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High folate levels in Aboriginal children after subsidised fruit and vegetables and mandatory folic acid fortification

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In Australia, as in many other countries, promotion of folate supplements and folic acid fortification of foods has been introduced to reduce the incidence of neural tube defects (NTDs). Voluntary folic acid fortification of a restricted number of foods started in Australia in 1995.¹ However, due to the limited effect of voluntary fortification on reducing the number of NTDs, mandatory folic acid fortification of bread-making flour was introduced in Australia in September 2009.² Mandatory folic acid fortification of staple foods, such as bread, has the potential to increase folate intake in the entire population, including the disadvantaged, who are less likely to follow public health recommendations regarding folic acid supplementation in early pregnancy. The effectiveness of this intervention was demonstrated by a reduction in the prevalence of folate deficiency after mandatory folic acid fortification in a large cohort study of patients at one Australian public hospital.³ In this study, the prevalence of low red blood cell (RBC) folate decreased from 3.4% in April 2009 to 0.5% in April 2010.

The increase in mean folate levels in the population has the potential to improve other health outcomes in addition to NTDs including reducing the risk of cardiovascular disease⁴ and cancer.⁵ However, unlike the substantial evidence of the role of folic acid fortification in reducing NTDs, the impact of folate on invasive cancer is less well

Abstract

Objective: To evaluate the impact of a fruit and vegetable (F&V) subsidy program for disadvantaged Aboriginal children in Australia, implemented alongside the introduction of mandatory folic acid fortification of bread-making flour.

Methods: A before-and-after evaluation was undertaken of a F&V subsidy program at three Aboriginal community-controlled health services in New South Wales. The program provided a weekly box of subsidised F&V linked to preventive health services and nutrition promotion for families. In this analysis, red blood cell (RBC) folate was assessed together with self-reported dietary intake at baseline and 12 months later in a cohort of 125 children (aged 0-17 years).

Results: No children had low RBC folate at baseline or at follow-up; however, 33 children (26%) exceeded the reference range of RBC folate at baseline and 38 children (30%) exceeded the reference range at follow-up. Mean RBC folate levels increased substantially in children at follow-up (mean RBC folate z-score increased +0.55 (95%CI 0.36-0.74). Change in F&V intake ($p=0.196$) and mean bread intake ($p=0.676$) were not statistically significant predictors for change in RBC folate levels.

Conclusions: RBC folate levels increased among these disadvantaged Aboriginal children following mandatory folic acid fortification and participation in a subsidised F&V program. Even before mandatory folic acid fortification, none of these children had low RBC folate.

Implications: The effect on health of mandatory fortification of foods with folate is not clear, hence, ongoing population-based monitoring of folate levels to assess the impact of mandatory folic acid fortification is important.

Key words: Aboriginal health, nutrition, biomarkers

established.^{6,7} There is also concern about other potential adverse impacts of high folate levels, including masking the anaemia of vitamin B12 deficiency or increasing cognitive impairment in older people with low vitamin B12.⁸ Thus, monitoring of folate levels in populations who may be more likely to consume large quantities of fortified folic acid has been advocated.⁹

In a recent evaluation of a fruit and vegetable (F&V) subsidy program for disadvantaged Aboriginal families in rural NSW, RBC folate was measured in a panel of biomarkers used to assess F&V intake. As this study involved baseline blood tests completed just before the introduction of mandatory folic acid fortification in Australia in 2009, as well as follow up tests completed after 3-9 months

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of folic acid fortification, it provided an opportunity to investigate, in an Aboriginal population at high risk of NTDs¹⁰ and invasive cancer,¹¹ the combined impact of subsidised F&V and folic acid fortification of bread on the folate levels of a cohort of children. The specific aim of this analysis was to investigate the extent to which any changes in RBC folate were related to either folic acid fortification or any change in F&V intake in these children.

Methods

The F&V program

The F&V subsidy program and the methods have been described previously.¹² Briefly, a before and after study was conducted of a cohort of low-income Aboriginal families who participated in a F&V subsidy program at three rural Aboriginal community-controlled health services: Bulgarr Ngaru Medical Aboriginal Corporation in the Clarence Valley, Galambila Aboriginal Health Service in Coffs Harbour and the Giingan Darrunday Marlaangu Health Clinic in the Nambucca Valley in northern NSW. The F&V subsidy program involved families with one or more young children attending annual health assessments, including dental and hearing check-ups, and then receiving a weekly box of subsidised F&V. Each family paid \$5 for a box containing \$40 of seasonal F&V (or \$60 if there were five or more children in the household) and collected the box from local greengrocers.

Participants

All children 0-16 years 9 (n=174) in each participating family (n=55) had a non-fasting blood sample taken and a 24-hour diet recall prior to commencement. The same assessments were repeated after 12 months participation in the program. The recruitment and baseline assessments were undertaken between December 2008 and September 2009, with follow-up assessments completed between December 2009 and September 2010. The response rate of participants at baseline and follow-up is shown in Figure 1.

Data collection and analysis

The 24-hour diet recalls were completed by participants face-to-face with a dietician or a trained Aboriginal health worker. For participants aged <10 years, these were completed together with a parent/carer. The data collection followed the multi-pass

method described in the 1995 Australian National Nutrition Survey.¹³ In addition, participants were asked about their usual consumption of F&V using validated short dietary questions based on the Dietary Intake Assessment Tool developed in Queensland, Australia.¹⁴

Pathology samples from the Clarence Valley and Coffs Harbour/Nambucca Valley were analysed for RBC folate in separate laboratories using different immunoassays on an Architect i2000 Immunoassay analyser (Abbott Diagnostics, Australia) and the ADVIA Centaur XP automated immunoassay platform (Siemens Diagnostics, Australia), respectively. To account for the differences between the results from each assay, the RBC folate data were standardised with a mean of 0 and standard deviation of 1 (i.e. converted to z-scores) for the combined before and after results at each location. After standardisation, the change in RBC folate before and after participation in the F&V program was compared using paired t-tests and linear model adjustments for gender, age and community, using SPSS Version 19 (IBM, New York, USA). The dietary recall data were analysed using the FoodWorks Professional 2009 dietary software programme (Xyris, Queensland, Australia) using the AusNut 2007 Australian food database. The correlation between the change in RBC folate and both bread intake and F&V intake was assessed with scatter plots and a linear regression model of the change in RBC folate against both mean bread intake and change in F&V intake.

Ethics

Ethics approval was obtained from the University of Melbourne, University of South Australia and the Aboriginal Health and Medical Research Council (New South

Wales) human research ethics committees. Community consent was obtained from the Boards of the three participating health services. Parents/carers provided written informed consent for their children to participate in this study and individual results were discussed with participants and/or parents at follow-up appointments.

Results

There were 55 Aboriginal families recruited to participate in the evaluation of the F&V program at the three Aboriginal Health Services which included 174 children. All assessments were of these children aged 0-17 years at baseline, with most between the ages of three and 12 years. The demographic characteristics of the participants at baseline (as shown in Table 1) indicates this was a cohort of low-income families, with the majority receiving Government benefits as their main source of income. The majority of adults in these households were smokers. There were differences between the cohorts of families at each location: the children were older and a lower proportion of adults smoked in Coffs Harbour, while the children were younger and a greater proportion were female in Nambucca Valley. However, the sample size at each location was not sufficient to examine the impact of these differences or differences in the implementation of the program between communities. In the Nambucca Valley, the mean RBC folate at baseline was significantly lower among those who only had a baseline value compared to those who returned for follow-up ($t=-2.8, p=0.008$), but not in the other two locations. However, there were no significant differences in F&V intake in those assessed only at baseline and those who underwent follow-up assessments (data not shown).

Figure 1: Response rate to baseline and follow-up assessments among F&V program participants.



Mean RBC folate levels increased in each community at the 12 month follow-up compared to the mean baseline levels, although only the 50% increase in the Clarence Valley (from 496 to 744 nmol/L, $p < 0.001$) was statistically significant (Table 2). Adjustment for age and sex did not affect the mean change in each community substantially or the statistical significance of the increase in the Clarence Valley. None of the children had low RBC folate at either baseline or follow-up, based on the reference ranges for the relevant assay. Interestingly, while only one of 76 children (1.3%) in the Clarence Valley exceeded the relevant reference range for folate at baseline, 13/25 (52%) in Coffs Harbour and 19/24 (79%) in the Nambucca Valley exceeded the reference range. A similar distribution was observed at follow-up, with no children exceeding the reference range in the Clarence Valley, but 15/25 (60%) in Coffs Harbour and 23/24 (96%) in the Nambucca Valley exceeding the upper bound of the reference range.

The mean change in RBC folate z-score (after-before) was 0.55 (95%CI 0.36-0.74) after adjustment for covariates (Figure 2). This mean change in RBC folate z-score is equivalent to a Cohen's d and thus represents a medium effect size. Among the individual communities, the mean RBC folate z-score increased significantly in the Clarence Valley and the Nambucca Valley (Figure 2).

Mean folate intake by age and sex at baseline exceeded the estimated average requirement (EAR) for all categories, except for girls aged 14-17 years, whose mean folate intake was less than the EAR (Table 3). However, there were small numbers of both 14-17 year old boys and girls. There were no children aged 2-3 years or 4-8 years whose folate intake was less than the estimated average requirement, while 5% of 9-13 year olds and 8% of 14-17 years olds consumed less than the EAR.

The scatterplots of mean F&V intake and change in F&V intake (from the two 24-hour recalls) versus the change in RBC folate z-score did not show a linear relationship between F&V intake and RBC folate

Table 1: Baseline demographic characteristics of participating children by individual community (n=174).

	All communities	Clarence	Coffs Harbour	Nambucca
Families, n	55	30	12	13
Children, n	174	90	36	48
Age (years) ^a	7.6 (4.2)	7.5 (3.8)	11.0 (3.3)	5.8 (4.3)
Sex, n (%)				
Male	82 (47%)	46 (51%)	18 (50%)	18 (37%)
Female	92 (53%)	44 (49%)	18 (50%)	30 (63%)
Children with smoker(s) in household, n (%) ^b	107 (66%)	62 (69%)	18 (50%)	27 (71%)
Families receiving unemployment benefits, pensions	51 (93%)	28 (93%)	10 (83%)	13 (100%)

^a Mean (SD)

^b Percentage of those with a valid response to smokers in household.

Table 2: Red cell folate before and after 12 months on the fruit and vegetable program for participating communities.^a

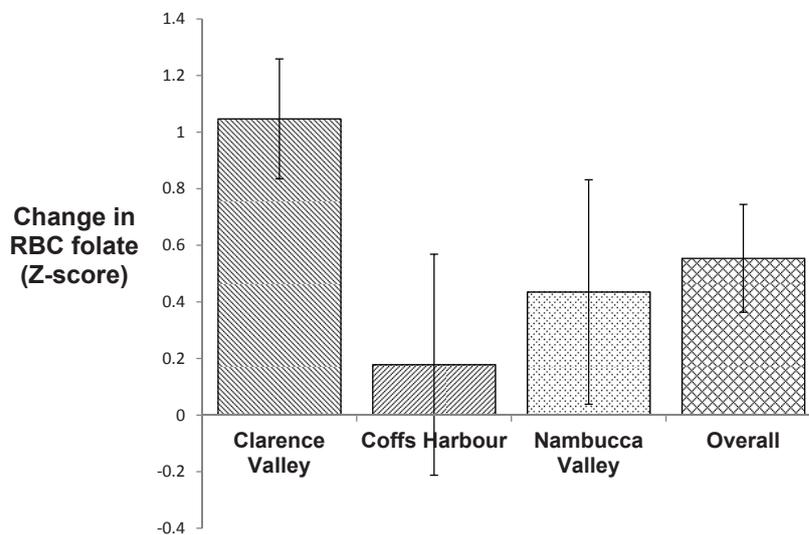
	RBC folate (nmol/L) Mean (SD)		Change in RBC Folate (95% CI)	Adjusted change in RBC Folate ^b (95% CI)
	Before	After		
Clarence Valley (n=76)	496 (224.2)	744 (185.4)	248 (195.5 – 301.3) [‡]	248 (197.2 – 299.6) ^c
Coffs Harbour (n=25)	1050 (247.2)	1161 (237.9)	111 (-9.1 – 231.1)	92 (-52.4 – 235.7)
Nambucca Valley (n=24)	1312 (376.4)	1414 (326.3)	102 (-39.2 – 243.3)	122 (-26.5 – 269.7)

^a Different assays were used in Clarence versus Coffs Harbour/Nambucca Valley. The reference ranges were 156-1452 nmol/L in Clarence and 218-820 nmol/L (<11 years old) and >630 nmol/L (>11 years old) in Coffs Harbour/Nambucca Valley

^b Adjusted for sex and age

^c Values significantly different from zero ($p < 0.05$)

Figure 2: Change in RBC folate z-scores by community before and after 12 months of the fruit and vegetable program (Mean, 95% CI).^{a,b}



^a Adjusted for gender, age and community

^b All changes in RBC folate significantly different from zero ($p < 0.05$) except for Coffs Harbour.

Table 3: Folate intake of fruit and vegetable program participants aged 2-17 years by age category and sex at baseline [Mean (SD), n=139].

	Age Category											
	2-3 years			4-8 years			9-13 years			14-17 years		
Mean (SD)	Male n=13	Female n=8	Total n=21	Male n=23	Female n=28	Total n=51	Male n=27	Female n=29	Total n=56	Male n=4	Female n=7	Total n=11
Folate (mcg)	225.0 (109.7)	262.5 (69.3)	239.3 (96.2)	318.0 (114.8)	235.4 (78.4)	272.6 (104.1)	291.2 (141.2)	313.8 (129.0)	302.9 (134.2)	440.8 (136.9)	179.7 (98.4)	274.6 (169.6)

(Supplementary File 1). Similarly, mean bread intake (from the two 24-hour recalls) versus the change in RBC folate z-score did not demonstrate a linear relationship (Supplementary File 1). In the linear regression models, change in F&V intake ($p=0.196$) (Table 4) and mean bread intake ($p=0.676$) (Table 5) were not statistically significant predictors of the change in RBC folate z-score. In both models, baseline RBC folate z-score was a significant predictor of the change in RBC folate z-score (Tables 4 and 5).

Discussion

We report a moderately large and statistically significant increase in RBC folate in the follow-up blood tests compared to baseline levels among a cohort of Aboriginal children who were part of a F&V subsidy program. This is consistent with the increase in other biomarkers of F&V intake reported previously in this study,¹² although the increase in RBC folate was larger than the increase in these other biomarkers. This additional increase was probably due to the impact of mandatory folic acid fortification of bread-making flour which was implemented during the evaluation of the program. Despite this, neither mean bread intake nor change in F&V intake were statistically significant predictors of the change in RBC folate in the linear regression models. Thus, it is difficult to draw meaningful conclusions as to whether F&V intake or the mandatory folic acid fortification of bread-making flour were more important in the increase in RBC folate observed.

There were no children with low RBC folate at either baseline or follow-up. However, 52% of children at Coffs Harbour and 79% of children at Nambucca exceeded the relevant reference range for RBC folate at baseline and this increased to 60% of children at Coffs Harbour and 96% of children at Nambucca at follow-up. Reported intake of F&V at baseline was low and mean folate intake at baseline exceeded EAR for boys and girls of all ages except 14-17 year old girls. These findings, together with the observation by staff at these health services of many children's high intake of breakfast cereal, particularly Sanitarium brand Weet-Bix®, suggest that folic acid fortification of foods including breakfast cereals contributed significantly to these high levels of RBC folate both before and after the completion of this evaluation study.

Table 4: Linear regression model of change in F&V intake and baseline RBC folate as predictors of change in RBC folate .

	B	Standard Error of B	β
Constant	0.57	0.09	
Baseline RBC folate z-score	-0.57	0.09	-0.52*
Change in F&V intake	-0.05	0.04	-0.11

Note: $R^2=0.30$ ($p<0.001$).
Dependent Variable: Change in RBC folate z-score
* $p<0.001$

Table 5: Linear regression model of mean bread intake and baseline RBC folate as predictors of the change in RBC folate.

	B	Standard Error of B	β
Constant	0.48	0.14	
Baseline RBC folate z-score	-0.66	0.07	-0.64*
Mean bread intake	0.02	0.05	0.03

Note: $R^2=0.41$ ($p<0.001$).
Dependent Variable: Change in RBC folate z-score
* $p<0.001$

In Australia, the voluntary folic acid fortification of foods, including breakfast cereals, began in June 1995.¹ Although breakfast cereals are the most common food voluntarily fortified with folic acid in Australia, folic acid fortification of breakfast cereals has not been universal.^{15,16} Folic acid fortified breakfast cereals include Sanitarium brand Weet-Bix®, but not some other common brands of wheat biscuit breakfast cereals (Uncle Toby's Vitabrits®, Coles Whole Wheat Biscuits). Thus food choices, including both the type of foods consumed and brand loyalty within specific food types¹⁷ appear to be important in determining the intake of folic acid fortified foods.

The absence of folate deficiency among the children in our study contrasts with the reported prevalence of low RBC folate (3.1%) in a large sample of Australian adults prior to mandatory folic acid fortification.³ Much higher levels of folate deficiency have previously been reported in Indigenous adults from remote communities. Lee et al.¹⁸ reported that 90% of adults had low RBC folate in one remote Northern Territory community in 1989, prior to voluntary folic acid fortification. More recently, after the introduction of voluntary folic acid fortification, a large cohort study among Indigenous adults in remote North Queensland reported high rates of low RBC

folate (15.9% in 1999 and 36.9% in 2007).¹⁹ No studies of RBC folate in Australian children were identified, however Gwynn et al. reported that 29% of Indigenous children aged 10-12 years consumed less than the EAR of folate in 2005-6.²⁰ In contrast, our study found only 5-8% of 9-17 year old children consumed less than the estimated average requirement of folate and none of the 2-8 year old children consumed less than the EAR. The findings are consistent with the low level of folate deficiency (<1%) reported in US²¹ and Canadian children²² (who have been exposed to mandatory folic acid fortification of cereal and grain products since 1998²³) and the low levels of folate deficiency reported in Australian adults after mandatory folic acid fortification of bread-making flour.³ Voluntary folic acid fortification and dietary folate appeared to be adequate to prevent folate deficiency in the disadvantaged Aboriginal children in this study. However, this needs confirmation in further studies and it is a concern that older girls reported the lowest intake of folate as they represent an important target group of folic acid fortification.

The high prevalence of RBC folate levels above the reference interval in this and other recent studies^{3,22} reflects the changing distribution of RBC folate in the population. RBC folate reference intervals have been determined by including the central 95% of values obtained from population samples. However, the introduction of voluntary and/or mandatory folate-fortification is shifting the distribution of RBC folate in the population.^{3,21} This has led at least one Australian pathology laboratory to change its folate reference intervals from a central 95% distribution to one based on the risk of folate deficiency with no upper limit specified (Chris Farrell, Laverty Pathology, personal communication). All of the high RBC folate results in this study were found using this laboratory assay, hence using the updated reference range would mean that none of these results would be classified as high. The removal of the upper limit of the folate reference range at this laboratory is based on the strong evidence of benefit from increased folate intake (and consequent increased RBC folate) and lack of clear evidence of harm from higher folate levels. Although the upper limit of RBC folate associated with safe levels of folate intake has not been clearly defined, the Australian and New Zealand nutrient reference values define an upper limit of

folate intake by age and gender.²⁴ This upper limit of folate intake represents the level below which adverse neurological effects due to increased folate in adults are very unlikely. Future work is needed to define the level of RBC folate equivalent to this upper limit of folate intake, analogous to the use of low RBC folate cut-offs to define inadequate folate intake.²⁵

The mean folate intake of these children at baseline above the EAR and the high proportion of RBC folate above the reference range in this and other studies^{3,22} has underlined the importance of monitoring the impact of folic acid fortification on populations. Despite the clear benefits of folic acid fortification on reducing NTDs,^{10,26} there remains uncertainty about the effects of folate fortification on cancer and cardiovascular disease risk.^{6,8,22} Although high folate levels have not been shown to be hazardous in children, Food Standards Australia New Zealand estimated that 9% of 2-3 year old children will consume greater than the upper limit of dietary folate after mandatory folic acid fortification. This is consistent with the finding that disadvantaged Aboriginal and Non-Indigenous children may be more likely to consume relatively larger amounts of folic acid fortified foods, such as bread or cereals.²⁰ The proposed monitoring plan of the impact of mandatory folic acid fortification in Australia and New Zealand identifies the importance of ensuring that those most likely to consume relatively higher amounts of fortified folic acid, particularly children, are included in these monitoring data.²⁷

A limitation of this study is the uncontrolled study design, which reduces the strength of the conclusions that can be drawn from these data. In addition, only 84% of participants had baseline assessments and 69-72% completed follow-up assessments. The majority of those who did not complete baseline assessments were aged three years or under. This may have biased the change in RBC folate as those aged under three who completed both assessments did not have a significant increase in RBC folate after standardisation. The two different RBC folate assays used at the different locations demonstrated marked variability and required standardisation prior to analysis. This variability between RBC folate assays is well recognised^{28,29} and is important to reduce given the increasing use of RBC folate to define adequate folate intake. The limitations of dietary self-report in Aboriginal

community studies³⁰ and in children³¹ have been described. In addition, the database used in the dietary analysis had not been updated to account for changes after the mandatory folic acid fortification of bread and thus could not be used to accurately assess folate intake at follow-up. Consequently this study emphasised RBC folate as a more objective biomarker, while recognising that folate reference intervals in children are often extrapolated from studies involving adults and that RBC folate is influenced by other factors, particularly smoking or exposure to passive smoke and age, as discussed further below.

Regarding the observed variation for RBC folate increases, it is difficult to know whether this might reflect the different assays, random variation, or residual confounding from unknown and unmeasured differences between participants at different locations. The study design, nature of analysis and different assays used place limitations on the conclusions that can be drawn. Our interpretation is that, overall, low RBC folate was not evident at baseline and, across communities, RBC folate tended to be greater at follow-up. That this increase was statistically significant for Clarence Valley, but not Coffs Harbour or Nambucca Valley, indicates a need for caution lest it be concluded that increased RBC folate was a function of the subsidy program.

In addition to the impact of folic acid fortification, serum and RBC folate have been reported to vary with age, with higher levels in 6-11 year olds compared to 12-19 year olds, in Canada²² and the US.^{21,32} This difference was evident both before and after mandatory folic acid fortification of enriched cereal grain products in 1998 in both countries. Thus, the impact of any decrease in RBC folate with age would have tended to bias towards the null any increase in RBC folate in this study. However, in both of these studies in the US and Canada, mandatory folic acid fortification had a more substantial impact on serum and RBC folate than any changes due to age.^{21,22,32} This emphasises the importance of identifying and monitoring the sources of folate in the diet, given the substantial impact of folic acid fortification on mean folate levels.³³

These data also highlight the difficulty of using RBC folate as a marker of F&V intake in populations exposed to folate fortification of the food supply. Folate may still have a role as a marker of F&V intake provided the intake

of folic acid from fortified sources does not change during a study. As noted above, this requires knowledge of the sources of folate in the diet. It also depends on no changes to the specific foods fortified with folate and constant levels of folic acid fortification during the study period, which was obviously not the situation during this study. Due to increasing impact of folic acid fortification, it appears that other biomarkers, particularly carotenoids and vitamin C, will be preferred to monitor changes in F&V intake.³⁴

In conclusion, this study has demonstrated increased RBC folate in Aboriginal children associated with the concurrent impact of an F&V subsidy program and mandatory folic acid fortification of bread-making flour. This finding, together with the high proportion of children in this study with RBC folate above the current reference range, reinforces the need to revise RBC folate reference ranges in children and for monitoring the long-term impact of folic acid fortification. It also highlighted the difficulty of using folate as a biomarker of F&V intake in populations exposed to folic acid fortification of their food supply.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Supplementary Figure 1: Scatterplot of change in fruit and vegetable intake by change in RBC folate z-score.

Supplementary Figure 2: Scatterplot of mean fruit and vegetable intake by change in RBC folate z-score.

Supplementary Figure 3: Scatterplot of mean bread intake versus change in RBC folate z-score.