

Serum and erythrocyte folate status of New Zealand women of childbearing age following a countrywide voluntary programme by the baking industry to fortify bread with folic acid

Abstract

Objective: To estimate the folate status of New Zealand women of childbearing age following the introduction, in 2010, of a new voluntary folic acid fortification of bread programme.

Design: The 2011 Folate and Women's Health Survey was a cross-sectional survey of women aged 18 to 44 years carried out in 2011. The survey used a stratified random sampling technique with the Electoral Roll as the sampling frame. Women were asked about consumption of folic acid fortified breads and breakfast cereals in a telephone interview. During a clinic visit, blood was collected for serum and erythrocyte folate measurement by microbiological assay.

Setting: A North Island (Wellington) and South Island (Dunedin) city centre in New Zealand.

Subjects: 288 women, of whom 278 completed a clinic visit.

Results: Geometric mean serum and erythrocyte folate concentrations were 30 nmol/L and 996 nmol/L, respectively. Folate status was 30-40% higher compared to women of childbearing age sampled as part of a national survey in 2008/09, prior to the introduction of the voluntary folic acid bread fortification programme. In the 2011 Folate and Women's Health Survey, reported consumption of fortified bread and fortified breakfast cereal in the past week was associated with 25% ($P = 0.01$) and 15% ($P = 0.04$) higher serum folate concentrations, respectively.

Conclusions: Serum and erythrocyte folate concentrations have increased in New Zealand women of childbearing age since the number of folic acid fortified breads was increased voluntarily in 2010. Consumption of fortified breads and breakfast cereals was associated with a higher folate status.

Introduction

To help reduce the rate of neural tube defects (NTD) in New Zealand, folic acid fortification of certain food categories, including bread and breakfast cereals, has been permitted but not mandated since 1996. Under these voluntary permissions, a number of breakfast cereals, but few breads, were fortified with folic acid¹. In 2007, the governments of New Zealand and Australia adopted joint legislation that required, at the end of a two year transition period, all bread to be fortified with folic acid^{2,3}. However, in August 2009, one month prior to the planned full implementation of the programme, the New Zealand Government deferred mandatory fortification until May 2012 and, instead, encouraged the bread baking industry to adopt more extensive voluntary fortification. The four major bread manufacturers in New Zealand, who together produce an estimated 90% of all bread in the country⁴, agreed to add folic acid – at a target of 200 µg folic acid per 100 g of bread – to approximately 30% of their range of breads by May 2010⁵.

Arguably the most important part of assessing the effectiveness of any folic acid fortification programme is to monitor its impact on the incidence rate of NTDs; however, this is problematic in New Zealand in the short-term, because the annual number of cases is small¹ and reliable documentation of a change in rates may require up to a decade. Furthermore, there is no mandatory requirement to record NTDs in terminated pregnancies. Thus total incidence rates are underestimated. A complementary approach is to monitor the blood folate status of women of childbearing age before and after implementation of a fortification programme. For this purpose, the New Zealand Ministry of Primary Industries commissioned a survey (the 2011 Folate and Women's Health Survey) to monitor the blood folate status of women of childbearing age after full implementation of the voluntary programme (May 2010). Change in folate status before and after voluntary fortification could be assessed by using as a baseline the results from the 2008/09 New Zealand (NZ) Adult Nutrition Survey which included information on the serum and erythrocyte folate concentrations of the New Zealand population⁶.

The objective of the 2011 Folate and Women's Health Survey was to estimate the folate status of New Zealand women of childbearing age following the introduction of the new (2010) voluntary folic acid fortification of bread programme. In addition, we examined the

influence of the consumption of folic acid fortified bread and other folic acid fortified foods on blood folate status.

Methods

Survey design

The 2011 Folate and Women's Health Survey was a cross-sectional survey of women of child bearing age (18 to 44 years) carried out from April to August 2011 in two city centres of New Zealand – a South Island centre, Dunedin, and a North Island centre, Wellington. We have previously shown that 12 months is sufficient time for serum and erythrocyte folate concentrations to reach a new steady state after a sustained increase in folic acid intake ⁷, thus we began recruitment for the 2011 Folate and Women's Health Survey in April 2011. The survey was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the [name of the ethics committee removed for blinding]. Written informed consent was obtained from all subjects. The survey was registered with the Australia New Zealand Clinical Trials Registry (ACTRN12611000463976). The 2008/09 NZ Adult Nutrition Survey was a nationwide multistage random survey of New Zealanders aged 15 years and older. A detailed description of the methods can be found elsewhere ⁸. For this paper, we include only results for women between the age of 18 and 44 years in order to match the ages of women who were recruited for the 2011 Folate and Women's Health Survey.

The 2011 Folate and Women's Health Survey used a stratified random sampling technique with the New Zealand Parliamentary Electoral Roll as the sampling frame. All New Zealand citizens and permanent residents 18 years or older are required by law to enrol to be registered on the Parliamentary Electoral Roll. Women listed on the electoral roll residing within the local authority boundary of Dunedin city (Dunedin centre), or Wellington city, Porirua city and Hutt city (Wellington centre) were stratified by age (18 to 19 years; 20 to 24 years; 25 to 29 years; 30 to 34 years; 35 to 39 years; and 40 to 44 years). The proportion of women randomly selected from each stratum was based on the proportion of New Zealand women in each of the age categories in the 2006 census ⁹. The aim was to recruit 300 women in total, 150 from each of the centres, assuming a 50% response rate.

Recruitment was carried out in the South Island centre – Dunedin – between April and June 2011 and in the North Island centre – Wellington – between June and August 2011.

Recruitment followed the four stage tailored method as recommended by Dillman *et al.*¹⁰, which involved an initial postal invitation to participate in the survey, a consent form, and a general questionnaire; 7-8 days later a reminder/thank-you postcard; 16 days after the initial mail out a second invitation pack; and 28 days after the initial mail out the telephone numbers of the non-respondents were obtained from the New Zealand white pages and they were phoned to invite them to participate one final time. If their number was not listed, or if they were unable to be reached, a final reminder postcard was sent. Due to a lower than anticipated response rate in the sample selected from the Wellington region, a second sample was selected from the Wellington region and contacted following the same procedures described above, with the aim to recruit 150 women in total from Wellington.

The self-administered general questionnaire was used to collect information on the following socio-demographic characteristics: birth date, ethnicity, education level, and household income level. The general questionnaire also collected information on factors that may affect folate status including health status, medical conditions, medication use, alcohol and coffee use, smoking status, and past pregnancies.

Participants also completed a telephone interview during which information about the specific brands of breakfast cereals, breads, and fortified spreads consumed over the past week was collected. Participants were at home when interviewed and were asked to read out the brand name of the packets of breakfast cereals and breads, if available, that they had consumed in the past week. The same was done with dietary supplements. The ingredients list on packets of breads, breakfast cereals, and supplements that were reported by the participants were checked at a supermarket in the local area of the participant for the presence of added folic acid. We classified bread as folic acid fortified if the ingredient list stated the bread contained folic acid or folate. Participant responses to the questions were recorded directly onto a standardised form. Participants who ate bread outside of their house, for instance at a café, were asked to name the café and describe the bread. Where possible, these eateries were contacted and questioned about the brand of breads they used, or the brand of ingredients if they made their own bread, to try to identify whether or not the bread consumed by the participant contained folic acid. If this was unable to be identified, the fortification status of the bread was specified as unknown. The phone interview was typically completed one week before the clinic visit. Information from the general questionnaire and the phone

interview was double-entered by KEB and CA; discrepancies were investigated and resolved by KEB.

Participants were asked to fast overnight before attending a morning clinic. During the clinic visit, height and weight were measured using standardised techniques. Participants were lightly clothed and were not wearing shoes. Blood was taken from an antecubital vein into a 6 mL tube containing EDTA, and a 6 mL tube that did not contain anticoagulant. The tube without anticoagulant was left for one hour at room temperature before it was centrifuged at 1650 g for 15 minutes at 4°C to isolate serum. After the blood draw, the EDTA tubes were placed immediately in a polystyrene container with an ice pack or in a refrigerator. Haematocrit was analysed from whole blood. The whole blood and serum samples were dispensed into aliquots and stored at -80°C within three hours of blood collection.

Laboratory methods

For both the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey, serum and whole blood folate was measured in the same laboratory by microbiological assay with the use of the test organism *Lactobacillus rhamnosus*, as described by O'Broin & Kelleher¹¹. For the 2011 Folate and Women's Health Survey folate analysis began and was completed in November 2011. Samples to be assayed were thawed just prior to use. Immediately after thawing, whole blood was first diluted one in ten in 1% ascorbic acid and incubated at 37°C for 30 minutes, then further diluted one in 40 in 0.5% sodium ascorbate. Serum was diluted one in 20 in 0.5% sodium ascorbate. Increasing amounts of folic acid (200 pg/ mL) were used for the standard curve; one standard curve was constructed per day. Plates were incubated for 42 hours at 37°C and then read on a microplate reader (Asys UVM 340, Biochrom, Cambridge, UK) with the wavelength set at 590 nm. Linear interpolation was used to generate the standard curve.

The accuracy of the microbiological assay was determined with the use of the NIST SRM (1955 Homocysteine and Folate in Frozen Human Serum) that was assayed in duplicate on one plate per day. The precision of the assay was monitored by analysing pooled plasma in duplicate on every plate. The analysed values ($n = 19$) of the three level NIST SRM (1955) from the 2008/09 NZ Adult Nutrition Survey folate analysis were: 6.2 nmol/L (uncertainty range: 4.9 to 6.3 nmol/L), 14.0 nmol/L (uncertainty range: 12 to 16 nmol/L), and 47.1 nmol/L

(uncertainty range: 37 to 51 nmol/L), with CVs of 9.8%, 12.5% and 8.9%, respectively. The mean (CV) for the pooled plasma ($n = 164$) was 19.2 nmol/L (16.5%). The analysed values ($n = 4$) of the three level NIST SRM (1955) from the 2011 Folate and Women's Health Survey folate analysis were: 7.5 nmol/L, 15.6 nmol/L, and 48.9 nmol/L, with CVs of 2.4%, 3.0% and 4.3%, respectively. The mean (CV) for the pooled plasma ($n = 29$) was 19.2 nmol/L (12.3%).

We used a cut-off of less than 6.8 nmol/L to indicate low serum folate concentrations and an erythrocyte folate concentration of less than 305 nmol/L to indicate deficiency¹²⁻¹⁴.

Statistical analysis

All statistical analyses were carried out using STATA statistical software, release 11 (StataCorp LP; College Station, USA). We used multiple linear regression to examine the relation between blood folate status and dietary variables in the 2011 Folate and Women's Health Survey. Because of their positive skew, serum and erythrocyte folate concentrations were log-transformed before statistical analysis and the differences between groups were presented as ratios. For the 2008/09 NZ Adult Nutrition Survey, survey commands were used to take into account the specific design of the survey. Survey weights based on the prioritized ethnicity of those who gave blood samples were used to set the survey design in STATA⁸. A two-sided Student's t-test for unpaired samples was used to test significant differences in blood folate status between females aged 18 to 44 years from the 2008/09 NZ Adult Nutrition Survey and the 2011 Folate and Women's Health Survey.

Results

The final weighted response rate of the 2008/09 NZ Adult Nutrition Survey for all participants (15 years and older) was 61%. The flow of participants through the 2011 Folate and Women's Health Survey is shown in **Figure 1**. Invitations to participate in the study were delivered to 724 women. In total, 296 women consented to participate; of these, eight completed neither a phone interview nor a clinic visit, leaving 288 included in the analysis. Of these 288 participants, 285 completed a telephone interview, and 278 completed a clinic visit. There were 97 participants who declined to take part, 57 non-deliveries of the information packs (i.e. returned to sender), and one woman who was ineligible because she

197 did not understand English. Two hundred and seventy three participants did not respond to
198 the invitation.

199
200 The overall response rate of the 2011 Folate and Women's Health Survey was 43%
201 [participants who completed a phone interview or clinic visit/(total selected – (non-deliveries
202 + ineligible)), 288/(724-58)]. The overall contact rate of the 2011 Folate and Women's
203 Health Survey was 59% (those contacted [included in analysis + total refused) as a
204 percentage of those eligible [included in analysis + total refused + non-contact]). Non-
205 contacts and refusals accounted for 72% and 28% of the overall non-response, respectively.

206
207 Characteristics of the female participants aged between 18 and 44 years from the 2008/09 NZ
208 Adult Nutrition Survey and the participants of the 2011 Folate and Women's Health Survey
209 are shown in **Table 1**. These groups were similar in BMI and age. However more participants
210 of the 2011 Folate and Women's Health Survey were of New Zealand European or other
211 ethnicity and a higher proportion were from less deprived areas.

212
213 One percent of participants in the 2011 Folate and Women's Health Survey had low serum
214 folate concentrations (less than 6.8 nmol/L); likewise, 1% had low erythrocyte folate
215 concentrations (less than 305 nmol/L). The geometric mean serum folate concentration was
216 31% (95% CI 19%, 45%) higher in women in the 2011 Folate and Women's Health Survey,
217 compared to women of the same age range in the 2008/09 NZ Adult Nutrition Survey.
218 Similarly, the geometric mean erythrocyte folate concentration was 38% (95% CI 29%, 48%)
219 higher in women in the 2011 Folate and Women's Health Survey, compared to women of the
220 same age range in the 2008/09 NZ Adult Nutrition Survey (**Table 2**).

221
222 Of the 285 participants in the 2011 Folate and Women's Health Survey who completed a
223 telephone interview, 93% (n=266), and 72% (n=205) reported consuming bread and breakfast
224 cereal in the past week, respectively (**Table 3**). Amongst the 285 participants, mean (SD)
225 consumption of bread and breakfast cereal was 12 slices (9 slices) and 3.4 bowls (2.8 bowls)
226 per week, respectively (data not shown). In the past week, only 18% (n=51) of participants
227 could be identified as having consumed at least one slice of bread that was folic acid fortified,
228 62% (n=177) could be identified as having consumed only non-fortified bread; the remaining
229 bread consumers, 13% (n=38), consumed at least one slice of bread of unknown fortificant
230 status (because they did not know the brand of bread eaten). In the past week, 41% (n=116)

of participants could be identified as having consumed at least one bowl of breakfast cereal that was confirmed as folic acid fortified, 26% (n=74) could be identified as having consumed only non-fortified breakfast cereal; the remaining consumers, 5% (n=15), consumed at least one bowl of breakfast cereal of unknown fortificant status (because they did not know the brand of cereal eaten). Fifty-three percent reported consuming a folic acid fortified yeast extract spread in the past week. Twenty-seven percent of participants reported the consumption of a folate-containing supplement.

The relation between folic acid fortified bread and breakfast cereal consumption and serum and erythrocyte folate concentrations in the 2011 Folate and Women's Health Survey is shown in **Table 4**. After adjustment for use of folate containing supplements, recruitment centre, and fortified breakfast cereal consumption, participants who we were able to confirm consumed folic acid fortified bread in the past week had significantly higher serum folate concentrations than participants who consumed either no bread, or only non-fortified or unidentified bread [adjusted ratio of the geometric means: 1.25 (95% CI 1.05, 1.48, $P = 0.012$)]. Erythrocyte folate concentrations were not significantly different between these two groups [adjusted ratio of the geometric means: 1.13 (95% CI 0.99, 1.28, $P = 0.079$)]. After adjustment for use of folate containing supplements, recruitment centre, and fortified bread consumption, participants who we were able to confirm consumed folic acid fortified breakfast cereal in the past week had significantly higher serum and erythrocyte folate concentrations than participants who consumed either no breakfast cereal, or only non-fortified or unidentified breakfast cereal (for serum folate, adjusted ratio of the geometric means: 1.15 [95% CI 1.01, 1.31, $P = 0.042$]; for erythrocyte folate, adjusted ratio of the geometric means: 1.13 [95% CI 1.02, 1.25, $P = 0.019$]).

Frequency of any breakfast cereal, but not any bread consumption, predicted serum and erythrocyte folate concentrations. For every additional bowl of breakfast cereal consumed per week, the adjusted ratio of the geometric mean serum folate concentrations was 1.036 (95% CI 1.012, 1.061, $P = 0.003$) and the adjusted ratio of the geometric mean erythrocyte folate concentrations was 1.031 (95% CI 1.013, 1.050, $P = 0.001$) (**Table 5**).

Discussion

Our results suggest that mean serum folate concentration among women of childbearing age in New Zealand increased by 8 nmol/L from before to after the introduction of a voluntary programme to fortify bread with folic acid. This is based on the differences between the present survey – the 2011 Folate and Women’s Health Survey – and the 2008/09 NZ Adult Nutrition Survey. Increased consumption of folic acid fortified bread is likely to have contributed to some of the difference because consumption of fortified bread was associated with 25% higher serum folate status. Increased consumption of folic acid fortified breakfast cereal probably also contributed to the difference in folate status between the two surveys. Breakfast cereal was an important predictor of serum folate status, and the number of folic acid fortified breakfast cereals in New Zealand increased from 49 in 2008 to 86 in 2011; in 2011 this represented 53% of the total breakfast cereals in New Zealand¹. Assuming total bread or breakfast cereal consumption did not change since 2008, the increase in folate status amongst women indicates a greater penetration of folic acid in the food supply. There is no evidence that the prevalence of folate supplement increased in women of childbearing age between 2008 and 2011. Twenty-six percent of female participants of childbearing age from the 2008/09 NZ Adult Nutrition Survey and 27% of participants of the 2011 Folate and Women’s Health Survey reported the consumption of a folate-containing supplement.

The dietary patterns of women in the 2011 Folate and Women’s Health Survey appear representative of New Zealand women. A New Zealand-wide market survey estimated that 81%, 69%, and 52% of New Zealand women aged 14 years or older had, in the past week, consumed bread, breakfast cereals, and yeast extract spreads respectively²³. Although the 2011 Folate and Women’s Health Survey encompassed a narrower age range, the results are comparable; we reported 93%, 72% and 53% of our participants had, in the past week, consumed bread, breakfast cereals, and yeast extract spreads, respectively.

Based on the work of Daly *et al.*¹⁵ and Wald *et al.*¹⁶ the change in mean serum folate concentration from the 2008/09 NZ Adult Nutrition Survey to the 2011 Folate and Women’s Health Survey can be used to predict the decline in NTD rate. An 18% reduction in NTD rates would be predicted from an increase in serum folate concentration from 28 nmol/L (2008/09 NZ Adult Nutrition Survey) to 36 nmol/L (2011 Folate and Women’s Health Survey).

Other countries have monitored blood folate status of the population before and after the introduction of a mandatory folic acid fortification programme. However, differences in the food fortification vehicle, target fortificant level, and different food consumption patterns in different countries, means that the effect of mandatory fortification programmes on the folate status of a population will vary from country to country. Blood folate was measured in the Australian Health Survey 2011-2013¹⁷, but it was not measured in national surveys that predate folic acid fortification of bread in 2009. Population-based surveys carried out in Iran¹⁸, Canada¹⁹, Costa Rica²⁰, Chile^{21,22}, and the United States²³ have documented increases in folate status of women of childbearing age following the introduction of a mandatory folic acid fortification programme, but comparing the relative impact of the fortification programmes across the countries is further complicated by the lack of international standardisation and calibration of folate measurement.

The serum and whole blood samples from the 2008/09 NZ Adult Nutrition Survey and the 2011 Folate and Women's Health Survey were analysed using the same microbiological assay in the same laboratory approximately two years apart. The mean value for the pooled sample, that was analysed 164 times in the 2008/09 NZ Adult Nutrition Survey and 29 times in the 2011 Folate and Women's Health Survey, was the same (19.2 nmol/L) in both surveys, suggesting excellent agreement of the laboratory analysis between the surveys. The NIST samples were only analysed four times in the 2011 Folate and Women's Health Survey, nevertheless the values were consistently higher than those obtained in the 2008/09 NZ Adult Nutrition Survey. However, the difference was less than 2 nmol/L for all three levels of the NIST SRM. Thus, differences across time in laboratory measurements may explain only a small part of the difference in folate status between the 2008/09 NZ Adult Nutrition Survey and the 2011 Folate and Women's Health Survey.

There were some differences in the way blood samples were collected in the two surveys, which may affect the comparison of blood folate status. Fasting blood samples were collected in the 2011 Folate and Women's Health Survey whereas non-fasting samples were collected in the 2008/09 NZ Adult Nutrition Survey. Folic acid consumed before blood sampling can increase slightly serum folate concentrations. Öhrvik *et al.*²⁴ has shown an increase in serum folate of approximately 3 nmol/L, in participants ($n = 8$) fed 200 µg folic acid in fortified bread (a dose similar to what one might receive through consumption of a folic acid fortified food product), with the maximum change occurring 150 minutes post-ingestion. In the

2008/09 NZ Adult Nutrition Survey some participants probably consumed a fortified food product before the blood draw, although blood collection of these participants was likely to have occurred throughout the range of the post-prandial time period. Thus, the effect on mean serum folate concentrations is likely to be quite small, probably around 1 to 2 nmol/L. Any temporary post-prandial increase in serum folate concentrations in the participants of the 2008/09 NZ Adult Nutrition Survey would tend to attenuate the magnitude of the true difference in serum folate status between the two surveys.

Both the 2008/09 NZ Adult Nutrition Survey and the 2011 Folate and Women's Health Survey used a random sampling technique. However, the response rate to the 2008/09 NZ Adult Nutrition Survey was slightly higher, and there were slight differences in some participant characteristics between the surveys. More of the 2011 Folate and Women's Health Survey participants were of New Zealand European and other ethnicity, and resided in areas of less deprivation. These factors were associated with higher serum and erythrocyte folate status in univariate analyses of the entire 2008/09 NZ Adult Nutrition Survey dataset (all $P < 0.05$, data not shown) and therefore this may also explain a small part of the difference in folate status between the two surveys.

Although the four major bread manufacturers agreed to add folic acid to a third of their range, the New Zealand Association of Bakers estimated in 2011 that only 12.5% of loaves produced annually were fortified with folic acid¹. The voluntary bread fortification strategy requested by the New Zealand government was partially successful in increasing the number of folic acid fortified breads on the market; however, in the absence of mandatory regulation, the decisions on which products to fortify and by how much are ultimately made by the manufacturers. The latest report from New Zealand Baking Industry stated that in 2014, 19% of New Zealand bread, by production volume, was fortified with folic acid²⁵.

In conclusion, although the introduction of the voluntary bread fortification programme in New Zealand did not achieve broad coverage of women of childbearing age – 18% of the participants reported consuming folic acid fortified bread in the past week – our results showed that women who consume folic acid fortified bread had higher folate status than those who do not. The voluntary programme to fortify bread with folic acid undoubtedly contributed towards the increase in folate status of women of child bearing age in New Zealand but the coincidental increase in the number of folic acid fortified breakfast cereal in

the food supply makes it difficult to estimate how much of the increase in blood folate status of childbearing women can be attributed to fortification of bread. In 2012, the New Zealand government announced that folic acid fortification of bread would remain voluntary in New Zealand, and together with the New Zealand Association of Bakers, developed a code of practice with a goal to fortify between 25% and 50%, by production volume, of bread with folic acid. Our results show that a voluntary fortification can improve folate status, but they also are a strong reminder to public health authorities – of the obvious – that the extent of change is more difficult to predict and control than with a mandatory programme. Given these results we believe that priority should be given to monitoring periodically the folate status of New Zealand women of childbearing age.

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Conflicts of interest

The authors have no conflicts of interest to declare.

Author contributions

KEB, CMS, SMW, JIM, IO, WP, LF, RCB designed research. KEB, CA conducted research; SMW, KEB, CMS performed statistical analysis; KEB, CMS wrote the paper, CMS had primary responsibility for final content, WP was the Nutrition Director of the New Zealand 2008/09 Adult Nutrition Survey; all authors read and approved the final manuscript.

Ethics committee

The University of Otago's human ethics committee approved the 2011 Folate and Women's Health Survey.

References

1. Ministry for Primary Industries (2012). *Voluntary folic acid fortification: Monitoring and evaluation report*. Wellington: Ministry for Primary Industries.
2. Food Standards Australia New Zealand (2007). *Australia New Zealand Food Standards Code - Amendment No. 93*. Canberra: Food Standards Australia New Zealand.
3. Food Standards Australia New Zealand (2007). *First review report - Proposal P295: Consideration of mandatory fortification with folic acid*. Canberra: Food Standards Australia New Zealand.
4. Ministry for Primary Industries (2012). *The future of folic acid fortification of bread in New Zealand*. Wellington: Ministry for Primary Industries.
5. Wilkinson K. Government defers folic acid fortification.
<http://www.beehive.govt.nz/release/government-defers-folic-acid-fortification>.
(accessed December 2012).
6. Bradbury KE, Williams SM, Mann JI, Brown RC, Parnell W, Skeaff CM. (2012) Estimation of Serum and Erythrocyte Folate Concentrations in the New Zealand Adult Population within a Background of Voluntary Folic Acid Fortification. *J Nutr* **144**, 68-74.
7. Bradbury KE, Williams SM, Green TJ, McMahon JA, Mann JI, Knight RG, et al. (2012) Differences in erythrocyte folate concentrations in older adults reached steady-state within one year in a two-year, controlled, 1 mg/d folate supplementation trial. *J Nutr* **142**, 1633-1637.
8. University of Otago, Ministry of Health (2011) *Methodology report for the 2008/09 New Zealand Adult Nutrition Survey*. Wellington: Ministry of Health.
9. Statistics New Zealand. 2006 Census data: Tables about New Zealand.
<http://www.stats.govt.nz/Census/2006CensusHomePage/Tables/AboutAPlace/SnapShot.aspx?id=9999999&type=region>. (accessed February 2012).
10. Dillman D, Smyth J, Christian L (2009). *Internet, mail, and mixed-mode surveys: The tailored design method*. 3rd ed. Hoboken: John Wiley & Sons, Inc.
11. O'Broin S, Kelleher B (1992) Microbiological assay on microtitre plates of folate in serum and red cells. *J Clin Pathol* **45**, 344-347.
12. Herbert V (1967) Biochemical and hematologic lesions in folic acid deficiency. *Am J Clin Nutr* **20**, 562-572.
13. IOM (Institute of Medicine). Dietary reference intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, D.C: 2000.
14. Gibson R (2005) Assessment of folate and vitamin B12 status. In *Principles of Nutritional Assessment*, 2nd ed., pp. 595-640. Oxford: Oxford University Press.
15. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM (1995) Folate levels and neural tube defects. Implications for prevention. *JAMA* **274**, 1698-1702.
16. Wald N, Law M, Morris J, Wald D (2001). Quantifying the effect of folic acid. *Lancet* **358**, 2069-2073.
17. Australian Bureau of Statistics (2014). Australian Health Survey: Biomedical Results for Nutrients, 2011-12. Canberra.
<http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4364.0.55.0062011-12?OpenDocument> (accessed August 2015)

18. Abdollahi Z, Elmadfa I, Djazayeri A, Golalipour MJ, Sadighi J, Salehi F, et al. (2011) Efficacy of flour fortification with folic acid in women of childbearing age in Iran. *Ann Nutr Metab* **58**,188-196.
19. Liu S, West R, Randell E, Longerich L, O'Connor K S, Scott H, et al. (2004) A comprehensive evaluation of food fortification with folic acid for the primary prevention of neural tube defects. *BMC pregnancy and childbirth* **4**, 20.
20. Chen LT, Rivera MA (2004) The Costa Rican experience: reduction of neural tube defects following food fortification programs. *Nutr Rev* **62**, S40-43.
21. Hertrampf E, Cortes F, Erickson JD, Cayazzo M, Freire W, Bailey LB, et al. (2003) Consumption of folic acid-fortified bread improves folate status in women of reproductive age in Chile. *J Nutr* **133**,3166-3169.
22. Hertrampf E, Cortes F (2004). Folic acid fortification of wheat flour: Chile. *Nutr Rev* **62**,S44-48
23. Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, et al. (2012) Estimation of trends in serum and RBC folate in the U.S. population from pre- to postfortification using assay-adjusted data from the NHANES 1988-2010. *J Nutr* **142**, 886-893.
24. Öhrvik V, Öhrvik H, Tallkvist J, Witthöft C (2010) Foliates in bread: retention during bread-making and in vitro bioaccessibility. *Eur J Nutr* **49**, 1365-1372.
25. Baking Industry Research Trust. Voluntary fortification of bread with folic acid. Annual Report 2014.
<http://www.bakeinfo.co.nz/files/file/620/Voluntary%20fortification%20of%20bread%20with%20folic%20acid%20Annual%20Report%202014%20%20May%202015%20Final.pdf> (accessed August 2015)

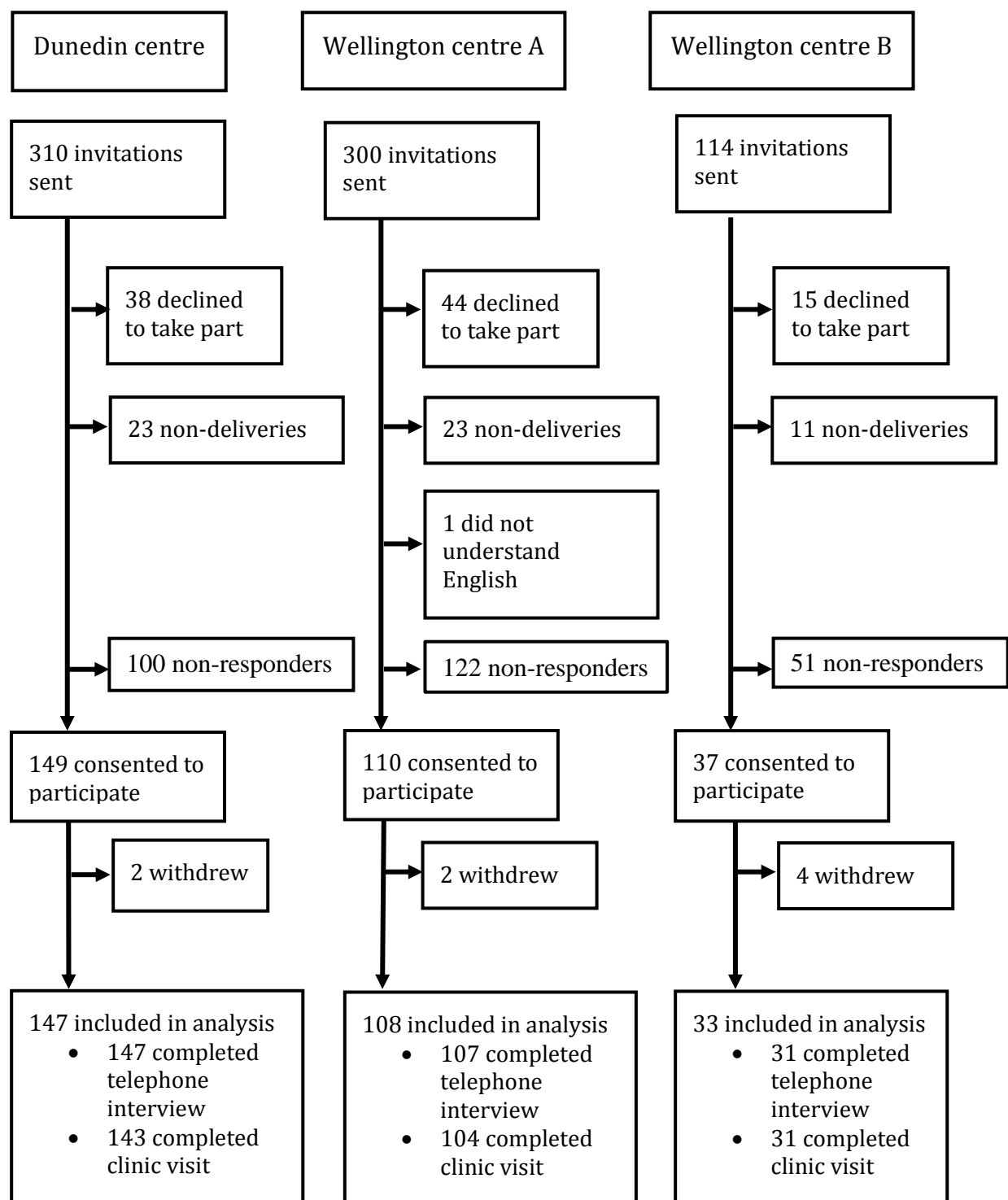


Table 1 A comparison of the characteristics of participants in the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey

	2008/09 New Zealand Adult Nutrition Survey	2011 Folate and Women's Health Survey
	<i>n</i> = 663	<i>n</i> = 288
BMI (kg/m ²)*	27 (9)	27 (6)
Age (years)*	32 (11)	33 (8)
Age category		
18 to 19 years	7%	7%
20 to 24 years	16%	14%
25 to 29 years	17%	11%
30 to 34 years	19%	19%
35 to 39 years	19%	23%
40 to 44 years	22%	26%
Ethnicity		
NZEO	78%	87%
Maori	16%	9%
Pacific	6%	4%
NZDep2006 score†		
1	5%	19%
2	8%	9%
3	8%	14%
4	12%	11%
5	9%	9%
6	15%	12%
7	11%	7%
8	12%	6%
9	9%	8%
10	12%	7%

NZEO, New Zealand European and others; NZDep2006, 2006 New Zealand Index of Deprivation.

Values are percent.

*Values are mean (SD).

†An NZDep2006 score of one represents a geographic area with the least deprivation.

Table 2 A comparison of the folate status of the 2008/09 New Zealand Adult Nutrition Survey participants and the 2011 Folate and Women's Health Survey participants

	2008/09 New Zealand Adult Nutrition Survey	2011 Folate and Women's Health Survey	Mean difference (95% CI)	Ratio* (95% CI)	<i>p</i> value
Serum folate (nmol/L)					
Mean (SEM)	28 (1)	36 (1)	8 (4 to 12)		< 0.001
Geometric mean (95% CI)	23 (21 to 24)	30 (28 to 32)		1.31 (1.19 to 1.45)	< 0.001
Erythrocyte folate (nmol/L)					
Mean (SEM)	794 (21)	1096 (30)	302 (229 to 374)		< 0.001
Geometric mean (95% CI)	720 (686 to 755)	996 (945 to 1049)		1.38 (1.29 to 1.48)	< 0.001
SEM, Standard error of the mean					

*Ratio of the geometric means of the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey.

Table 3 Consumption of folic acid fortified or non-fortified bread and breakfast cereal, folic acid fortified yeast spread, or folate supplements amongst participants in the 2011 Folate and Women's Health Survey

	Consumers	Any fortified	All unfortified	Unknown or combination of unfortified and unknown
Bread*	266 (93%)†	51 (18%)‡	177 (62%)	38 (13%)
Breakfast cereal*	205 (72%)†	116 (41%)‡	74 (26%)	15 (5%)
Fortified yeast spread*	152 (53%)	-	-	-
Folate-containing supplement¶	77 (27%)	-	-	-

Values are number (percentage) and are based on 285 women who completed the dietary questionnaire

*Consumption in the past week

†For bread, consumed at least one slice of any bread in past week; for breakfast cereal, consumed at least one bowl of any breakfast cereal in past week

‡For bread, consumed at least one slice of folic acid fortified bread in past week; for breakfast cereal, consumed at least one bowl of folic acid fortified breakfast cereal in past week

¶Regular consumption

Table 4 Serum and red blood cell folate concentration (nmol/L) according to consumption of folic acid fortified bread or breakfast cereal

Measurement	Bread				Breakfast cereal			
	Fortified*	Non-fortified, unidentified, or no bread*	Adjusted ratio (95% CI)†	P value	Fortified*	Non-fortified, unidentified, or no bread*	Adjusted ratio (95% CI)‡	P value
Serum folate	37 (32, 44)	28 (26, 31)	1.25 (1.05, 1.48)	0.012	33 (29, 36)	28 (25, 31)	1.15 (1.01, 1.31)	0.042
Erythrocyte folate	1124 (990, 1276)	969 (916, 1026)	1.13 (0.99, 1.28)	0.079	1079 (991, 1176)	942 (883, 1006)	1.13 (1.02, 1.25)	0.019

*Values are geometric mean (95% CI)

†Ratio of the geometric means of blood folate concentrations of participants who consumed at least one slice of folic acid fortified bread relative to participants who consumed only non-fortified or unidentified bread or no bread adjusted for use of folic acid containing supplements (Y/N), city of residence (Wellington or Dunedin), and fortified breakfast cereal consumption (Y/N).

‡Ratio of the geometric means of blood folate concentrations of participants who reported consuming at least one bowl of folic acid fortified cereal relative to participants who consumed only non-fortified or unidentified breakfast cereal or no breakfast cereal adjusted for use of folic acid containing supplements (Y/N), city of residence (Wellington or Dunedin), and fortified bread consumption (Y/N).

Table 5 The relation between frequency of consumption of bread or breakfast cereal and serum or red blood cell folate

Measurement	Adjusted ratio per increment of bread (slice per week)*	<i>P</i> value	Adjusted ratio per increment of breakfast cereal (bowl per week)†	<i>P</i> value
Serum folate	1.005 (0.998, 1.013)	0.18	1.036 (1.012, 1.061)	0.003
Erythrocyte folate	1.005 (0.999, 1.012)	0.08	1.031 (1.013, 1.050)	0.001

*Values are adjusted ratio (95% CI) per incremental difference in frequency of bread consumption; adjusted for use of folic acid containing supplements (Y/N), city of residence (Wellington or Dunedin), and bowls of breakfast cereal consumption.

†Values are adjusted ratio (95% CI) per incremental difference in frequency of breakfast cereal consumption; adjusted for use of folic acid containing supplements (Y/N), city of residence (Wellington or Dunedin), and slices of bread consumption. For example, 1.036 can be interpreted as a 3.6% higher geometric mean serum folate concentration per incremental (one more bowl per week) difference in breakfast cereal consumption.