Monitoring voluntary fortification of bread with folic acid

MAF Technical Paper No: 2011/103

Prepared for the Ministry of Agriculture and Forestry
by K. E.Bradbury, C. M. Skeaff, I. Oey, S. Williams, J. Mann.
Department of Human Nutrition, Department of Food Science, Department of Preventive and Social Medicine

ISBN 978-0-478-38766-7(online)
ISSN 2230-2794 (online)

December 2011

Ministry of Agriculture and Forestry
Te Manatū Ahuwhenua, Ngāherehere

BIOSECURITY NEW ZEALAND

NEW ZEALAND FOOD SAFETY AUTHORITY

Disclaimer

While every effort has been made to ensure the information in this publication is accurate, the Ministry of Agriculture and Forestry does not accept any responsibility or liability for error or fact omission, interpretation or opinion which may be present, nor for the consequences of any decisions based on this information.

Any view or opinions expressed do not necessarily represent the official view of the Ministry of Agriculture and Forestry.

Requests for further copies should be directed to:

Publication Adviser
MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
Facsimile: 04-894 0300

This publication is also available on the MAF website at
http://www.maf.govt.nz/news-resources/publications

Monitoring voluntary fortification of bread with folic acid

Folic acid is the synthetic form of the B vitamin folate and may be added to manufactured foods and drinks, or taken as a vitamin supplement.

Women who don't get enough folic acid before and during pregnancy have a higher risk of their baby developing abnormalities known as neural tube defects (NTDs). The most common NTDs are spina bifida and anencephaly. There is considerable scientific evidence showing that increased dietary folic acid intakes can reduce the risk of NTDs.

To support women in achieving a higher dietary folic acid intake, the New Zealand Government issued a New Zealand Food Standard in 2007 requiring the fortification of almost all types of bread at a level of 80-180 micrograms of folic acid per 100 grams of bread. Bread was chosen because surveys had suggested that it was widely consumed by women of childbearing age. Combined with existing health promotion and education strategies including the promotion of folic acid supplements, the aim is to improve the blood folate levels of women of childbearing age.

The New Zealand Food Standard's implementation was subsequently deferred until May 2012 with the focus being on bread companies taking up modified voluntary folic acid fortification permissions in a wider range of bread products. Bread companies agreed to add folic acid at a level of 200 micrograms of folic acid per 100 grams of bread to approximately one third of all breads by April/May 2010.

The Ministry of Agriculture and Forestry (MAF) is responsible for monitoring these actions and assessing whether or not the desired folic acid content in breads was being achieved and its usefulness at improving the blood folate status of women of childbearing age.

An examination of the blood folate status of women and the folic acid content of folic acid fortified breads was carried out by the University of Otago for MAF between October 2010 and November 2011.

The survey reports on the blood folate status of women of childbearing age living in Dunedin and Wellington (n = 288) between April 2011 and November 2011 following the expanded uptake of voluntary folic acid fortification permissions by the major bread companies. It compares results from the 2008/09 New Zealand Adult Nutrition Survey Report and results from the 1999 Dunedin Australia New Zealand Food Authority Survey with this survey to determine if there has been a change over time in blood folate status of women of childbearing age. It should be noted that the survey was not designed to measure any change in the rate of NTD-affected pregnancies.

It also reports on the folic acid content of the top ranked folic acid fortified breads for sale in the North (n = 7) and South Islands (n = 10) between 27 October 2010 and 8 February 2011.

Fifty-nine percent of women returned a red blood cell folate measurement of 906 nanomoles per litre or higher, a level associated with a very low risk of NTD. This was up from 26% of women as reported in the 2008/09 New Zealand Adult Nutrition Survey.

There was a non-significant trend for average red blood cell folate levels to be higher in those women who consumed folic acid fortified breads.

These improvements in red blood cell folate status cannot be statistically attributed to the wider availability of folic acid fortified breads. However, after accounting for other factors, it does appear to have made a contribution to the increased folate status of women of childbearing age.

The survey found variable levels of folic acid in the breads tested, within and across companies. Median folic acid content of the 17 folic acid fortified breads tested was 144 micrograms per 100 grams bread. Five breads, including three from the North Island, were found to contain less than 50 micrograms folic acid per 100 grams bread, twelve contained between 105 and 452 micrograms folic acid per 100 grams bread.

MAF acknowledges that different bread manufacturing processes are used by bread companies, which could contribute to the variation in folic acid levels. It is working closely with the bread companies and their industry organisation, the Baking Industry Research Trust (BIRT) to learn more about bread manufacturing processes, current monitoring systems and test methods to explain the variability found in the folic acid fortified breads.

The findings from this report will be consolidated with other monitoring information and used by MAF to inform a review of its current risk management activities for the fortification of bread with folic acid.

Updated 20 December 2011

Monitoring voluntary fortification of bread with folic acid

K. E. Bradbury[1], C. M. Skeaff[1], I. Oey[2], S. Williams[3], J. Mann[1].

[1]Department of Human Nutrition, [2]Department of Food Science, [3]Department of Preventive and Social Medicine

December 2011

A report prepared for the Ministry of Agriculture and Forestry

Project Number: 1185

Summary

Consumption of folic acid before conception and through the early stages of pregnancy reduces the risk of having a foetal neural tube defect. The baking industry, in response to a request from the New Zealand government, agreed to the voluntary addition of folic acid (200 micrograms folic acid per 100 grams of bread) to approximately one third of all breads. The programme has been in place since April/May 2010.

The purpose of the research project was to analyse the folic acid content of the top ten ranked folic acid fortified breads, by sales volume, in both the North Island and the South Island. Another purpose of the research was to assess, in a representative sample of 300 women, aged 18 to 44 years in Wellington and Dunedin, the consumption of folic acid fortified bread, as well as women's serum and red blood cell folate status.

Seventeen folic acid fortified breads, seven from the North Island and ten from the South Island, were collected for analysis of folic acid content. Five breads, three from the North Island and two from the South Island, were found to contain less than 50 micrograms folic acid per 100 grams bread, twelve contained between 105 and 452 micrograms folic acid per 100 grams bread. Median (interquartile range) folic acid content of the 17 folic acid fortified breads we tested was 144 (41, 189) micrograms per 100 grams bread.

Geometric mean serum folate concentration amongst women in the survey was 30 nanomoles per litre (95 percent confidence interval: 28, 32 nanomoles per litre); geometric mean red blood cell concentration was 996 nanomoles per litre (95 percent confidence interval: 945, 1049 nanomoles per litre). Median serum and red blood cell folate concentrations were 29 nanomoles per litre (interquartile range: 20, 47 nanomoles per litre) and 989 nanomoles per litre (interquartile range: 744, 1316 nanomoles per litre). Fifty-nine percent of women had a red blood cell folate concentration 906 nanomoles per litre or higher, a concentration associated with a very low risk of neural tube defect. Mean serum and red blood cell folate concentrations were not significantly higher in Dunedin compared with Wellington women, although there was a trend for higher folate status in Dunedin. Mean serum and red blood cell folate concentrations in participants of the present survey, conducted after the

voluntary folic acid fortification of bread programme, were higher than in women 18-44 years from the 2008/09 New Zealand Adult Nutrition Survey and from the 1999 Dunedin ANZFA Survey.

The voluntary addition by the bread industry of folic acid to a range of breads has coincided with increased serum and red blood cell folate status of women.

Contents

List of Tables and Figures

Introduction

The results of randomised controlled trials conducted in the mid-1980s showed that periconceptional supplementation with folic acid reduced the risk of neural tube defect (NTD)-affected pregnancies [1,2]. Furthermore, results from observational studies have shown that women who have red blood cell folate concentrations above 905 nanomoles per litre (nmol/L) or plasma folate above 15.8 nmol/L have a risk of NTD similar to women taking a daily 400 micrograms (μg) folic acid supplement [3]. The majority of women do not take a daily 400 μg folic acid supplement before conception and through the early stages of pregnancy [4,5]. For this reason, in 2006, the New Zealand and Australian governments regulated mandatory fortification of bread with folic acid – to take effect in September 2009 [6]. The food standard would require bread manufacturers to produce bread containing 80 to 180 ug of folic acid per 100 grams (g) of bread. Predictions based on modelling bread consumption – as reported in the 1997 New Zealand National Nutrition Survey – indicated that mandatory fortification of bread would deliver, on average, 140 μg of folic acid per day to women of childbearing age in New Zealand [7].

In August 2009, the New Zealand government announced that mandatory fortification would be deferred until May 2012. In the interim, the major bread producers in New Zealand were requested by the government to adopt greater voluntary fortification, agreeing to add around 200 μg of folic acid per 100 g of bread to approximately one third of their range of breads by April/May 2010 (Personal communication, Jenny Reid, January 2010).

To inform the New Zealand government's decision in 2012 about mandatory fortification the New Zealand Ministry of Agriculture and Forestry (MAF) commissioned research that would document the effectiveness of the bread manufacturers' voluntary folic acid fortification programme. Effectiveness would best be measured by a decline in the rate of NTD-affected pregnancies, but the reliability of any estimate before 2012 would be poor given the small population size of New Zealand. The next best measure of effectiveness would be to monitor the change in blood folate status of women of childbearing age before and after voluntary fortification. The Ministry of Agriculture

and Forestry also wanted the folic acid content of fortified bread to be measured as part of monitoring the voluntary fortification programme.

Results from the 2008/09 New Zealand Adult Nutrition Survey would be the primary and best source of information about the blood folate status of women of childbearing age before voluntary fortification of bread. A population-based survey conducted in 1999 in Dunedin [8] would also provide relevant information about blood folate status of women of childbearing age pre-voluntary fortification. The major purpose of this report is to gather information on blood folate status after voluntary fortification by conducting a small population-based survey of women.

There is good evidence in New Zealand that carefully conducted small surveys employing representative population sampling techniques can provide estimates of nutritional status similar to those of much larger national surveys. For example, the iodine status of 300 school-children in Dunedin and Wellington [9] was virtually the same as that of 1796 New Zealand school-age children who participated in the nationwide 2002 Children's Nutrition Survey [10].

In this report, we present the results of work commissioned by MAF. There are two major sections of original results: first, the folic acid content of a range of folic acid fortified breads purchased in Wellington and Dunedin is reported; and secondly, the blood folate status and consumption of folic acid fortified bread amongst women of childbearing age in Wellington and Dunedin is reported.

Contract service requirements:

The studies and analysis which this report includes are as outlined in the Service Requirements of the contract:

1. Sample and analytically determine the folic acid concentration in a range of fortified breads available in the New Zealand market;

2. Carry out a shelf-life study to determine folic acid stability in fortified breads;

3. Determine the blood folate status of women of childbearing age following the commencement of fortification and compare results from the 2008/09 New Zealand Adult Nutrition Survey Report and 1999 Dunedin Australia New

Zealand Food Standards Authority (ANZFA) survey with this 2011 Folate and Women's Health survey to determine if there has been a change over time in blood folate status of women of childbearing age;

4. Determine the frequency of consumption of folic acid fortified foods in women of childbearing age and determine, within the 2011 Folate and Women's Health Survey, the relation between bread consumption – total or fortified – and blood folate status;

5. Undertake comparative analysis of results with data from previous work on the blood folate status of women of childbearing age conducted prior to fortification.

Methods

Analysis of folic acid content in bread

Procedure for selecting and collecting bread samples in Wellington and Dunedin

The selection of folic acid fortified brands of bread for inclusion in the survey was based on a top-ten ranking of folic acid fortified breads by sales volume. The Baking Industry Research Trust gathered confidential information from the four main bread companies in New Zealand to generate two lists of the top-ten folic acid fortified breads, one for North Island and one for South Island breads, ranked by sales volume in each Island. All breads on the two lists were analysed, with one exception; two of the breads in the top-ten list for the North Island from the same brand differed only in the usage, one for "toast" and the other for "sandwich". Folic acid was only analysed in the "toast" version. Therefore, between 27/10/2010 and 8/02/2011, nine brands of bread were selected for analysis in the North Island and ten in the South Island.

Breads were purchased on the morning of folic acid extraction. In the South Island, three loaves of each brand were purchased from retail outlets in Dunedin city centre. In the North Island, breads were purchased from retail outlets in Wellington city centre. Where possible, each of the three loaves was purchased from a different outlet. Within a brand, loaves with the same "best before date" were purchased if feasible. In instances where there were a variety of batches available for purchase, the loaves with the longest time between date of purchase and the expiry date (the freshest loaves) were selected. The packet of each new bread we tested was checked to confirm that folic acid or folate was listed as an ingredient. The Baking Industry Association of New Zealand (BIANZ) website (http://www.bianz.co.nz/industry-news/folic-acid-fortified-breads-list.html) provides a list of folic acid fortified breads, and was accessed for additional confirmation that the breads were fortified with folic acid. All breads that were collected were listed as fortified on the BIANZ website. Subsequent to folic acid analysis, if was confirmed that two of the North Island breads, W 8 and W 9, did not list folic acid in the ingredient list. The results for breads W 8 and W 9 are excluded from this report; therefore, this report includes results for seven North Island and ten South Island breads.

For each brand, a combined loaf was created from the three loaves by choosing every alternate slice from a different loaf, i.e. crust from loaf 1, 2nd slice from loaf 2, 3rd slice from loaf 3, 4th slice from loaf 1 and so on. Each combined loaf was homogenised and extracted.

Procedure for collecting bread used to study the stability of folic acid fortified in bread during shelf-life

The top ranked folic acid fortified bread in the South Island – a white bread – and the top ranked folic acid fortified bread in the North Island – a whole wheat bread – were used for the shelf-life study. Four loaves of the South Island bread were collected from a Dunedin outlet on the morning of extraction. Four loaves of the North Island bread were couriered to Dunedin immediately after they came off the packaging line in the afternoon and received the following morning (3/02/11) in Dunedin.

Two combined loaves were made from the four South Island loaves; the same was done with the four North Island loaves. The first combined loaf was made by selecting the crust from loaf 1, 2nd slice from loaf 2, 3rd slice from loaf 3, 4th slice from loaf 4, 5th slice from loaf 1, and so forth. Once this first combined loaf was created, the second combined loaf was made in the same fashion, starting with the crust from loaf 2.

The first combined loaf from each Island was homogenised and extracted on the first day it would have appeared on retail shelves (Time 0), whereas the second combined loaf was stored according to the manufacturer's instructions in the original bread bag and homogenised and extracted on the morning of the "best-before" date (Time end).

Sample preparation and extraction of bread samples

Folic acid extraction and isolation was conducted based on the method of Konings [11] modified for folic acid fortified bread. Overviews of the extraction and purification processes are presented in **Figure 1** and **Figure 2**, respectively.

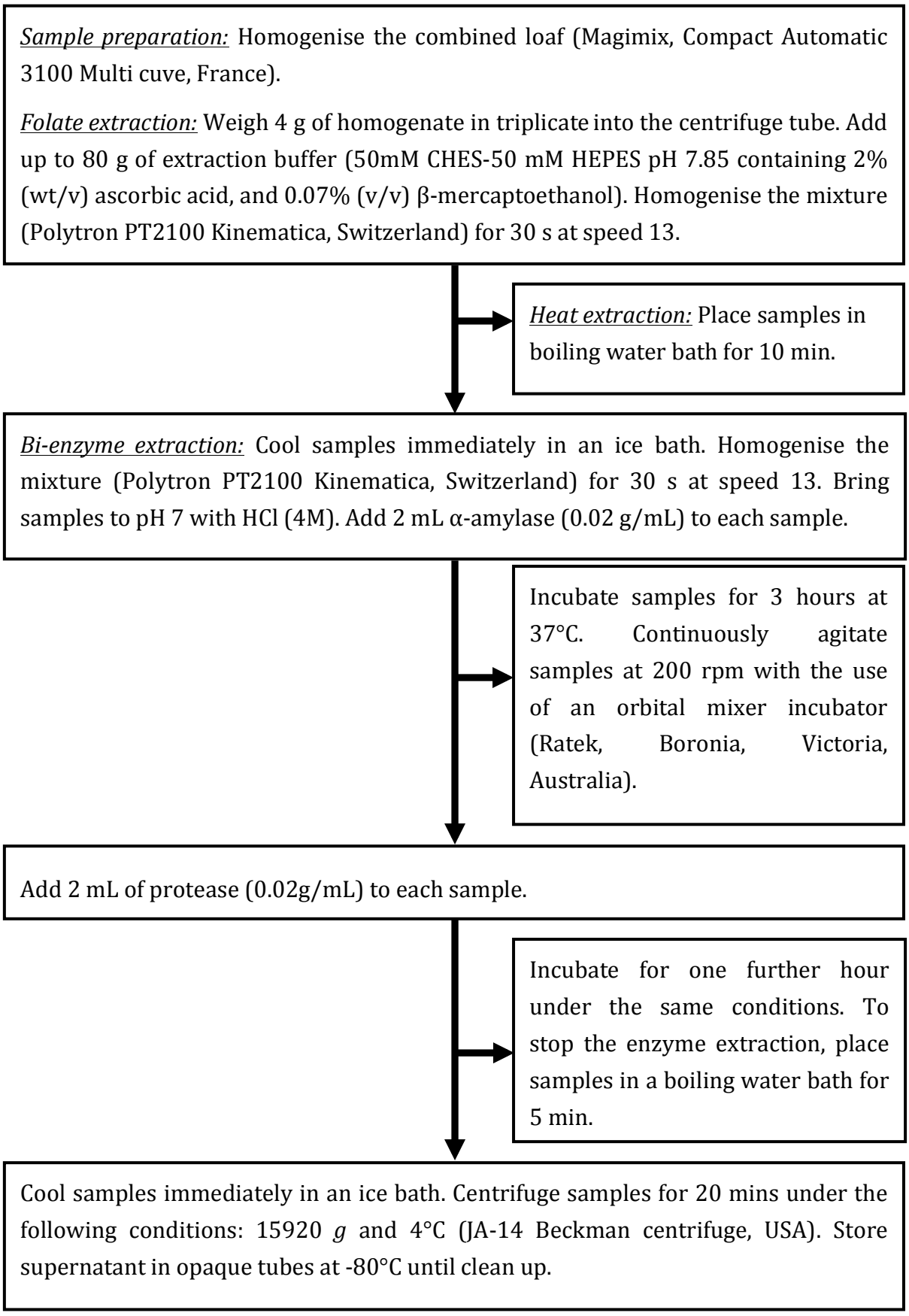


Figure 1: Overview of folic acid extraction process

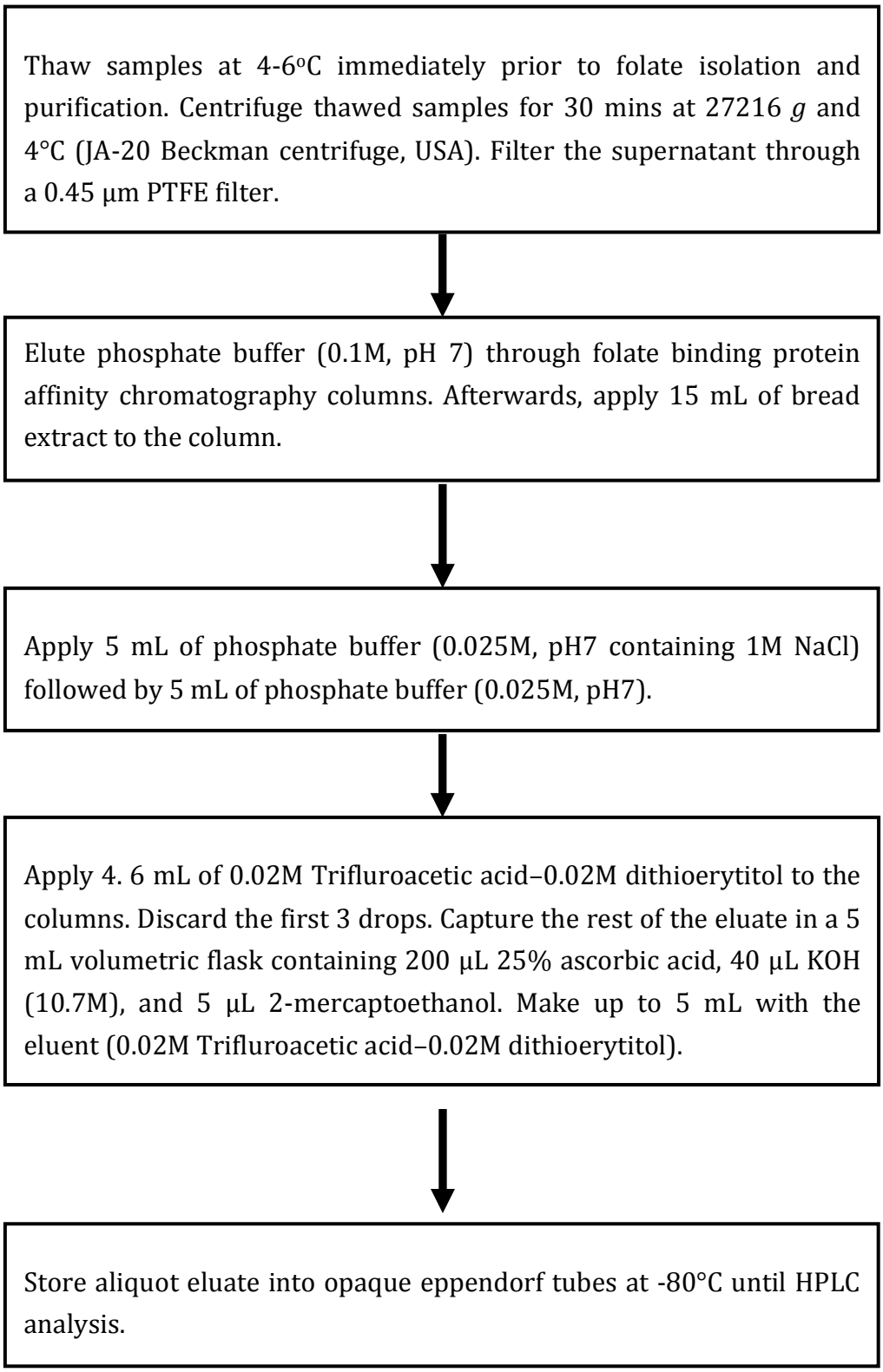


Figure 2: Overview of the folic acid isolation and purification process

High-performance liquid chromatographic analysis of folic acid extracts from bread

The high-performance liquid chromatography (HPLC) method for folic acid was conducted based on the method described by Verlinde et al. [12]. A 1200 series HPLC (Agilent Technologies, Germany) equipped with a UV-DAD detector and EZChrom Elite 3.3 software was used to identify and quantify the concentration of folic acid in the samples. Folic acid identification was done based on peak purity of commercially available purified folic acid standard. A Prevail RP C_{18} column (250 × 4.6 mm, 5 µm particle size; Grace Davison Discovery Sciences, Deerfield, Illinois, USA) was used to separate folic acid. The conditions for elution of folic acid were as follows: flow rate, 1mL/min; column temperature, 25°C; UV detection, 283 nm. A gradient of HPLC grade methanol and 0.1% (v/v) formic acid was used as mobile phase. The run time was 39 minutes; the gradient started at 88% 0.1% (v/v) formic acid, 12% methanol, and was maintained isocratically for 4.2 minutes, after which the methanol concentration was raised linearly to 56% within 12.6 minutes (thus until 16.8 min). Subsequently, the condition with 56% methanol was maintained isocratically until 29.6 minutes before equilibration to the initial condition of 12% methanol at 30.6 minutes where it was maintained for 4 minutes before the next sample injection. The retention time for folic acid was 16.2 minutes. Prior to HPLC injection, the samples were filtered with 0.45 µm PTFE filter; 100 µL of each sample was injected into the HPLC.

The standard curve was constructed with folic acid working solution (10 µg/mL) as the external standard, which was prepared as follows: 0.1 mL of folic acid standard stock solution (1 mg/mL), 400µL 25% ascorbic acid (wt/v), 80 µL KOH (10.7M), 10 µL β-mercaptoethanol, made up to 10 mL with the elution solution (0.02M trifluoroacetic acid-0.02M dithioerytritol). The following injection volumes: 1 µL, 2 µL, 3 µL, 4 µL, 5 µL, 6 µL and 7 µL were used to construct the standard curve of folic acid ranging from 10 to 70 pg (bound on column). Folic acid content in the working solution was determined using the spectrophotometer at 283 nm and 25^{o}C as described by Konings [11]. Peak height and peak area were used to quantify folic acid content of the bread extract. A linear correlation with r^2 >0.99 between peak height or peak area and the concentration of folic acid standards was obtained. An average of the amount calculated from the standard curves constructed using the peak area and the peak height of the folic acid

working standard was used. The concentration of folic acid in the bread samples was expressed per 100 g edible portion.

Survey design to assess folate status of women

The 2011 Folate and Women's Health Survey was a cross-sectional survey of women of childbearing age (18–44 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. The survey used a stratified random sampling technique with the electoral role as the sampling frame. The University of Otago's human ethics committee approved the survey, and all participants gave informed written consent. The survey was registered with the Australia New Zealand Clinical Trials Registry (ACTRN12611000463976).

Sample size

A sample size of 300 participants (150 in each city centre) would provide 90% power (alpha = 0.05, two sided) to detect a 64 nmol/L change in red blood cell folate concentrations – about one quarter of a standard deviation – and a change in the proportion of women with red blood cell folate concentrations above 905 nmol/L from 33% to 46%. An additional consideration in choosing the sample size was the size of the confidence interval around the estimate of the proportion of women with a red blood cell folate concentration above 905 nmol/L. For a prevalence of 50% of women above 906 nmol/L, 300 participants would give a confidence interval ± 5-6%.

Electoral role sample selection

The New Zealand Parliamentary Electoral Roll was used as the sampling frame to select women from Dunedin and Wellington. 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.

The New Zealand elections office approved our application for an electronic copy of the Parliamentary Electoral Roll for the purposes of selecting our sample. STATA (version 11) was used to select the sample. Participants who were eligible for selection were those women who were between the ages of 18–44 years on the 25/03/11. The New Zealand Parliamentary Electoral Roll does not list the sex of those enrolled, however, it does list their honorific. Those with the following titles that were deemed to be

exclusively male – 'Baron', 'Brother', 'Count', 'Father', 'Lord', 'Master', 'Monsignor', 'Mr', and 'Sir' – were excluded from the sample. Local authority boundaries were used to restrict the samples to our South Island and North Island centres. For our South Island centre – Dunedin – the sample was restricted to those living within the local authority boundary of Dunedin city. For our North Island centre – Wellington – the sample was restricted to those living within the boundaries of three local authorities: Wellington city, Porirua city and Hutt city.

Those listed were categorised into the following age groups: 18–19; 20–24; 25–29; 30–34; 35–39; and 40–44 years. For each of the city centres, a random number was generated for potential participants and within each age category the random number was used to create an ordered list.

The census 2006 information on the proportion of women in each age category from 18–44 years was used to determine the proportion of women in our final sample in each age category. For example, from the census 2006 data, 17% of women in the age range 18–44 years were in the age category 20 to 24, therefore 17% of our sample of 300 women in each centre was selected from this age range. The census 2006 data lists women into five-year age categories. We assumed that 40% of women in the census age category of 15–19 years were 18 or 19.

The ordered lists within each age categories were examined to remove ineligible participants; firstly, those with given names that were clearly male, were removed from the list; secondly, those with a mailing address greater than 25 km from the main intersection in the respective city centres were removed. We used the main intersection in each city centre as defined by the New Zealand Automobile Association, in Dunedin, the intersection of George Street and the north side of The Octagon outside the Council Offices, in Wellington, the intersection of Willis Street and Mercer Street outside Cigana House. A map of each city, showing the 25 km radius from the city centre is presented in the Appendix A. Where a selected person was removed during the screening of the sample, the next person on the ordered list in the same age category replaced them.

Participant recruitment procedure

Recruitment followed the four stage tailored method as recommended by Dillman [13]. Firstly, the final sample of 300 women in each city centre were mailed out an initial invitation to participate. This postal invitation included the cover letter inviting them to

participate, a coloured brochure, a consent form, a general questionnaire, a freepost return envelope, a study pen, and an opt out form if they decided not to participate. Secondly, all women in the sample were sent, seven to eight days after the first mailout, a postcard which served as a thank-you to those that had already returned their consent form and general questionnaire, and a reminder to those that had not. Thirdly, 16 days after the initial mail out, a second invitation with replacement information pack was sent to the non-responders. Finally, 28 days after the initial mail out, the non-respondents telephone numbers were obtained from the white pages and they were phoned to invite them to participate one final time. If their number was not listed, or if they could not be reached, a final reminder postcard was sent. Due to a lower than anticipated response rate in the Wellington sample, a second sample was selected from Wellington and contacted following the same procedures described above. One hundred and fourteen women were invited to participate in the second sample round, which was based on the response rate of the first Wellington sample at the time of the decision to recruit a second sample (around 33%), with the aim to recruit 150 women in total from Wellington.

General questionnaire

A self-administered questionnaire was used to collect information on the following socio-demographic characteristics: birth date, ethnicity, education level, and income level. The general questionnaire also collected information on factors that may affect folate status: health status, whether the participant was on a gluten-free diet, medical conditions, medication use, alcohol and coffee use, smoking status, past pregnancies, and overseas travel within the last year.

Dietary assessment

Participants completed a telephone interview during which information about their frequency of consumption in the past week of breakfast cereals, breads, fortified spreads, and supplements was collected. Participant responses to the questions were recorded directly onto a standardised form. The phone interview was pilot tested with women in the target age range of our survey to check the participant's comprehension of the questions, and based on feedback from the pilot testing, the phone interview was modified prior to use in the survey. 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 bread was specified as unknown. They were also asked how often in the past week they had consumed marmite, vegemite or vegemite cheesybite, and whether they took any supplements. 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 local supermarket for the presence of added folic acid or added folate. We classified bread as folic acid fortified if the ingredients list stated the bread contained folic acid; we did not alter this classification according to our analysis of the folic acid content of breads. The phone interview was typically completed one week before the clinic visit, participants were told at the beginning about the importance of being honest and trying to remember at best as they could, and at the end of the interview they were reminded to eat as they normally would and that any questions they had about folate would be answered at the clinic visit.

Clinic visit

Anthropometric assessment

Participants were asked to attend a morning clinic at which their height and weight were measured using standardised techniques. Participants were lightly clothed and not wearing shoes.

Blood sample collection

Participants were asked to not consume food or beverages 10 hours before they came to the clinic to give blood. Two six-millilitre (mL) tubes of blood were drawn by veni-puncture in the ante-cubital fossa; one tube contained EDTA for the collection of whole blood, the other tube contained no anticoagulant and was used to isolate serum. The serum tube was left for one hour at room temperature before it was centrifuged at 3000 revolutions per minute for 15 minutes at 4°C and serum removed. The EDTA tubes were placed immediately in a polystyrene container with an ice pack when blood was collected. Haematocrit was analysed from whole blood. The whole blood, plasma, and

serum samples were dispensed and stored at -80°C within three hours of blood collection.

Measurement of serum and whole blood folate concentrations

Serum and whole blood folate was measured by microbiological assay with the use of the test organism *Lactobacillus rhamnosus*, as described by O'Broin [14]. Samples to be assayed were thawed just prior to use. Once thawed, 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. Serum and whole blood from the same participant were assayed in quadruplicate on the same plate. 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 microplate reader with the wavelength set at 590 nm. Linear interpolation was used to quantify the concentration of folate in the blood samples.

Red blood cell folate concentrations were calculated according to the following equation :

$$Red\ blood\ cell\ folate = \frac{whole\ blood\ folate - [serum\ folate\ x\ (1 - haematocrit)]}{haematocrit}$$

The accuracy of the microbiological assay was determined with the use of the three level standard reference material 1955 (SRM 1955) for serum folate from the National Institute of Standards Technology (NIST, USA) that was assayed in duplicate on one plate per day. The analysed values (n = 4) were 7.5 nmol/L (certified range: 4.9 to 6.3 nmol/L), 15.6 nmol/L (certified range: 12 to 16 nmol/L), and 48.9 nmol/L (certified range: 37 to 51), with CVs of 2.4%, 3.0% and 4.3%, respectively. The precision of the assay was monitored by analysing pooled plasma in duplicate on every plate. The mean and CV for the pooled plasma (n = 29) was 19.2 nmol/L and 12.3%, respectively. We used the same cut-offs as the National Health and Nutrition Examination Survey (NHANES) to indicate low serum folate concentrations (< 6.8 nmol/L) and folate deficiency (a red blood cell folate concentration of < 317 nmol/L) [15,16]**.** To relate red blood cell folate concentration to risk of NTD-risk, cut-offs proposed by Daly et al. [3] were used, whereby a red blood cell folate concentration < 339 nmol/L was used to categorise women at high risk of having a NTD-affected pregnancy, and a red blood cell

folate conentration of 906 nmol/L or higher was used to categorise women at very low risk of having a NTD-affected pregnancy.

The reduction in the prevalence of NTDs was predicted as described by Wald et al. [17] and was based on the change in serum folate concentrations between the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey.

Statistical analysis

All statistical analysis were carried out using STATA (version 11.2, Stata Corp, College Station, TX). Differences in the characteristics of participants between the two cities, or of participants and non-participants of the 2011 Folate and Women's Health Survey were examined using simple regression for continuous variables and chi-squared test for categorical variables. We used multiple linear regression to examine the relation between blood folate status and diet. Because of their positive skew, serum and red blood cell folate concentrations were log-transformed before statistical analysis and the differences between groups were presented as ratios. A two sided Student's t-test for unpaired samples was used to test significant differences in blood folate status between the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey.

Results

Folic acid content of folic acid fortified breads

The details about the breads selected for folic acid analysis are presented in **Table 1.** In the North Island, four of the seven breads tested contained 100–300 µg folic acid per 100 g edible portion **(Table 2)**, the other three breads contained less than 50 µg per 100 g edible portion. In the South Island, two of the ten breads tested had folic acid concentrations that exceeded 400 µg per 100 g edible portion, six breads contained 100–300 µg folic acid per 100 g edible portion. The other two breads (D 8 and D 9) contained less than 50 µg per 100 g edible portion. The mean (SD) and median (interquartile range) for all 17 breads was 151 µg (131) and 144 µg (41, 189), respectively.

Folic acid stability over shelf-life of folic acid fortified bread

The stability of folic acid during the five to six day shelf-life of fortified white and whole-wheat bread is shown in **Table 3.** The folic acid content was marginally lower, by 1 µg (0.4%) – in white bread at the end of the shelf-life (i.e. best before date), but in whole-wheat bread was decreased by 18 µg (9.1%) compared to the content of folic acid at Time 0.

Response rate to survey recruitment

The flow of participants through the 2011 Folate and Women's Health survey is shown in **Figure 3.** Invitations to participate in the study were delivered to 310 Dunedin women. In total, 149 women in Dunedin consented to participate; of these, two completed neither a phone interview nor a clinic visit, leaving 147 included in the analysis. All 147 participants completed a telephone interview, and 143 completed a clinic visit. There were 38 participants who declined to take part, and 23 non-deliveries (i.e. returned to sender) of the information packs. One hundred participants did not respond to the invitation. The total response rate for Dunedin was 51% [participants who completed at a phone interview or clinic visit/(total selected – non-deliveries), 147/(310-23)].

In the first Wellington sample, 300 women were invited to participate in the study. One hundred and ten women consented to participate, of these two completed neither a

phone interview nor a clinic visit. Of these 108 participants, one did not complete a telephone interview and four people did not complete a clinic visit. There were 44 participants who declined to take part, 23 non-deliveries, and one person who was not eligible to participate as she did not understand English. Therefore, out of the 300 women selected there were 122 women who did not respond to the invitation. The response rate for the first Wellington sample was 39% [participants who completed at least one of a phone interview or clinic visit/(total selected – (non-deliveries + ineligible), 108/(300-24)].

In the second Wellington sample, 114 women were invited to participate in the study. Thirty-seven consented to participate, four completed neither a phone interview nor a clinic visit, leaving 32 included in the analysis. Of these two participants, one did not complete a phone interview, and one did not complete a clinic visit. There were 15 participants who declined to take part, and 11 non-deliveries. Therefore, out of the 114 women selected there were 52 who did not respond to the invitation. The response rate for the second Wellington sample was 32% [33/(114-11)]. The overall response rate for the Wellington samples combined was 37%.

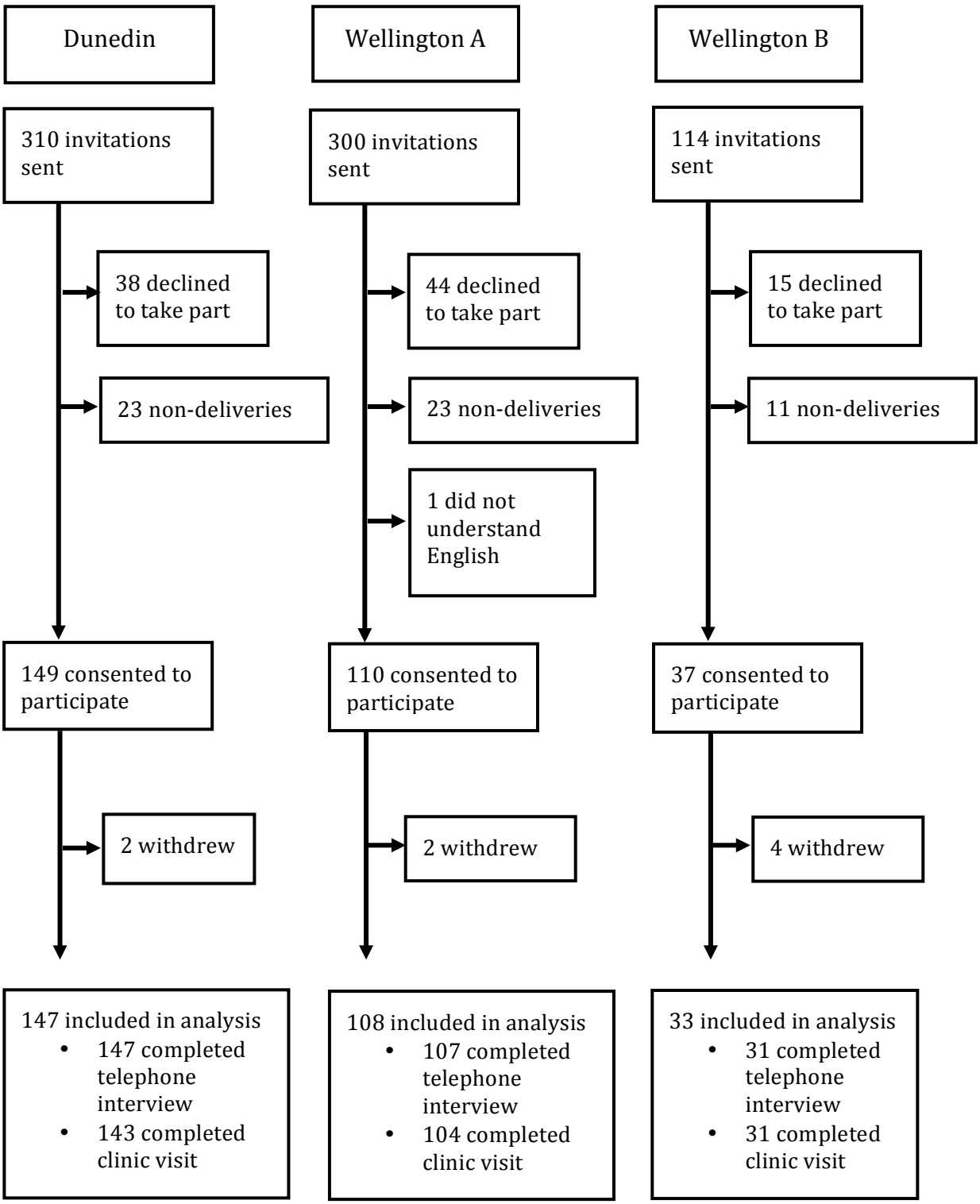


Figure 3: Flow of participants through the 2011 Folate and Women's Health Survey

Characteristics of survey participants

The mean age of participants was 33 years **(Table 4)** and their mean (SD) body mass index (BMI) was 27 (6) kilograms per metre-squared (kg/m^2). The most common ethnicity was New Zealand European and other (81%). Almost half of the women had some tertiary education and more than 80% said they were in good or excellent health; one quarter of the women used oral contraceptive agents and a third used prescription medications; three quarters of the women drank alcohol and a similar proportion drank coffee. Almost one quarter of women had visited, in the past year, a country with mandatory folic acid fortification of food.

In general, the age, BMI, educational, health, and income characteristics of women living in Wellington did not differ from those of women living in Dunedin; however, there were statistical differences in ethnicity, use of prescription medication, and history of pregnancy between women recruited from the two cities. There was a slightly higher proportion of Maori and Pacific women in Wellington than in Dunedin (p=0.001); more women in Dunedin were using prescribed medication (39% compared with 25%, p=0.01) and more women in Dunedin had previously been pregnant (65% compared with 54%, p=0.048).

From the information included on the electoral roll, we were able to compare a limited number of characteristics between the participants and non-participants of the 2011 Folate and Women's Health Survey (**Table 5**). The proportion of women with Maori descent was similar for participants and non-participants. However, younger women and those from areas of more deprivation were less likely to participate in our survey (p = 0.001, $p < 0.001$, respectively).

Consumption of folic acid fortified bread, breakfast cereal, and spreads

Bread was consumed by 93% of the participants in the week prior to completing the telephone food frequency questionnaire **(Table 6)**. Women were only classified as consumers of folic acid fortified bread if they reported eating one or more slices of a folic acid fortified brand of bread (identified as breads with folic acid on the ingredient list). Eighteen percent of women consumed at least one slice of folic acid fortified bread in the past week. Seventy-five percent of women consumed bread that was either not fortified with folic acid or could not be identified as fortified because the participant could not recall the brand name of the bread; of this group, 38 participants (13%)

consumed one or more slices of bread of unknown brand. About half of women consumed a folic acid fortified spread. There was a significant correlation between total slices of bread consumed in the previous week and frequency of using folic acid fortified spreads (r=0.28, $p<0.001$). There was an inverse association between smoking (Y/N) and frequency of consumption of breakfast cereals in the past week (r=-0.21, $p<0.001$).

Breakfast cereal was consumed by 72% of the participants in the week prior to completing the telephone food frequency questionnaire **(Table 6)**. Forty-one percent of women consumed at least one bowl of folic acid fortified breakfast cereal in the past week and 31% consumed breakfast cereal that was either non-fortified or could not be identified as such; of this latter group, 15 (5%) consumed one or more bowls of breakfast cereal of unknown brand.

There were no significant differences ($p>0.05$) between Wellington and Dunedin in the proportion of women consuming folic acid fortified bread, folic acid fortified breakfast cereal, or folic acid fortified spreads. The proportion of women who ate any bread or any breakfast cereal also did not differ significantly between cities (data not shown).

Amongst all women, the mean (SD) consumption of bread and breakfast cereal, was 12 (9) slices and 3.4 (2.8) bowls, respectively, in the past week **(Table 7)**. This frequency of consumption did not differ between Wellington and Dunedin ($p=0.296$ for bread and $p=0.737$ for breakfast cereal). Amongst consumers of any bread, mean frequency of consumption was 13 (8) slices in the past week; this did not differ between the two cities ($p=0.636$) **(Table 8)**. Amongst consumers of any breakfast cereal, mean frequency of consumption was 4.8 (2.2) bowls in the past week; this did not differ between the two cities ($p=0.528$) **(Table 8)**. Consumers of folic acid fortified bread reported a mean frequency of all bread consumption of 13 (8) slices in the past week, this did not differ between the cities ($p=0.887$) **(Table 9)**. Consumers of folic acid fortified breakfast cereal reported a mean frequency of all breakfast cereal consumption of 4.8 (2.3) bowls in the past week, this did not differ between the cities ($p=0.179$) **(Table 9)**.

Serum and red blood cell folate concentrations of participants

Women in the survey had a mean (SEM) serum folate concentration of 36 (1) nmol/L and red blood cell folate concentration of 1096 (30) nmol/L **(Table 10)**. The distribution of serum and red blood folate concentrations was positively skewed. To stabilise the variance, the data were log-transformed giving a geometric mean for serum

folate of 30 nmol/L (95%CI: 28, 32 nmol/L) and geometric mean for red blood cell folate of 996 nmol/L (95%CI: 945, 1049) nmol/L). The median serum folate concentration was 29 nmol/L (interquartile range: 20, 47 nmol/L) and the median red blood cell folate concentration was 989 nmol/L (interquartile range: 744, 1316). The prevalence of women with deficient serum and red blood cell folate concentrations, defined as less than 6.8 nmol/L for serum and less than 317 nmol/L for red blood cell, was 1%. Almost 60% of women in the survey had a red blood cell folate concentration 906 nmol/L or higher, a concentration associated with NTD risk similar to women taking a 400 μg daily folic acid supplement.

Mean serum folate and red blood cell folate concentrations were significantly higher in Dunedin compared with Wellington; however, after adjustment for folic acid supplement use (Y/N) and consumption of folic acid fortified breakfast cereals (Y/N) the trend for slightly higher concentrations in Dunedin was not significant. The adjusted ratio of the geometric means of serum folate in Dunedin relative to Wellington women was 1.13 (95%CI: 1.00, 1.29; $p<0.068$) and for red blood cell folate it was 1.11 (95%CI: 1.00, 1.22; $p<0.052$).

In simple linear regression, serum and red blood cell folate concentrations were unrelated ($p>0.05$), to antibiotic use (Y/N), having visited in the last year a country with mandatory folic acid fortification (Y/N), use of prescription medication (Y/N), ethnicity, education, NZDep2006 score, and age. Use of dietary supplements containing folic acid (Y/N) and smoking (Y/N) were significantly associated with serum and red blood cell folate concentrations ($p<0.05$). Frequency of use of folic acid fortified spreads was significantly associated with serum folate but not red blood cell folate concentrations. Given the association between smoking and breakfast cereal consumption and the association between folic acid fortified spread use and bread consumption, the final multiple linear regression model controlled for bread consumption, breakfast cereal consumption, folic acid supplement use, and city of residence. Adjustment for any other potential confounder had negligible influence on the overall results or on the predictive ability of the statistical model.

The relation between bread and breakfast cereal consumption and serum and red blood cell folate concentrations

Participants who reported consuming folic acid fortified bread had a significantly higher mean serum folate concentration compared with participants who did not consume folic acid fortified bread **(Table 11)**. The ratio of geometric means after adjustment for use of folic acid supplements, consumption of folic acid fortified breakfast cereal, and city of residence was 1.24 (95%CI: 1.05, 1.48; p=0.012). The same trend in serum folate concentration was apparent for consumers of fortified breakfast cereal relative to non-consumers with an adjusted ratio of 1.15 (95%CI: 1.00, 1.31; p=0.042). Mean red blood cell folate concentrations were not significantly higher in consumers of folic acid fortified bread, however, there was a trend for higher concentrations amongst consumers; the adjusted ratio was 1.12 (95%CI: 0.99, 1.28; p=0.079). Participants who ate folic acid fortified breakfast cereals had significantly higher red blood cell folate concentrations compared with those who did not consume folic acid fortified breakfast cereal; the adjusted ratio was 1.13 (95%CI: 1.02, 1.25; p=0.019).

There was no relation between frequency of total bread consumption (slices per week) and serum or red blood cell folate concentrations **(Table 12)**. Frequency of total breakfast cereal consumption was significantly associated with serum and red blood cell folate concentrations. Per incremental difference (one more bowl per week) in frequency of breakfast cereal consumption the adjusted ratio for serum folate was 1.036 (95%CI: 1.012, 1.061; p=0.003) and for red blood cell folate was 1.031 (95%CI: 1.013, 1.050; p=0.001).

Comparison with serum and red blood cell folate status pre-voluntary folic acid fortification of bread

The blood folate results from the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey are compared in **Table 13**. Mean and geometric-mean serum and red blood cell folate concentrations were significantly higher (all $p < 0.001$) in the 2011 Folate and Women's Health Survey compared to participants aged 18-44 years in the 2008/09 New Zealand Adult Nutrition Survey. The magnitude of difference for serum folate concentrations was 7.9 nmol/L (95% CI: 4.3-11.4 nmol/L), and for red blood cell folate concentration was 302 nmol/L (95% CI: 229-374 nmol/L). The proportion of women in the 2011 Folate and Women's Health Survey

with red blood cell folate concentrations 906 nmol/L or higher was 33% (95%CI: 26, 40) higher compared with women of similar age in the 2008/09 New Zealand Adult Nutrition Survey. The age, body mass index, ethnicity, and New Zealand Deprivation 2006 (NZDep2006) characteristics of participants aged 18-44 y in the 2008/09 New Zealand Adult Nutrition Survey and the 2011 Folate and Women's Health Survey are shown in **Table 14**; information from the two surveys about education are not shown in the **Table 14** because the questions used to gather the information in the 2008/09 New Zealand Adult Nutrition Survey were not readily comparable to the 2011 Folate and Women's Health Survey. Fifty-nine percent of women aged 18-44 y in the 2008/09 New Zealand Adult Nutrition Survey had a qualification beyond high school. Those women with incomplete qualifications, or qualifications that took less than three month to finish were excluded from this category. Sixty-nine percent of participants in the 2011 Folate and Women's Health Survey had a certificate or diploma, or at least some tertiary study. Therefore, although the education categories in the two surveys are not directly comparable, the participants appear to have similar education levels.

A population-based survey of 212 women, 18-45 y living in Dunedin was conducted in 1999 and showed that median (interquartile range) red blood cell folate concentration was 787 nmol/L (616, 1073 nmol/L) [8] (data not shown). This is lower than the results of the present survey which show a median (interquartile range) red blood cell folate concentration of Dunedin women was 1042 nmol/L (778, 1381 nmol/L).

Prediction of change in Neural Tube Defect rate following the introduction of the voluntary folic acid fortification of bread programme

Based on the work of Daly et al. [3] and Wald et al. [17], the change in arithmetic mean serum folate concentration from the 2008/09 New Zealand 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 rate would be predicted from an increase in serum folate concentration from 27.8 nmol/L (2008/09 New Zealand Adult Nutrition Survey) to 35.6 nmol/L (2011 Folate and Women's Health Survey).

Discussion

Folic acid content of folic acid fortified bread

The most striking result from our analysis of the folic acid fortified breads was that five out of the 17 breads had folic acid contents less than 50 μg per 100 g edible portion; three of the brands with very low folic acid contents were from the North Island and two were from the South Island. We are confident in the accuracy and precision of the results. All breads that were purchased in Wellington were baked in North Island factories, and all breads purchased in Dunedin were baked in South Island factories.

We assessed the accuracy of the analytical assay by spiking samples of unfortified bread with folic acid; recovery of folic acid from spiked samples exceeded 90%. The standard curve was linear and gave excellent reproducibility, based on low standard deviations **(Table 2)**.

There were five breads containing less than 50 μg/100 g edible portion, nine breads containing 100 –200 μg/100 g edible portion, one containing 200–300 μg/100 g edible portion, and two containing 400–500 μg/100 g edible portion. The mean (SD) folic acid content of the 17 folic acid fortified breads was 151 (131) μg/100 g edible portion; median (interquartile range) was 144 (41, 189) μg/100 g edible portion. The high standard deviation of the mean shows a high variability in the folic acid content of different brands. Personal communication from the Folic Acid Working Group suggested that the bread manufacturers were encouraged as part of the voluntary folic acid fortification of bread programme to add 200 μg/100 g edible portion.

It is noteworthy that the breads we received directly from the bread manufacturers for the shelf-life study contained remarkably close to 200 μg/ edible portion (198 and 256 μg). The results of the shelf-life study showed that folic acid is quite stable during the five to six days of shelf-life with negligible loss in the white bread (<1%) and about 9% loss in the whole-wheat bread. It is noteworthy that the North Island brand of bread used for the shelf life study was the same brand as W 5. The latter was found to contain negligible amounts of folic acid. The breads were purchased on different dates (W 5 on 17/1/11 and NI 1 on 2/2/11) and manufactured in different North Island bakeries.

It is important to note that breads for our study were collected from supermarkets on one occasion; therefore, our analysis can only reflect the folic acid content at that point in time and cannot automatically reflect folic acid levels in bread at all times. The three North Island brands of bread that we found had very low folic acid contents were the top three North Island folic acid fortified breads ranked brands by sales volume (ranking not shown, to preserve confidentiality); the two Dunedin brands with undetected folic acid were ranked fifth and sixth in the South Island. Thus, if our analysis of bread reflects the usual folic acid content of these brand during the six to twelve months prior to collecting blood from the participants in our study, one would expect the folate status of women residing in Dunedin to be slightly higher compared with women residing in Wellington because the food supply in Dunedin during that period would have more folic acid from bread. The trend for higher folate status in Dunedin compared with Wellington women is consistent with this proposition. Though the adjusted ratio of mean serum concentrations in the two cities (1.13, p =0.068), as well as that for red blood cell folate (1.11, p =0.052), did not differ statistically from 1, the lower limit of the confidence interval was 1.00 for serum and red blood cell folate concentrations **(Table 10)**.

Ten of the 17 breads tested contained 100–300 μg folic acid/100 g edible portion, and were therefore close to the agreed target of 200 μg folic acid/ 100 g edible portion. There was little evidence of excessive folic acid in the folic acid fortified breads that we analysed, with only two of the 17 breads tested containing more than 400 μg/100 g edible portion. The most common problem was inadequate folic acid content with five breads containing less than 50 μg folic acid per 100 g edible portion.

The 2011 Folate and Women's Health Survey participants

We had a limited number of characteristics available from the electoral roll to compare those who participated in our survey and those who did not. The percentage of women of Maori descent did not differ between the two groups, however a higher percentage of non-participants were younger, and came from more deprived areas. It is possible that some of these women did not receive the invitation to participate, as younger women or those from more deprived areas may be more likely to move. If the invitation was delivered to an out-of-date address, we relied on the new residents to return the invitation pack to us. Women who did not want to participate were encouraged to return an 'opt-out' slip to us. For women that we never heard from, it is therefore

difficult to know whether they did not want to participate, or whether they never received the invitation to participate. Because our participants were more likely to be older and less deprived than the sample from which they were drawn, it is possible that our participants reflect a slightly healthier group compared with that of all New Zealand women of childbearing age. However, the fact that age and NZDep score were not predictors of serum of red blood cell folate concentration in the 2011 Folate and Women's Health Survey suggests that the slightly older age and less deprivation index score of the participants is unlikely to bias the results.

Consumption of folic acid fortified bread, breakfast cereal and spread

The vast majority, 93%, of the survey participants reported consuming one or more slices of bread in the last week. A recent report by the Australian Institute of Health and Welfare reported, based on results compiled by Food Standards Australia New Zealand, that between 2001 and 2008 about 80-85% of New Zealand women, 14 years or older, consumed bread [18]. Eighteen percent of the participants in the 2011 Folate and Women's Health Survey were able to confirm having consumed one or more slices of fortified bread in the last week. The actual proportion of women consuming folic acid fortified bread was likely to have been higher because 13% of women were unable to recall at least one brand of bread they consumed, which made it impossible to confirm if it was fortified or not. In addition, the participants were asked about their consumption of foods in the last week, and thus the phone interview may not have captured all of the brands of bread and breakfast cereal regularly consumed by participants. The corollary is that some women who regularly consumed folic acid fortified bread were classified as non-consumers. The possible underestimation in our survey of the proportion of women consuming folic acid fortified bread and over-estimation of the proportion of women not consuming fortified bread would attenuate differences in the blood folate status between women classified as consumers or non-consumers of folic acid fortified bread.

Seventy-two percent of women in our survey reported consuming one or more bowls breakfast cereal in the last week. The report by the Australian Institute of Health and Welfare [18] showed that about 70% of New Zealanders 14 years or older consumed breakfast cereal between 2001 and 2008. We were able to confirm that 41% of women consumed folic acid fortified breakfast cereal in the previous week; again this is likely to be an underestimation of the proportion of women who regularly consume folic acid

fortified breakfast cereal, but probably not as much as that for bread because only 5% of women were unable to identify at least one brand of breakfast cereal consumed.

Fifty-three percent of women in our survey reported consuming folic acid fortified spread in the previous week. The report by the Australian Institute of Health and Welfare [18] showed that just over 50% of New Zealand women 14 years or older consumed yeast containing spreads in 2007 and 2008.

The proportion of women consuming any bread or breakfast cereal, whether fortified or not, did not differ between Wellington and Dunedin. Furthermore, the frequency of consumption (i.e. slices per week or bowls per week) of bread and breakfast cereals amongst all consumers, consumers of bread or breakfast cereals, or consumers of fortified bread or breakfast cereals did not differ between the cities. This suggests that the differences in mean blood folate status of women residing in Wellington compared with those residing in Dunedin did not result from a different prevalence or frequency of bread and breakfast cereal consumption between the two cities.

The relation between consumption of folic acid fortified bread or breakfast cereal and blood folate status

Consumption of folic acid fortified bread (Y/N) was associated with higher serum folate concentrations **(Table 11)**. The difference in geometric mean serum folate concentration between consumers of folic acid fortified and non-fortified bread geometric was 9 nmol/L (p = 0.012). This difference was adjusted for folic acid fortified breakfast cereal consumption (Y/N), folic acid supplement use (Y/N) and city of residence. Quinlivan and Gregory [19] in reviewing controlled intervention trials to establish the dose response relation between dietary folate equivalents (DFE; 1 DFE = 0.6 μg folic acid with meals) and change in serum folate concentrations, reported that 86 μg/d of folic acid increases serum folate concentrations by 5 nmol/L. Accordingly, an 9 nmol/L difference in serum folate concentration would require a 155 μg/d difference in folic acid intake. Two slices of bread (approximately 60-70 g) fortified with 200 μg per 100 edible portion would provide 120 μg folic acid. Women who were classified as consumers of folic acid fortified bread ate 1.9 slices of bread per day (13 slices per week, **Tables 7 & 9**). Thus, for consumption of folic acid fortified bread to account for all of the difference in serum folate concentration between consumers and non-consumers of folic acid fortified bread, women in each group would have consumed exclusively the

corresponding type of bread; this seems unlikely. Consumption of folic acid fortified breakfast cereal was associated with a 5 nmol/L higher mean serum folic acid concentrations than non-consumers after adjustment for folic acid fortified bread consumption, folic acid supplement use, and city.

Mean red blood cell folate concentration was higher in consumers compared with non-consumer of folic acid fortified breakfast cereals; the adjusted ratio of the geometric mean (1.13) was similar to that for serum folate (1.15). However, consumption of folic acid fortified bread was not associated with significantly higher mean red blood cell folate concentration. The reason for this is not readily apparent. It may be as simple as the number of women classified as consumers of folic acid fortified bread being too small a group to give the analysis adequate statistical power. Furthermore, some of the breads we classified as folic acid fortified – on the basis of the ingredient list – may have contained little folic acid. Attenuation bias of misclassifying folic acid fortified bread consumer might have played a role, but one would expect this to also apply for the serum folate results. The upper level of the confidence interval for the adjusted difference in red blood cell folate concentration between folic acid fortified and non-fortified bread consumers was 1.28, a value sufficiently large to make it difficult to completely discount the possibility of an association.

It is interesting to note that frequency of breakfast cereal consumption was significantly associated with serum and red blood cell folate concentrations **(Table 12)**; however, frequency of bread consumption was not significantly associated with either serum or red blood cell folate concentrations. It is possible the stronger association of blood folate status with breakfast cereal compared with bread consumption reflects a higher penetration of folic acid fortified products in the breakfast cereal food category compared with the bread category; a situation that may have been exacerbated by the very low folic acid content of breads that were supposed to be fortified at 200 µg/100 g edible portion. Thus, frequency of breakfast cereal consumption is more likely to be associated with higher folic acid intakes than frequency of bread consumption, even though women in our survey consumed more servings per week of bread (6-7 servings per week) than servings of breakfast cereal (4-5 servings per week). A higher amount of folic acid fortification per serve in breakfast cereal compared with bread may also have an influence. Furthermore, we did not test the folic acid content of the most popular folic acid fortified breakfast cereals, and it is possible that it is common for the actual

folic acid content of fortified breakfast cereals to be somewhat higher than that reported on the label – a phenomenon referred to as 'overages' [20].

The accuracy, precision, and validity of the telephone food frequency questionnaire was not tested prior to the survey, therefore, it is impossible to know to what extent measurement error may have influenced the results. In general, dietary measurement error would tend to increase misclassification of participants into categories of fortified or non-fortified food consumption, thus, our results are likely to be an underestimation of the true association between consumption of folic acid fortified breads and breakfast cereals.

Comparison of folate status pre- and post the voluntary folic acid fortification of bread programme

Compared to women 18-44 years of age in the 2008/09 New Zealand Adult Nutrition Survey, the blood folate concentrations of participants in the 2011 Folate and Women's Health Survey were significantly higher **(Table 13)**. For red blood cell folate the difference between the two surveys was 302 nmol/L, and for serum folate, the difference was 7.9 nmol/L. The proportion of women with red blood cell folate concentration 906 nmol/L or higher was 33 percentage points higher in the 2011 Folate and Women's Health Survey compared with the 2008/09 New Zealand Adult Nutrition Survey; however, the 95% confidence intervals around this estimate ranged from 26% to 40%. Women in the two surveys were of similar age and body mass index; however, there was a lower proportion of Maori and Pacific women in the 2011 Folate and Women's Health Survey as well as a higher proportion of women with NZDep2006 scores indicating "less deprivation" **(Table 14)**. We were unable to make a direct comparison of the education characteristic of women in the two surveys; however, given that ethnicity, education, and NZDep2006 scores were not associated with serum of red blood cell folate status in the 2011 Folate and Women's Health Survey, the difference in ethnicity and any differences in the education and NZDep2006 characteristics of participants in the two surveys is unlikely to introduce bias.

According to the dose response relation reported by Quinlivan and Gregory [19] an 8 nmol/L difference in serum folate concentration would arise if folic acid intake was 140 µg/d higher in women in our survey compared with in the 2008/09 New Zealand Adult Nutrition Survey. It is likely that the voluntary folic acid fortification of bread,

introduced in April/May 2010, provided some of the additional dietary folic acid; but how much, is difficult to establish. One argument for linking the voluntary fortification of bread with improved folate status of women, was the non-significant trend for higher serum and red blood cell folate concentrations of women in Dunedin compared with Wellington; after adjustment for folic acid supplement use and consumption of folic acid fortified breakfast cereals. Given that the top three North Island ranked (by sales volume) folic acid fortified breads were found to have little folic acid, it is probable that the folic acid intake from bread was slightly higher in women residing in Dunedin than in Wellington. The demographic characteristics of women in Dunedin differed slightly from women in Wellington **(Table 4)**. In Dunedin, there was a higher proportion of New Zealand European and Others and lower proportion of Maori and Pacific, a higher proportion of prescription medication users, and higher proportion of women with a past pregnancy. However, in regression analysis, none of these factors predicted serum or red blood cell concentrations in the participants. Therefore, the slight differences in the demographic characteristics of women in the two cities probably did not contribute to the differences in mean serum and red blood cell folate concentrations. Given that women consumed, on average, 1.7 slices of bread per day and that a significant proportion of women did not eat folic acid fortified bread, it is unlikely that the difference in serum folate concentration between the 2011 Folate and Women's Health Folate and the 2008/09 New Zealand Adult Nutrition Survey can be attributed entirely to consumption of folic acid fortified bread.

It is interesting to note that a convenience sample of Australian inpatients and outpatients pre- and post- mandatory folic acid fortification showed a 5.4 nmol/L higher serum folate concentration after the introduction of mandatory folic acid fortification of bread flour [21].

Attributing the difference in folate status of women pre- and post-voluntary fortification to differences over time in the intake of folic acid requires that the blood collection methods and microbiological assay gave equivalent values when the samples for the 2008/09 New Zealand Adult Nutrition Survey and 2011 Folate and Women's Health Survey 2001 were analysed. Blood samples in the 2011 Folate and Women's Health Survey and the 1999 Dunedin ANZFA Survey were taken after a 10 hour overnight fast whereas non-fasting samples were collected from participants in 2008/09 New Zealand Adult Nutrition Survey. Pfeiffer *et al.* [22] found no measurable difference in serum or red

blood cell folate concentrations between fasting and non-fasting samples collected for the National Health and Nutrition Examination Survey (NHANES) 1999-2000. The National Institutes of Standards and Technology (NIST) certified reference material for serum folate (SRM 1955) was used as quality control during the serum and red blood cell folate analysis of the 2008/09 New Zealand Adult Nutrition Survey samples, as was a pooled plasma sample. The analysed values (n = 19) of the three level NIST certified reference material from the 2008/09 New Zealand Adult Nutrition Survey folate analysis were: 6.2 nmol/L (certified range: 4.9–6.3 nmol/L), 14.0 nmol/L (certified range: 12–16 nmol/L), and 47.1 nmol/L (certified range: 37–51 nmol/L), with Coefficients of Variation (CVs) of 9.8%, 12.5% and 8.9%, respectively. The mean and CV for the 2008/09 New Zealand Adult Nutrition Survey pooled plasma (n = 164) was 19.2 nmol/L and 16.5%, respectively. These analytical values, measured during the 2008/09 New Zealand Adult Nutrition Survey, for the SRM 1955 as well as the pooled plasma did not differ significantly from the values we obtained (see methods section, and Appendix B) in the 2011 Folate and Women's Health Study for the exactly the same SRM 1955 and pooled plasma quality controls. The close agreement shows that the accuracy of the microbiological assay did not differ between the surveys and that any difference in the mean serum and red blood cell folate concentrations of participants in the surveys is the result of real change in folate status over time rather than analytical change.

We have conducted a number of folate intervention trials over the last ten years in which the baseline folate status of women of childbearing age has been reported. Unfortunately, women in these studies were not recruited using random or population-based methods, furthermore, there were a number of participant selection criteria (e.g. excluded if red blood cell folate 906 nmol/L or higher) that biased the sample in comparison with the 2011 Folate and Women's Health Study, the 2008/09 New Zealand Adult Nutrition Survey and the 1999 Dunedin ANZFA Survey. For these reasons it is not appropriate to assess the change in folate status pre- and post-voluntary fortification by reference to these intervention study results.

Conclusions

The median (interquartile range) folic acid content of the 17 breads we tested was detected was 144 (41, 189) µg per 100 g of bread. The variation in folic acid content of the folic acid fortified breads was considerable. Most folic acid fortified breads contained between 100–200 µg per 100 g edible portion, however, five breads contained less than 50 µg per 100 g edible portion, and two breads contained 400–500 µg per 100 g edible portion.

Folic acid added to white and whole-wheat bread is stable over the shelf-life of the bread.

A small proportion (18%) of women were able to recall consuming folic acid fortified bread. Bread was consumed by more than 90% of women in the survey and mean consumption was twelve slices in the previous week. There was no difference in the prevalence or frequency of bread consumption between women residing in Wellington or Dunedin.

Consumption (Y/N) of folic acid fortified bread was associated with significantly higher serum folate concentration but not higher red blood cell folate concentrations. Frequency (slices per week) of bread consumption was not associated with serum or red blood cell folate concentrations. These associations may have been attenuated, as some breads that were classified as folic acid fortified, on the basis of the ingredient list, contained little folic acid.

Blood folate status of women in the survey was good with almost 60% having a red blood cell folate concentration 906 nmol/L or higher. This concentration is associated with a risk of NTDs as low as that when taking a 400 µg daily folic acid supplement.

Serum and red blood cell folate concentrations did not differ significantly between women in Wellington and Dunedin; however, there was a trend of borderline significance for higher concentrations in Dunedin. This may reflect lower folic acid content of folic acid fortified breads in Wellington than in Dunedin; the three top ranked folic acid fortified breads in Wellington were found to have little folic acid.

Serum and red blood cell folate status of women in the 2011 Folate and Women's Health Survey was higher than women of the same age in the 2008/09 New Zealand Adult

Nutrition Survey and the 1999 Dunedin ANZFA Survey. This suggests that voluntary fortification of bread has contributed to the increased folate status of women.

References

1. Anonymous. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. Lancet 1991;338:131-7.

2. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. New Engl J Med 1992;327:1832-5.

3. Daly LE, Kirke PN, Molloy A, Weir DG, Scott JM. Folate levels and neural tube defects. Implications for prevention. Jama 1995;274:1698-702.

4. Dobson I, Devenish C, Skeaff CM, Green TJ. Periconceptional folic acid use among women giving birth at Queen Mary Maternity Hospital in Dunedin. Aust N Z J Obstet Gynaecol 2006;46:534-7.

5. Schader I, Corwin P. How many pregnant women in Christchurch are using folic acid supplements in early pregnancy? N Z Med J 1999;112:463-5.

6. Annette King Minister for Food Safety. New Zealand (Mandatory Fortification of Bread with Folic Acid) Food Standard 2007.

7. Food Standards Australia New Zealand. Proposal P295: Consideration of mandatory fortification with folic acid. Canberra and Wellington; 2006.

8. Ferguson EL, Skeaff CM, Bourn DM, Nixon M, Parnell WR. Folate status of representative populations in Dunedin, issues for folate fortification. Research: Ministry of Health; 2000.

9. Skeaff SA, Thomson CD, Gibson RS. Mild iodine deficiency in a sample of New Zealand schoolchildren. Eur J Clin Nutr 2002;56:1169-75.

10. Ministry of Health. NZ Food NZ Children: Key results of the 2002 National Children's Nutrition Survey. Wellington: Ministry of Health; 2003.

11. Konings EJ. A validated liquid chromatographic method for determining folates in vegetables, milk powder, liver, and flour. J AOAC Int 1999;82:119-27.

12. Verlinde PH, Oey I, Deborggraeve WM, Hendrickx ME, Van Loey AM. Mechanism and related kinetics of 5-methyltetrahydrofolic acid degradation during combined high hydrostatic pressure-thermal treatments. J Agric Food Chem 2009;57:6803-14.

13. Dillman DA. Mail and Internet Surveys: The Tailored Design Method. 2nd ed. New York: Wiley; 2000.

14. O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. Journal of Clinical Pathology 1992;45:344-7.

15. Gibson RS. Principles of Nutritional Assessment. 2nd ed. Oxford: Oxford University Press; 2005.

16. Senti FR, Pilch SM. Analysis of folate data from the second National Health and Nutrition Examination Survey (NHANES II). J Nutr 1985;115:1398-402.

17. Wald NJ, Law MR, Morris JK, Wald DS. Quantifying the effect of folic acid. Lancet 2001;358:2069-73.

18. Australian Institute of Health and Welfare. Mandatory folic acid and iodine fortification in Autralia and New Zealand: supplenent to the baseline report for monitoring. Canberra: AIHW; 2011.

19. Quinlivan EP, Gregory JF, 3rd. Reassessing folic acid consumption patterns in the United States (1999 2004): potential effect on neural tube defects and overexposure to folate. Am J Clin Nutr 2007;86:1773-9.

20. Rader JI, Weaver CM, Angyal G. Total folate in enriched cereal-grain products in the United States following fortification. Food Chem 2000;70:275-89.

21. Brown RD, Langshaw MR, Uhr EJ, Gibson JN, Joshua DE. The impact of mandatory fortification of flour with folic acid on the blood folate levels of an Australian population. Med J Aust 2011;194:65-7.

22. Pfeiffer CM, Caudill SP, Gunter EW, Osterloh J, Sampson EJ. Biochemical indicators of B vitamin status in the US population after folic acid fortification: results from the National Health and Nutrition Examination Survey 1999-2000. Am J Clin Nutr 2005;82:442-50.

Table 1 Information about folic acid fortified breads analysed in this survey

	Bread Code No.[1]	Date of purchase	City of purchase	Loaf sample no	Best before date
North Island					
	W 1	9/11/10	Wellington	1	
				2	11/11/10
				3	
	W 2	17/01/11	Wellington	1	
				2	21/01/11
				3	
	W 3	9/11/10	Wellington	1	
				2	12/11/10
				3	
	W 4	9/11/10	Wellington	1	
				2	12/11/10
				3	
	W 5	17/01/11	Wellington	1	
				2	21/01/11
				3	
	W 6	17/01/11	Wellington	1	
				2	21/01/11
				3	
	W 7	17/01/11	Wellington	1	
				2	21/01/11
				3	
	W 8	9/11/10	Wellington	1	
				2	13/11/10
				3	
	W 9	9/11/10	Wellington	1	
				2	13/11/10
				3	

[1]Bread code assigned for this study

	Bread Code No.[1]	Date of purchase	City of purchase	Loaf sample no	Best before date
South Island					
	D 1	27/10/10	Dunedin	1	
				2	31/10/2010
				3	
	D 2	27/10/10	Dunedin	1	
				2	31/10/2010
				3	
	D 3	27/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 4	27/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 5	27/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 6	28/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 7	28/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 8	28/10/10	Dunedin	1	
				2	1/11/10
				3	
	D 9	28/10/10	Dunedin	1	
				2	2/11/10
				3	
	D 10	8/02/11	Dunedin	1	
				2	
				3	
Shelf-life study					
	W 5	3/02/11	Wellington	1	
				2	7/02/11
				3	
				4	
	D 7	3/02/11	Dunedin	1	
				2	8/02/11
				3	
				4	

[1]Bread code assigned for this study

Table 2 Folic acid content of folic acid fortified bread

Bread Code No.	Mean (SD) folic acid in bread (μg/100 g edible portion)[1]
North Island	
W 1	156 (5)
W 2	41 (23)
W 3	133 (7)
W 4	119 (4)
W 5	6 (2)
W 6	8 (4)
W 7	246 (15)
South Island	
D 1	189 (25)
D 2	158 (19)
D 3	452 (39)
D 4	420 (21)
D 5	105 (4)
D 6	144 (6)
D 7	190 (9)
D 8	4 (1)
D 9	4 (1)
D 10	184 (8)

[1]Based on triplicate measurement from three loaves

Table 3 Folic acid content of bread from start to end of shelf-life (best before date)

Bread code no.	Type of bread	Mean (SD) folic acid in bread (µg/100 g edible portion)[1]	
		Sale date	Shelf-life date
NI 1	Brown	198 (12)	180 (15)
SI 1	White	256 (9)	255 (30)

[1] Based on triplicate measurement from four loaves

Table 4 Characteristics of participants in the survey

	All	Wellington	Dunedin	*p* value[1]
Number	288	141	147	
Age[2]	33 (8)	33 (8)	33 (8)	0.635
BMI[2]	27 (6)	26 (6)	27 (6)	0.493
Ethnicity				0.001
NZEO	233 (81%)	101 (73%)	132 (90%)	
Maori	26 (9%)	16 (12%)	10 (7%)	
Pacific	11 (4%)	8 (6%)	3 (2%)	
Asian	16 (6%)	14 (10%)	2 (1%)	
Education				0.154
Less than high school	53 (18%)	22 (16%)	31 (21%)	
High school graduate	37 (13%)	15 (11%)	22 (15%)	
Certificate or diploma	16 (6%)	5 (4%)	11 (8%)	
Some tertiary	39 (14%)	22 (16%)	17 (12%)	
Tertiary	142 (49%)	77 (55%)	65 (45%)	
Gluten-free diet	9 (3%)	5 (4%)	4 (3%)	0.687
Health				0.596
Excellent	89 (31%)	47 (34%)	42 (29%)	
Good	159 (55%)	74 (53%)	85 (58%)	
Fair	38 (13%)	18 (13%)	20 (14%)	
Poor	1 (0%)	1 (1%)	0 (0%)	
Antibiotics[3]	41 (14%)	21 (15%)	20 (14%)	0.754
Household income				0.106
≤ $20 000	69 (24%)	41 (30%)	28 (19%)	
$20 001 to $50 000	66 (23%)	35 (25%)	31 (22%)	
$50 001 to $100000	74 (26%)	30 (22%)	44 (31%)	
>$100000	74 (26%)	33 (24%)	41 (28%)	
User of oral contraceptive agent	75 (26%)	36 (26%)	39 (27%)	0.928
User of prescription medication	92 (32%)	35 (25%)	57 (39%)	0.010
Alcohol drinker	211 (74%)	100 (71%)	111 (76%)	0.455
Drinks per week[2,4]	4 (4)	5 (4)	4 (3)	0.312
Coffee drinker	201 (70%)	97 (69%)	104 (71%)	0.748
Drinks per week[2,4]	14 (12)	13 (11)	15 (12)	0.319
Smoker	34 (12%)	12 (9%)	22 (15%)	0.086
Currently pregnant	10 (3%)	5 (4%)	5 (3%)	0.956
Past pregnancy[5]	171 (60%)	75 (54%)	96 (65%)	0.048
Visited country with mandatory fortification[6]	67 (23%)	38 (27%)	29 (20%)	0.494

Values are number (%) unless otherwise stated

Numbers within a group may not add up to total number of participants due to missing data

[1] *p* values are for the difference between Wellington and Dunedin participants. Simple linear regression was used to test for differences in continuous variables, and chi-squared test for categorical variables

[2] Mean (SD)

[3] Taken in last month

[4] Restricted to consumers

[5] Currently pregnant participants not included

[6] During last year, visited a country with mandatory folic acid fortification

Table 5 Characteristics of participants and non-participants in the survey

	Participants	Non-participants	*p* value[1]
Number	288	725	
Age category			
18-19 y	19 (7%)	36 (8%)	
20-24 y	41 (14%)	83 (19%)	
25-29 y	32 (11%)	86 (20%)	0.001
30-34 y	54 (19%)	80 (18%)	
35-39 y	67 (23%)	78 (18%)	
40-44 y	75 (26%)	74 (17%)	
Maori descent	26 (9%)	56 (13%)	0.115
NZDep2006[2]			
1	54 (19%)	63 (14%)	
2	26 (9%)	58 (13%)	
3	41 (14%)	32 (7%)	
4	31 (11%)	31 (7%)	
5	25 (9%)	31 (7%)	< 0.001
6	33 (12%)	33 (8%)	
7	19 (7%)	39 (9%)	
8	16 (6%)	52 (12%)	
9	22 (8%)	42 (10%)	
10	21 (7%)	56 (13%)	

Values are number (%)

[1] *p* values are for the difference between participants and non-participants, tested using chi-squared test

[2] A New Zealand Deprivation Index 2006 (NZDep2006) score of one represents a geographic area with the least deprivation

Table 6 Prevalence of bread, breakfast cereal, and spread consumption in the previous week

	No. recruited	Bread consumers			Breakfast cereal consumers			Fortified spread consumers
		Fortified[1]	Non-fortified/ unidentified[2]	Any	Fortified[3]	Non-fortified/ unidentified[4]	Any	
All	288	18% (51)	75% (215)	93% (266)	41% (116)	31% (89)	72% (205)	53% (152)
Wellington	141	14% (20)	75% (106)	89% (126)	36% (50)	36% (50)	72% (100)	49% (69)
Dunedin	147	21% (31)	74% (109)	95% (140)	45% (66)	27% (39)	71% (105)	56% (83)

Values are percent of participants recruited (n)

[1]Participants who consumed any folic acid fortified bread. To be included in this category participants reported eating a brand of bread (at least one slice) known to be fortified with folic acid.

[2]Participants who did not consume folic acid fortified bread. Participants in this category reported eating only non-fortified brands of bread, unidentified brands of bread or a combination of both.

[3]Participants who consumed any folic acid fortified breakfast cereal. To be included in this category participants reported eating a brand of breakfast cereal (at least one bowl) known to be fortified with folic acid.

[4]Participants who did not consume folic acid fortified breakfast cereal. Participants in this category reported eating only non-fortified brands of breakfast cereal, unidentified brands of breakfast cereal or a combination of both.

Percentages may not match to total recruited due to missing data

Table 7 Frequency of consumption[1] of all bread and breakfast cereals in the previous week

Place	Bread (slices per week)	*p* value[2]	Breakfast cereal (bowls per week)	*p* value[2]
All	12 (9)		3.4 (2.8)	
Wellington	12 (9)	0.296	3.4 (2.8)	0.737
Dunedin	13 (8)		3.5 (2.9)	

[1]Mean (SD) consumption amongst all participants

[2]*p* values are for the difference between Wellington and Dunedin partipants, tested using simple linear regression

Table 8 Frequency of consumption[1] of all bread and breakfast cereals amongst consumers in the past week

Place	Bread (slices per week)[2]	*p* value[3]	Breakfast cereal (bowls per week)[4]	*p* value[3]
All	13 (8)		4.8 (2.2)	
Wellington	13 (9)	0.636	4.7 (2.1)	0.528
Dunedin	13 (8)		4.9 (2.2)	

[1]Mean (SD) consumption amongst consumers

[2]There were 266 (93%), 126 (91%), and 140 (95%) consumers of bread in All, Wellington and Dunedin, respectively

[3]*p* values are for the difference between Wellington and Dunedin partipants, tested using simple linear regression

[4]There were 205 (72%), 100 (72%), and 105 (71%) consumers of breakfast cereals in All, Wellington and Dunedin, respectively

Table 9 Frequency of consumption[1] of bread and breakfast cereals in the past week amongst consumers of folic acid fortified bread or breakfast cereal

Place	Bread (slices per week)[2]	*p* value[3]	Breakfast cereal (bowls per week)[4]	*p* value[3]
All	13 (8)		4.8 (2.3)	
Wellington	13 (7)	0.887	4.5 (2.3)	0.179
Dunedin	13 (8)		5.0 (2.3)	

[1]Mean (SD) consumption of all bread amongst folic acid fortified product consumers

[2]There were 51 (18%), 20 (14%), and 31 (21%) consumers of any fortified bread in All, Wellington and Dunedin, respectively

[3]*p* values are for the difference between Wellington and Dunedin partipants, tested using simple linear regression

[3]There were 116 (41%), 50 (36%), and 66 (45%) consumers of any fortified breakfast cereals in All, Wellington and Dunedin, respectively

Table 10 Serum and red blood cell folate status of participants

	All	Wellington	Dunedin	Difference[3] (95%CI)	Ratio[4] (95%CI)	Adjusted ratio[5] (95%CI)
Serum folate (nmol/L)						
n	271	129	142			
Mean (SEM)	36 (1)	33 (2)	38 (2)			
Geo mean (95%CI)	30 (28, 32)	27 (25, 30)	32 (29, 35)		1.17 (1.01, 1.35) p = 0.033	1.13 (1.00, 1.29) p = 0.068
Median (IQR)[1]	29 (20, 47)	25 (19, 37)	33 (20, 49)	8 (2, 13) p = 0.004		
< 6.8 nmol/L[2]	1% (0, 2)	1% (0, 2)	1% (0, 2)			
Erythrocyte folate (nmol/L)						
n	271	129	142			
Mean (SEM)	1096 (30)	1034 (44)	1152 (42)			
Geo mean (95%CI)	996 (945, 1049)	935 (866, 1010)	1054 (982, 1131)		1.13 (1.01, 1.25) p = 0.025	1.11 (1.00, 1.22) p = 0.052
Median (IQR)[1]	989 (744, 1316)	897 (675, 1251)	1042 (778, 1381)	157 (37, 276) p = 0.011		
< 317 nmol/L[2]	1% (0, 2)	1% (0, 2)	1% (0, 2)			
≤ 339 nmol/L[2]	1% (0, 2)	1% (0, 2)	1% (0, 2)			
≥ 906 nmol/L[2]	59% (53, 65)	49% (40, 58)	68% (61, 76)			

[1]Interquartile range (IQR), 1st and 3rd quartile

[2]Values are % (95%CI)

[3]Difference between medians of Dunedin and Wellinton

[4]Ratio of the geometric means of Dunedin relative to Wellington

[5]Ratio of the geometric means of Dunedin relative to Wellington adjusted for use of folic acid containing supplements (Y/N), and breakfast cereal consumption (Y/N)

Table 11 Serum and red blood cell folate concentration (nmol/L) according to consumption of folic acid fortified bread or breakfast cereal

	Bread				Breakfast cereal			
Measurement	Fortified[1]	Non-fortified, unidentified, or no bread[1]	Adjusted ratio (95%CI)[2]	*p* value	Fortified[1]	Non-fortified, unidentified, or no bread[1]	Adjusted ratio (95%CI)[3]	*p* value
Serum folate	37 (32, 44)	28 (26, 31)	1.24 (1.05, 1.48)	0.012	33 (29, 36)	28 (25, 31)	1.15 (1.00, 1.31)	0.042
Red blood cell folate	1124 (990, 1276)	969 (906, 1026)	1.12 (0.99, 1.28)	0.079	1079 (991, 1176)	942 (883, 1006)	1.13 (1.02, 1.25)	0.019

[1]Values are geometric mean (95%CI)

[2]Ratio of the geometric means of folic acid fortified relative to non-fortified, unidentified 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). For example, 1.24 can be interpreted as a 24% higher geometric mean serum folate concentration in consumers of fortified bread compared with non-fortified or unknown bread.

[3]Ratio of the geometric means of folic acid fortified relative to non-fortified, unidentified 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 12 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)[1]	*p* value	Adjusted ratio per increment of breakfast cereal (bowl per week)[2]	*p* value
Serum folate	1.005 (0.998, 1.013)	0.176	1.036 (1.012, 1.061)	0.003
Red blood cell folate	1.005 (0.999, 1.012)	0.082	1.031 (1.013, 1.050)	0.001

[1]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.

[2]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.

Table 13 Comparison of 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	Mean difference (95% CI)	Ratio (95%CI)	*p* value
Serum folate (nmol/L)					
Mean (SEM)	27.8 (1.1)	35.6 (1.4)	7.9 (4.3, 11.4)		<0.001
Geo-mean (95%CI)	23 (21, 24)	30 (28, 32)		1.31 (1.19, 1.45)	<0.001
Red blood cell folate (nmol/L)					
Mean (SEM)	794 (21)	1096 (30)	302 (229, 374)		<0.001
Geo-mean (95%CI)	720 (686, 755)	996 (945, 1049)		1.38 (1.29, 1.48)	<0.001
≥ 906 nmol/L (95%CI)	26% (22, 31)	59% (53, 65)	33% (26, 40)		<0.001

Table 14 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
Number	663	288
BMI (kg/m^2)[1]	27 (9)	27 (6)
Age (y)[1]	32 (11)	33 (8)
Age category[2]		
18-19 y	7%	7%
20-24 y	16%	14%
25-29 y	17%	11%
30-34 y	19%	19%
35-39 y	19%	23%
40-44 y	22%	26%
Ethnicity[2]		
NZEO	78%	87%
Maori	16%	9%
Pacific	6%	4%
NZDep2006[3]		
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%

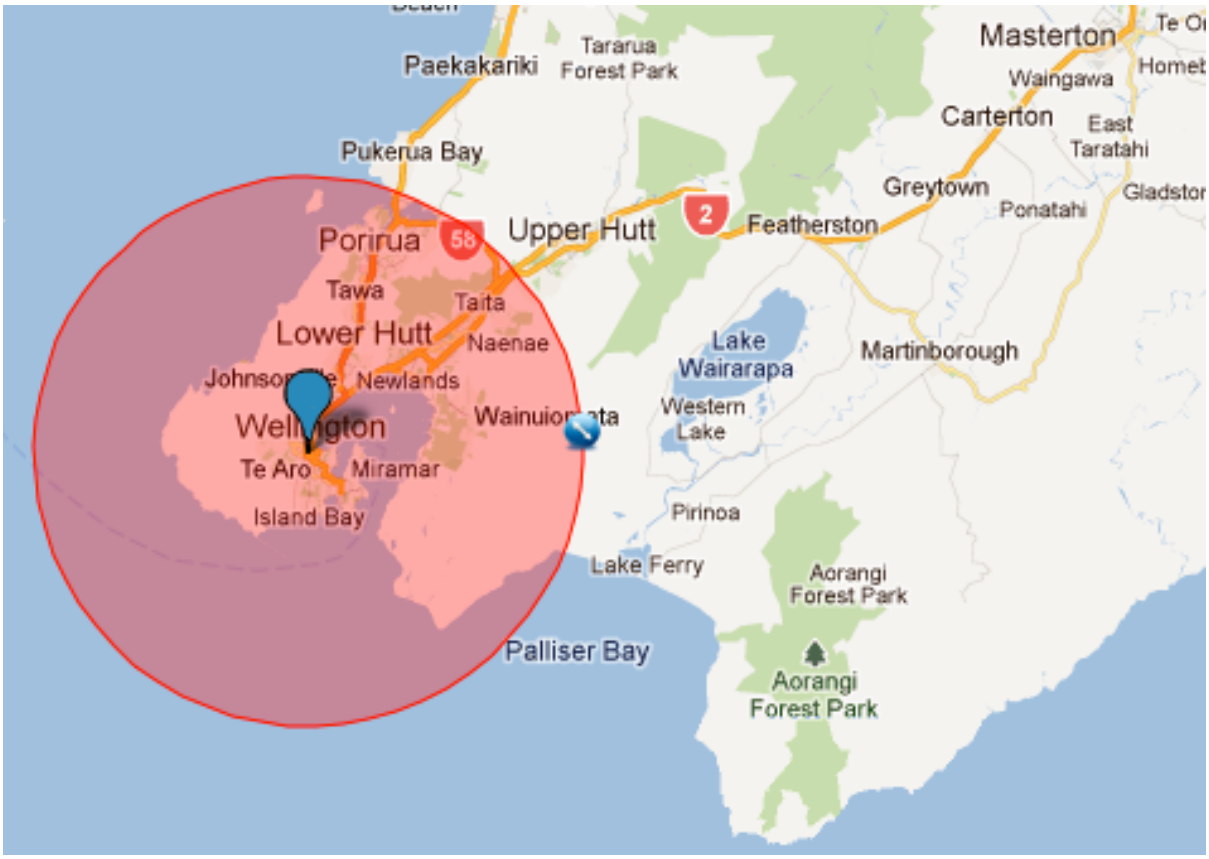
[1]Values are mean (SD)

[2]Values are percent

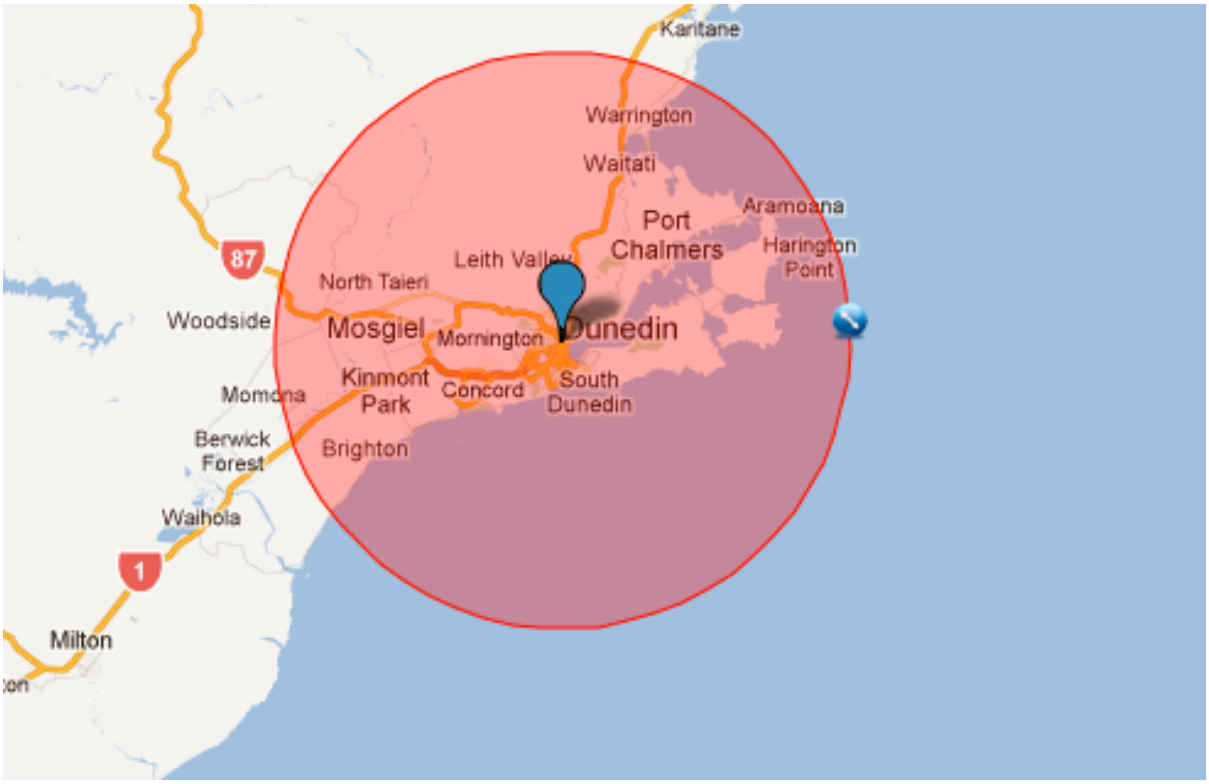
[3]A New Zealand Deprivation Index 2006 (NZDep2006) score of one represents a geographic area with the least deprivation

Appendix A

Map showing 25 km radius from Wellington city centre



Map showing 25 km radius from Dunedin city centre



Appendix B

Folate concentration (nmol/L) for external standard reference material and internal pooled plasma.

		NIST SRM 1955[1]			
		Level I	Level II	Level III	ANS pooled plasma
NIST SRM 1955[1] "information values"	mean ± 2SD (±2 SD range)	5.6 ± 0.7 (4.9 to 6.3)	14 ± 2 (12 to 16)	44 ± 7 (37 to 51)	
2011 Women's Folate and Health Survey	mean ± SD (CV)	7.5 ± 0.2 (2.4) n = 4	15.6 ± 0.5 (3.0) n = 4	48.9 ± 2.1 (4.3) n = 4	19.2 ± 2.4 (12.3) n = 29
2008/09 New Zealand Adult Nutrition Survey	mean ± SD (CV)	6.2 ± 0.6 (9.8) n = 19	14.0 ± 1.8 (12.5) n = 19	47.1 ± 4.2 (8.9) n = 19	19.2 ± 3.2 (16.5) n = 164

[1]National Institute of Standards and Technology Standard Reference Material 1955: NIST SRM 1955. The NIST standard reference material 1955 for folate in human serum is provided as three samples each with a different concentration (i.e. level).