly selected from the Perth electoral roll. The cohort was surveyed in 1995/96 before widespread introduction of folate fortification of a variety of foods, and followed up on two occasions, firstly in 1998/99 and again in 2001, when a moderate number of folate fortified foods were available. Subjects with abnormal serum creatinine concentrations at baseline were excluded from this analysis.

Main results: Repeated measures analysis of variance was used to determine changes in serum folate and tHcy over the three surveys and to assess whether time trends were related to age, sex, *MTHFR* C677T genotype, or consumption of folate fortified foods. An increase (38%) in mean serum folate ($p < 0.0005$) and a decrease (21%) in mean tHcy ($p < 0.0005$) were seen after introduction of the voluntary folate fortification policy in Australia. Serum folate was consistently higher ($p = 0.032$) and tHcy was consistently lower ($p = 0.001$) in subjects who consumed at least one folate fortified food compared with subjects who did not consume any folate fortified foods in the previous week. The time related changes in serum folate and tHcy were affected only by intake of folate fortified foods ($p < 0.0005$) and not by any other measured variables including age, sex, or *MTHFR* genotype.

Conclusion: Voluntary fortification of foods with folate in Australia has been followed by a substantial increase in serum folate and decrease in tHcy in the general population.

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Voluntary folate fortification was recommended by an expert panel of Australia's National Health and Medical Research Council in 1994 in response to the convincing body of evidence that folate supplementation reduced the incidence of neural tube defects (NTDs).¹ In June 1995 Australia's National Food Authority permitted the voluntary fortification of flour, bread, savoury biscuits, breakfast cereals, pasta, yeast extracts (a popular spread for bread in Australia), fruits and vegetable juices, and meal replacements with folate to 100 µg folate per serving.² Manufacturers are not required to fortify their products. In 1998, supplemental foods such as folate fortified milk products were added to the list and temporary permission was given for a health claim describing the link between adequate maternal dietary folate intake and a reduction in risk of NTDs developing in pregnancy. This is currently the primary means by which folate fortified products are identified and promoted nationally.³

There has been a recent and sustained decrease in the prevalence of NTDs in Western Australia that is thought to be attributable to increased peri-conceptual intake of folate supplement in response to health promotion campaigns and the voluntary folate fortification policy. The total frequency of NTDs (births plus terminations) was comparatively constant for the period 1980–1995 (average of 1.96 per 1000 births) but decreased by 30% to 1.38 per 1000 for 1996–2000.⁴

Increased intake of folate may have a wider impact beyond reducing the risk of NTDs. There is sound epidemiological evidence of an independent, dose related association between

total plasma homocysteine (tHcy) and morbidity and mortality from vascular disease.^{5–7} Folate decreases tHcy effectively at doses that could be achieved by increased intake of folate from folate fortified and folate rich foods.^{8–12} If widely shared in the community, modest decreases in tHcy could have a large impact on the population wide burden of vascular disease, assuming the relation between tHcy and vascular disease is causal.

A mandatory folate fortification policy in the USA was associated with a substantial increase in serum folate and decrease in tHcy in a population of middle aged and older adults.⁸ Other countries, including Australia and the United Kingdom have adopted a voluntary folate fortification policy but it is not known whether this is adequate to increase serum folate and reduce plasma homocysteine values significantly. We therefore investigated the effect of the voluntary folate fortification policy in Australia on serum folate and tHcy in a population based cohort. The cohort was surveyed in 1995/96 before widespread introduction of folate fortification, and followed up on two occasions, firstly in 1998/99, when some folate fortified foods were available, and again in 2001, when about 50% of breakfast cereals sold, 18% of breads sold, the two leading brands of yeast extract, and small proportions of other foods, including milk, were fortified with folate.^{3 13}

Abbreviations: NTD, neural tube defect; tHcy, total plasma homocysteine

Table 1 Serial changes in serum folate and total plasma homocysteine concentrations after voluntary folate fortification in Australia in a population based cohort

Variable	Baseline	Follow up 1	Compared with baseline*	Follow up 2	Compared with follow up 1*	Trend over time
Serum folate ($\mu\text{g/l}$) (SD)	7.4 (4.5)	9.8 (5.1)	$p < 0.0005$	10.2 (4.5)	$p = 0.124$	$p < 0.0005$
Serum folate $< 6 \mu\text{g/l}$	39%	21%	$p < 0.0005$	17%	$P = 0.005$	$p < 0.0005$
tHcy ($\mu\text{mol/l}$) (SD)	11.7 (3.6)	9.6 (3.3)	$p < 0.0005$	9.3 (3.7)	$p = 0.004$	$p < 0.0005$
tHcy $> 13 \mu\text{mol/l}$	29%	11%	$p < 0.0005$	10%	$P = 0.755$	$p < 0.0005$
Pearson correlation between serum folate and tHcy	$r = -0.26$	$r = -0.32$		$r = -0.31$		
% Use of folate supplements	15.4	10.7	$p = 0.020$	20.7	$p < 0.0005$	$p < 0.0005$

$n = 468$. *Paired sample t test. tHcy, total plasma homocysteine.

METHODS

Participants and study design

The participants were men and women aged 27 to 77 years from Perth, the capital city in Western Australia. Subjects were originally recruited for the 1989 Australian National Heart Foundation Perth risk factor prevalence survey.¹⁴ This was a stratified random survey of 2000 people drawn from the electoral roll for Perth. Enrolment to vote is compulsory for Australian citizens. Repeat electoral roll and death record matching in May 1995 established a current address for 1807 living subjects, and of these 1111 (61%) agreed to take part in the carotid ultrasound disease assessment study (CUDAS) between June 1995 and December 1996.¹⁵ Subjects were followed up on two occasions, firstly between September 1998 and December 1999, and again from October to December 2001. Subjects with abnormal serum creatinine ($>120 \mu\text{mol/l}$ men, $>100 \mu\text{mol/l}$ women) concentrations at baseline were considered likely to have chronic renal disease and were excluded from this analysis. Data presented here are for 468 subjects who participated in all three surveys.

Ethical issues

The human research ethics committee at the University of Western Australia approved the study. Informed consent was obtained from all participants.

Biochemical analyses

At each survey venous blood samples were collected from all participants after an overnight fast. Plasma and serum were separated by centrifugation shortly after venipuncture and transported to the laboratory in ice cooled containers. Total plasma homocysteine concentration was determined by high performance liquid chromatography using the method of Araki and Sako.¹⁶ As there is no definition of "high" tHcy concentration we used the often used threshold of $>13 \mu\text{mol/l}$, which approximately defines the top quartile of tHcy in our population.¹⁵ Serum folate was determined by immunoassay using an ACS:180 (Bayer Diagnostics) in the first two surveys. In the final survey serum folate was determined by immunoassay using a DPC Immulite 2000 as the ACS:180 method was no longer available. A random subset of samples ($n = 98$) from the first two surveys were re-measured via the DPC Immulite 2000 method. Results from the two methods agreed well with analysis yielding the following regression equation: y (ACS:180) = $0.98x$ (DPC Immulite 2000) + $1.444 \mu\text{g/l}$; $r = 0.95$; $p < 0.0005$. This calibration equation enabled comparison of results across all three surveys. A serum folate of $<6 \mu\text{g/l}$ was defined as "low".¹⁷ DNA was extracted by the salt phenol chloroform method from the cells of the buffy coat. Methylene tetrahydrofolate reductase *MTHFR* C677T genotype was determined by *HinfI* digestion of the PCR products.¹⁸

Dietary and lifestyle assessment

Dietary intakes of naturally occurring folate, vitamin B6, and vitamin B12 were estimated at baseline (1995/96) and follow up 1 (1998/99) by a self administered semi-quantitative food frequency questionnaire.^{19, 20} At follow up 2 (Dec 2001) dietary intake of folate was estimated using a previously validated rapid dietary assessment tool that collected information on the use of the types and brand of foods that may be fortified with folate.²¹

Statistical analyses

Pearson's correlation was used to determine the relation between serum folate and tHcy. Paired sample t tests were used to determine if there was a difference in mean tHcy, serum folate, and dietary intake of naturally occurring folate, vitamin B6, and vitamin B12 between surveys. Repeated measures analysis of variance was used to determine if there were changes in serum folate and tHcy over the three surveys and (via interaction terms) whether time trends were related to age, sex, *MTHFR* genotype, or consumption of folate fortified foods. Frequencies of categorical variables were compared using McNemar's test and Cochran's Q test. We used logistic regression to explore associations between use of folate fortified foods and age, sex, use of folate supplements, smoking, and socioeconomic status as estimated from the SEIFA index,²² and adopted a two tailed α 0.05 as showing significance.

RESULTS

Study population

The study population consisted of 219 women and 249 men, ranging in age at baseline from 27 to 77 years, with a mean age of 53 years. They were a sub-cohort of the overall CUDAS general population, whose clinical characteristics have been described previously.¹⁵

Serum folate and total plasma homocysteine

Table 1 shows the folate and tHcy data at baseline and follow up visits. The Pearson correlation coefficient (r) for serum folate and tHcy was consistent across the surveys at about -0.3 ($p < 0.0005$). Repeated measures analysis showed an increase (38%) in mean serum folate ($p < 0.0005$) and a decrease (21%) in mean tHcy ($p < 0.0005$) after introduction of the voluntary folate fortification policy in Australia (table 1). The percentage of subjects with low serum folate ($<6 \mu\text{g/l}$) decreased from 39% at baseline to 17% at follow up 2 ($p < 0.0005$). The percentage of subjects with high tHcy ($>13 \mu\text{mol/l}$) decreased from 29% at baseline to 10% at follow up 2 ($p < 0.0005$).

Figure 1 shows the distribution of serum folate concentration at baseline, and follow up visits 1 and 2 for the whole cohort (A) and when the 152 subjects who reported taking folate supplements at any of the surveys are excluded (B).

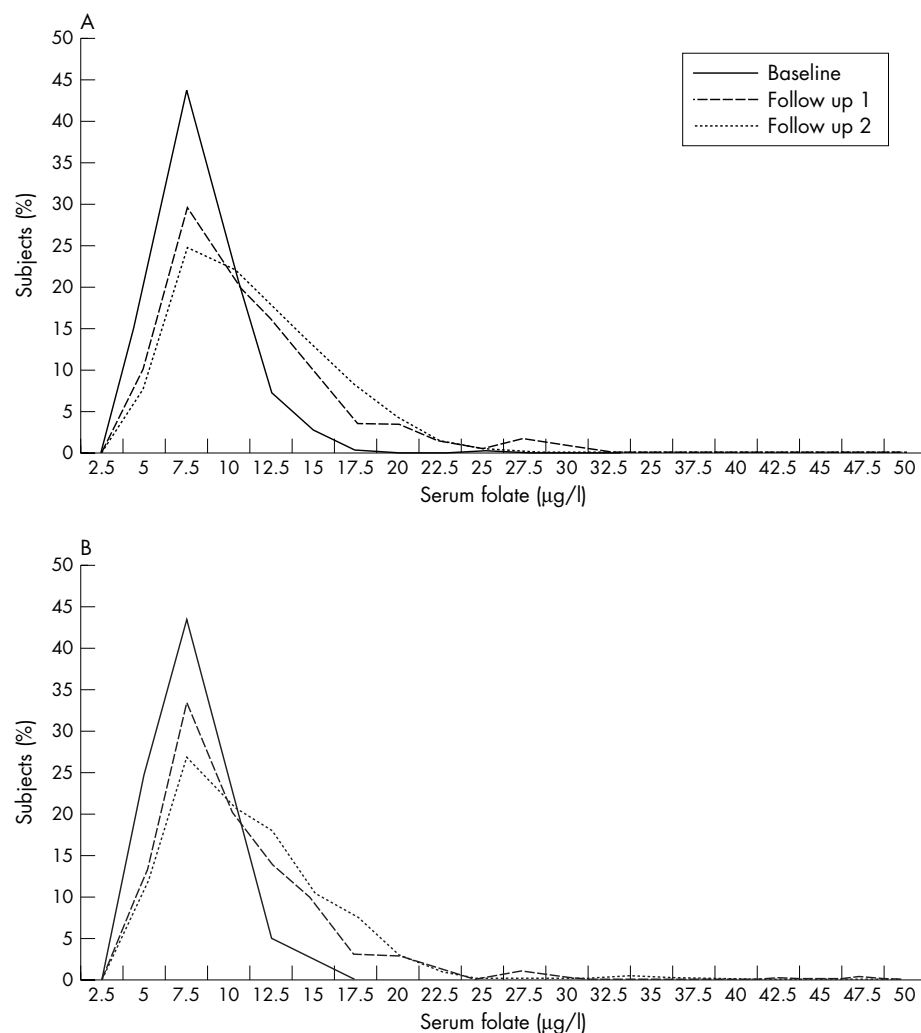


Figure 1 Serum folate concentrations after voluntary folate fortification in Australia in a population based cohort (A) and in the cohort when supplement users are excluded (B).

Figure 2 shows similar distributions of tHcy for the whole cohort (A) and for the cohort excluding users of supplements (B). The figures show the clear upward shift in the distribution of serum folate from baseline to the follow up surveys, and the downward shift in tHcy across the whole cohort and for the cohort excluding users of supplements.

The use of dietary supplements containing folate was greater at follow up 2 compared with baseline (table 1). However, we did not determine how long subjects had been using these vitamin supplements and only 13 subjects (2.8%) reported using supplements at all three surveys.

Intake of folate fortified foods

The estimated mean dietary folate from naturally occurring sources was about 300 µg/day and did not differ significantly from the baseline survey to follow up 1 (paired sample *t* test, $p = 0.65$). Similarly, the dietary intake of naturally occurring vitamin B6 and vitamin B12 did not differ significantly from the baseline survey to follow up 1 (paired sample *t* test: B6 $p = 0.43$, B12 $p = 0.99$). The estimated dietary intake of folate from fortified sources only at follow up 2 was 65 µg/day. Over half (57%) of the cohort reported consumption of a folate fortified breakfast cereal and 52% reported consumption of a folate fortified yeast extract (for example, Vegemite and Marmite) in the week before the survey. Only 1% of the cohort reported consuming folate fortified bread and 7% of the cohort reported consuming folate fortified milk.

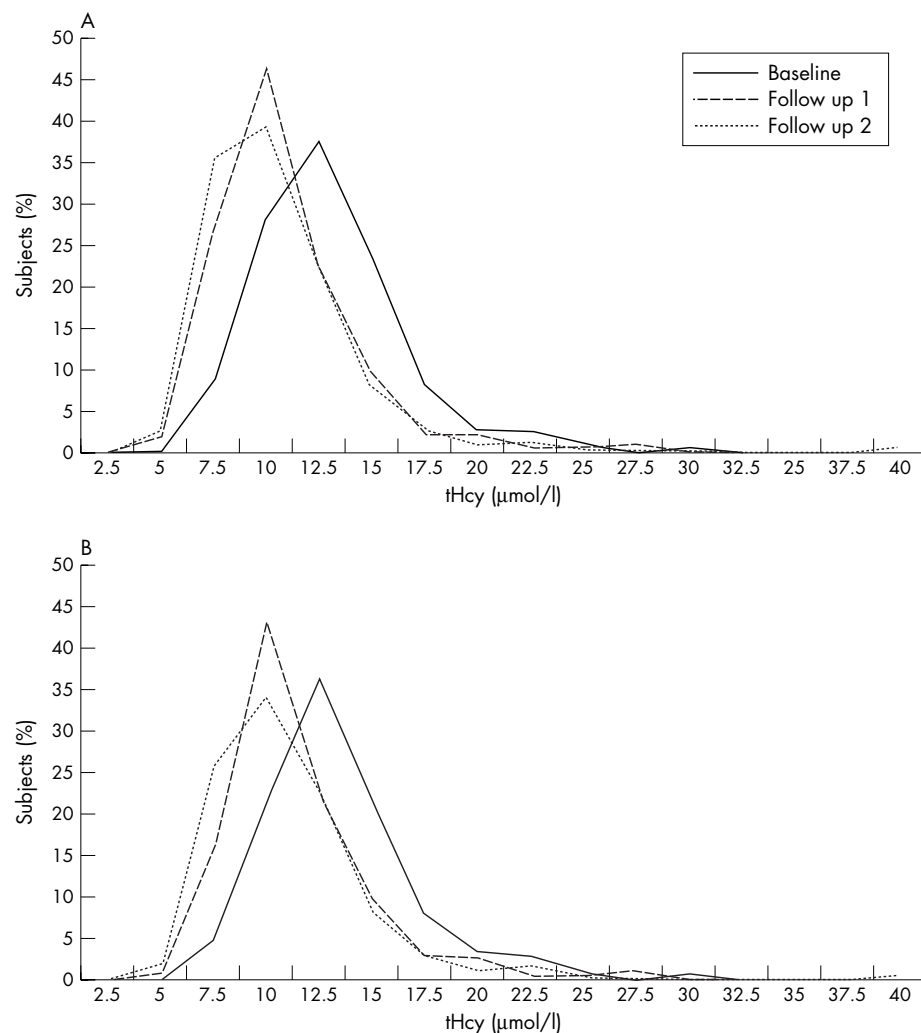
Subgroup analyses

There was a consistently higher mean serum folate for women compared with men ($p = 0.001$). Conversely, the tHcy was higher for men than women ($p < 0.0005$). The survey by sex interaction was not significant for either serum folate or tHcy.

Mean serum folate was not significantly different for different age groups ($p = 0.126$). However, mean tHcy increased consistently with increasing age ($p < 0.0005$). The survey by age interaction was not significant for either serum folate or tHcy.

There was a significant difference in serum folate concentrations across *MTHFR* genotypes ($p = 0.017$) with the homozygous subjects (TT—comprising 10% of the cohort) having a consistently lower serum folate than the heterozygotes (CT—comprising 48% of the cohort) or subjects with the wild type gene (CC—comprising 42% of the cohort). However, the *MTHFR* genotype-time interaction was not significant for serum folate with a comparable increase in mean serum folate among the three genotypes. Similarly, the TT subjects had a consistently higher tHcy than the CT or CC subjects ($p = 0.021$) but had a comparable decrease in mean tHcy concentrations over the survey time. The *MTHFR* genotype-time interaction was of borderline significance for tHcy ($p = 0.079$).

Serum folate was consistently higher ($p = 0.032$) and tHcy was consistently lower ($p = 0.001$) in subjects who consumed



at least one folate fortified food compared with subjects who did not consume folate fortified foods in the previous week. The time by use of folate fortified foods interaction was highly significant for serum folate ($p < 0.0005$) and tHcy ($p < 0.0005$).

Factors related to the use of folate fortified foods

We explored, by binary logistic regression, the relation between certain demographic, lifestyle, and socioeconomic factors and use of folate fortified foods. None of the variables sex, age, smoking status, use of folate supplements, and socioeconomic status was significantly related to the use of folate fortified foods.

DISCUSSION

Our findings suggest that voluntary fortification of foods with folate in Australia has had a significant beneficial effect on serum folate and tHcy concentrations in the population. In our cohort of 468 men and women, mean serum folate increased significantly, by 38%, and mean tHcy decreased significantly, by 21%, after voluntary fortification was implemented. The proportion of subjects with low serum folate or high tHcy decreased by more than half. As compared with the successful results of mandatory folate fortification policy in the USA,⁸ this is the first study, to our knowledge, that has shown that even a voluntary folate fortification policy can be followed by a substantial increase in serum folate and decrease in tHcy in the general population.

Key points

- The mandatory folate fortification policy in the USA has been followed by increased blood folate concentrations and decreased plasma homocysteine in middle aged and older persons.
- Australia, New Zealand, the United Kingdom, and other European countries currently have a voluntary folate fortification policy, but it is not known whether this is sufficient to increase folate status and reduce plasma homocysteine in the general population.
- Voluntary fortification of foods with folate in Australia has been followed by a significant increase in serum folate and decrease in plasma homocysteine in this community.
- Assuming a causal relation between plasma homocysteine and vascular disease, the observed changes might eventually translate into reductions in ischaemic heart disease and stroke of 13% and 20% respectively.

The estimated mean dietary intake of folate measured at baseline was similar to that seen in the 1995 Australian national nutrition survey (NNS)²³ and did not differ significantly from the equivalent estimate at follow up 1.

No adjustments were made to the nutrient database between our surveys to account for fortification of foods with folate. Thus our data show that there was no significant difference in folate intake from naturally occurring sources and suggest that any change in folate status is the result of folate added to the diet as part of the voluntary fortification policy in Australia. Indeed, subjects who consumed at least one folate fortified food per week had a greater increase in serum folate and a greater decrease in tHcy than subjects who did not use folate fortified foods. It is important to emphasise here that subjects did not identify themselves as users of folate fortified foods; rather they were asked to complete a rapid food frequency questionnaire and to show the type and brand names of the foods they ate. The questionnaire made no reference to folate but captured information that allowed us to identify which subjects were users of folate fortified foods. The time related changes in serum folate and tHcy were related only to intake of folate fortified foods ($p < 0.005$) and not to age, sex, or *MTHFR* genotype.

We explored the relation between use of these foods and a number of sociodemographic and lifestyle factors, but none of the factors we examined (age, sex, socioeconomic status, use of folate supplements, and smoking status) was related to the use of folate fortified foods. This suggests that such use is widespread in the community and not restricted to any particular age or socioeconomic group, although it remains possible that other factors that we did not explore may differentiate users of folate fortified food from non-users.

The reported use of dietary supplements containing folate was irregular. While the proportion using supplements at follow up 2 was greater than at baseline only 2.8% reported using supplements at all three surveys. The small number of subjects reporting regular use of supplements may explain why there is little difference in serum folate when the distribution for the entire cohort is compared with the distribution for the cohort with subjects using supplements containing folate at any of the three surveys are removed.

While voluntary folate fortification in Australia came into effect in June 1995, the response by food manufacturers to the new policy has been modest.¹³ We found the greatest increase in serum folate and decline in tHcy from baseline to follow up 1, at a time when only some folate fortified foods were available. However, the first foods to be fortified with folate were breakfast cereals and Lawrence *et al*¹³ reported that in June 1999 (towards the end of follow up 1) 50% of breakfast cereals sold were fortified with folate. Breakfast cereal was the most frequently used folate fortified food in

our cohort, which may explain why we observed a greater increase in serum folate and decline in tHcy between baseline and follow up 1 than with between follow up 1 and follow up 2.

The reported increases in serum or plasma folate under a mandatory folate fortification policy in the USA (5.4 µg/l), Canada (3.8 µg/l), and Chile (7.2 µg/l) are appreciably greater than the measured increase in serum folate under the voluntary folate fortification policy in Australia.^{8 24 25} However, the decrease in plasma homocysteine in Australia has been comparable to that reported in the other countries, which may suggest a threshold effect on homocysteine lowering from increased folate intake in the general population. A mandatory folate fortification policy in Australia is currently being considered by Food Standards Australia New Zealand (the bi-national independent statutory authority that develops food standards that apply to all foods produced or imported for sale in Australia and New Zealand). Under the voluntary folate fortification policy this study observed a greater increase in serum folate in users of folate fortified foods. A mandatory policy, which should reach a greater proportion of the population, is likely to result in a wider spread increase in serum folate. It will be interesting to see whether this results in a further decrease in homocysteine concentrations.

A daily supplement of 400 µg folate is reported to achieve maximum lowering of tHcy.¹² Dietary modelling based on data from the 1995 NNS predicted that mandatory fortification to 50% of the recommended daily intake of cereals, juice, and yeast extract would result in women aged 15–49 years consuming an additional 250 µg of folate per day,²⁶ with men expected to consume even more. Only a limited proportion of the permitted foods have been fortified under the voluntary folate fortification policy in Australia, and our cohort consumed less than a third of the anticipated mean increase in daily intake of folate from fortified foods predicted from the modelling of mandatory fortification described above. Thus there is even greater potential to raise serum folate in our population with wider adoption of folate fortification in Australia.

Although the cardiovascular benefit of reducing tHcy is unconfirmed, on the basis that the association between tHcy and vascular disease is causal, Wald *et al* estimated that a 3 µmol/l decrease in tHcy would result in a reduction of 16% in ischaemic heart disease and of 24% in stroke.⁷ In this study a decline in mean tHcy of 2.4 µmol/l was seen after the introduction of voluntary folate fortification in Australia. If homocysteine proves to be a cause of vascular disease, then the voluntary folate fortification policy in Australia would be expected eventually to have a measurable impact on cardiovascular disease. The growing availability of folate fortified foods points to the potential of folate fortification to decrease homocysteine in the community still further.

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Policy implications

- Australia, New Zealand, the United Kingdom, and other European countries currently have a voluntary folate fortification policy.
- This paper shows that the voluntary fortification of foods with folate in Australia has been followed by a significant increase in serum folate and decrease in plasma homocysteine in this community. The increase in serum folate and decrease in tHcy was greater in subjects who consumed at least one folate fortified food per week than subjects who did not use folate fortified foods.
- Only a limited proportion of the permitted foods have been fortified under the voluntary folate fortification policy in Australia. Thus, there is even greater potential to raise serum folate in our population with wider adoption of folate fortification in Australia.

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