National Diet and Nutrition Survey Rolling Programme (NDNS RP)
Supplementary report: blood folate results for the UK as a whole, Scotland, Northern Ireland (Years 1 to 4 combined) and Wales (Years 2 to 5 combined)

A survey carried out on behalf of Public Health England, the Food Standards Agency in Scotland the Food Standards Agency in Northern Ireland and the Food Standards Agency in Wales
National Diet and Nutrition Survey: Blood folate results for the UK, Scotland, Northern Ireland (Years 1 to 4 combined) and Wales (Years 2 to 5 combined)
About Public Health England

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Notes to text and tables

1. The data used in the report have been weighted. The weighting strategy is described in Appendix B of the Years 1 to 4 (combined) reports for the UK, Scotland and Northern Ireland and the forthcoming Years 2 to 5 (combined) report for Wales. Unweighted sample sizes are shown at the foot of each table.

2. The NDNS RP requires weights to adjust for differences in sample selection and response. The weights adjust for:
   o differential selection probabilities of addresses, households and individuals
   o non-response to the individual questionnaire
   o non-response to the nurse visit
   o non-response to providing a blood sample

3. The data were analysed with the “survey”\textsuperscript{1,2} package in the statistical software R (version 3.0.2)

4. The following conventions have been used in tables:
   non-zero values of less than 0.5% and thus rounded to zero
   [ ] unless stated otherwise data and bases for a variable with a cell size between 30-49 are presented in square brackets.

5. Values for means, medians, percentiles, standard deviations and standard errors are shown to an appropriate number of decimal places. For reasons of space, standard error has been abbreviated to s.e. and standard deviation to sd.

6. The group to whom each table refers is stated at the upper left corner of the table.

7. The term ‘significant’ refers to statistical significance (at the 95% or 99% level) and is not intended to imply substantive importance

\textsuperscript{1} T. Lumley (2014) “survey: analysis of complex survey samples”. R package version 30.2.
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Executive summary

This report presents results for blood folate concentrations for the UK and separately for Scotland, Northern Ireland and Wales based on data collected in Years 1 to 4 of the NDNS Rolling Programme (RP) and Years 2 to 5 for Wales. This is a supplementary report to the UK NDNS RP Years 1 to 4 report published in May 2014.¹

Folate in the diet comes from naturally occurring folates in foods and folic acid from fortified foods such as some breakfast cereals and from dietary supplements.

Results are reported for serum total folate, red blood cell folate and free (unmetabolised) folic acid for the standard NDNS age/sex groups and for women of child-bearing age. Due to small cell sizes, especially in children under 10 years and adults aged 65 years and over, results are not presented for all age/sex groups in Scotland, Northern Ireland and Wales.

Statistical comparisons have been performed for mean serum total and red blood cell folate concentrations between Scotland, Northern Ireland and Wales and the UK as a whole. No sub-group analyses are presented for population folate status in England as the great majority of the UK participants (84%) were resident in England and therefore results for the UK as a whole broadly indicate population folate status in England.

Results are based on 1769 adults (aged 19 years and over) and 902 children in the UK who gave a blood sample, representing 51% of adults and 27% of children who completed a food and drink diary for at least three days. Of this total:

- in Scotland: 440 adults and 216 children aged 4 to 18 years, representing 51% of adults and 27% of children who completed a diary
- in Northern Ireland: 264 adults and 96 children aged 11 to 18 years, representing 56% of adults and 41% of children who completed a diary
- in Wales: 228 adults and 60 children aged 11 to 18 years, representing 49% of adults and 34% of children aged 11 to 18 years who completed a diary

Serum total and red blood cell folate were measured using research methods selected as providing the most accurate quantitation possible, as determined by an international expert review and workshop in 2008 on methods for assessing folate status.² Laboratory analyses were provided by the Centres for Disease Control and Prevention (CDC), Atlanta, Georgia, US. Serum total folate was measured by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS), a state of the art method which captures the individual forms of folate including free (unmetabolised) folic acid. Whole blood folate was measured by the long-established microbiological assay method. Both methods are the same as those currently used for the US National Health
and Nutrition Examination Survey (NHANES). These assays do not give the same results as the clinical assays used to measure folate status in previous NDNS. Therefore, comparisons of blood folate concentrations between the RP and previous NDNS cannot be made.

Results are compared with the World Health Organization (WHO) thresholds for serum total folate and red blood cell folate indicating risk of biochemical folate deficiency (see section 1.5 for more details).

**Serum total folate**

In the UK as a whole, 16.9% of boys aged 11 to 18 years and 21.8% of girls aged 11 to 18 years had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L). Less than 4% of children under 11 years of age had a serum total folate concentration below the threshold. The proportion of adults who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L) was 15.5% for men aged 19 to 64 years, 13.9% for women aged 19 to 64 years, 8.5% for men aged 65 years and over and 12.4% for women aged 65 years and over.

For women of childbearing age, 22.1% of those aged 16 to 24 years, 17.7% of those aged 25 to 34 years and 13.1% of those aged 35 to 49 years had levels below the threshold.

In Scotland and Northern Ireland mean serum total folate levels were significantly lower than in the UK as a whole for adults aged 19 to 64 years (both for men/women combined and separately for women) and for women of childbearing age (16 to 49 years). In women of childbearing age, 24.4% in Scotland and 30.6% in Northern Ireland had levels below the WHO threshold; these are higher proportions than in the same group in the UK as a whole (16.5%).

In Wales, mean serum total folate for adults aged 65 years and over was significantly lower than for the UK as a whole, though the proportion below the WHO threshold was similar in Wales and the UK as a whole. There was no significant difference in mean serum total folate between Wales and the UK as a whole for women of childbearing age.

**Red blood cell folate**

In the UK as a whole, 9.3% of boys and 19.7% of girls aged 11 to 18 years had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L). Less than 4% of children under 11 years of age had a red blood cell folate concentration below the threshold.
The proportion of adults in the UK as a whole who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L) was 6.8% for men aged 19 to 64 years, 8.6% for women aged 19 to 64 years, 7.3% for men aged 65 years and over and 10.8% for women aged 65 years and over.

For women of childbearing age in the UK as a whole, mean red blood cell folate was significantly lower amongst those aged 16 to 24 years than those aged 35 to 49 years. The proportion of women of childbearing age (16 to 49 years) with a red blood cell folate concentration below the WHO threshold was 11.3% with the highest proportion being those aged 16 to 24 years (15.6%).

In Scotland, mean red blood cell folate levels were significantly lower than in the UK as a whole for women aged 19 to 64 years and adults aged 65 years and over. In Scotland 13.9% of women aged 19 to 64 years and 16.5% of adults aged 65 years and over were below the WHO threshold; these proportions are higher than in the UK as a whole (8.6% and 9.3% respectively). For women of childbearing age the proportion in Scotland with a red blood cell folate concentration below the WHO threshold was 14.8%.

In Northern Ireland, mean red blood cell folate levels were significantly lower than in the UK as a whole for women aged 19 to 64 years, adults aged 19 to 64 years and women of childbearing age. For women of childbearing age (16 to 49 years) the proportion with a red blood cell folate concentration below the WHO threshold was 20.2% in Northern Ireland; higher than in the UK as a whole (11.3%).

There were no significant differences in mean red blood cell folate levels between Wales and the UK as a whole for any age group. For women of childbearing age in Wales the proportion with a red blood cell folate concentration below the WHO threshold was 10.3%, similar to that for the UK as a whole.

**Free folic acid**

Descriptive statistics for free folic acid concentrations for each age/sex group are presented in tables 1 to 4 (see chapter 2 for more details).

**Comparisons between NDNS and NHANES folate concentrations**

Mean serum total and red blood cell folate concentrations in the US (2005-2010; post-fortification) as assessed in NHANES are approximately twice the concentrations in the UK. This is likely to be due to mandatory fortification of bread flour with folic acid in the US (see section 3.3 for more details).


4 Cross-over comparison studies have not been conducted for the LC-MS/MS assay used for the current RP and those assays used previously to measure folate in the past NDNS. Direct comparisons cannot therefore be made between these current and past NDNS datasets. CDC have conducted comparison studies for different assays used in NHANES for folate measurement to enable comparison over time. NDNS RP methods are the same used in NHANES currently. The results of NDNS RP therefore can be compared with NHANES folate data pre- and post- fortification (see section 3.3).


Chapter 1. Introduction

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

1.1 Background

This supplementary report presents the results for serum total folate, red blood cell folate and free (unmetabolised) folic acid in blood samples taken for the NDNS Rolling Programme (RP) during the nurse visit for the following participants:

- those aged 1.5 years and over in the UK as a whole
- those aged 4 years and over in Scotland
- those aged 11 years and over in Northern Ireland and Wales

The blood samples included in this report were collected in England, Scotland and Northern Ireland between February 2008 and July 2012 (Years 1 to 4 of the NDNS RP), and in Wales between July 2009 and July 2013 (Years 2 to 5 of the NDNS RP).

Serum total and red blood cell folate were measured by research methods which were selected as providing the most accurate quantitation currently possible following an international expert review and workshop commissioned by the Food Standards Agency in 2008 on methods for assessing folate status. The Expert Workshop recommended that liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) should be used as the method of choice for the NDNS RP, with the microbiological method being an acceptable alternative for red blood cell folate. The LC-MS/MS method used to measure serum total folate in the RP is a state of the art method, developed at the Centers for Disease Control and Prevention (CDC), US and used since 2007 for the US National Health and Nutrition Examination Survey (NHANES). LC-MS/MS captures the individual forms of folate in serum including free (unmetabolised) folic acid; serum total folate represents the sum of all forms including free folic acid. LC-MS/MS is not currently used for whole blood folate in NHANES because there are unresolved differences between LC-MS/MS and the microbiological assay. Thus the microbiological assay, long established as the "gold standard" for folate measurement is used in NDNS RP for measurement of whole blood folate.

Methods used for folate measurement in previous NDNS and the current RP are tabulated in Table A. The methods used for the measurement of folate in the RP do not give the same results as the clinical assay methods used for previous NDNS because there are substantial differences between the analytical methods, and therefore results presented in this report cannot be compared with previous results. Comparisons are, however, made between the RP results for Scotland, Wales and Northern Ireland and the UK as a whole.
Table A. Methods used to measure blood folate in NDNS RP and the previous NDNS

<table>
<thead>
<tr>
<th>Survey</th>
<th>Serum total/plasma folate</th>
<th>Red blood cell folate</th>
<th>Free folic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previous NDNS of children aged 1.5-4.5 years (1992/93)</td>
<td>Biorad Quantaphase I (radio-protein-binding method)</td>
<td>Biorad Quantaphase I (radio-protein-binding method)</td>
<td>Not measured separately</td>
</tr>
<tr>
<td>Previous NDNS of adults aged 65 years and over (1994/95)</td>
<td>Biorad Quantaphase II (radio-protein-binding method)</td>
<td>Biorad Quantaphase II (radio-protein-binding method)</td>
<td>Not measured separately</td>
</tr>
<tr>
<td>Previous NDNS of children aged 4-18 years (1997)</td>
<td>Abbott IMx (microparticle enzyme immunoassay)</td>
<td>Abbott IMx (microparticle enzyme immunoassay)</td>
<td>Not measured separately</td>
</tr>
<tr>
<td>Previous NDNS of adults aged 19-64 years (2000/01)</td>
<td>Abbott (microparticle enzyme immunoassay)</td>
<td>Abbott IMx (microparticle enzyme immunoassay)</td>
<td>Not measured separately</td>
</tr>
<tr>
<td>NDNS RP Years 1-4; UK, Scotland and Northern Ireland</td>
<td>LC-MS/MS</td>
<td>Microbiological assay</td>
<td>LC-MS/MS</td>
</tr>
<tr>
<td>NDNS RP Years 2-5; Wales</td>
<td>LC-MS/MS</td>
<td>Microbiological assay</td>
<td>LC-MS/MS</td>
</tr>
</tbody>
</table>

Dietary intake of folate is presented in the main NDNS RP UK\textsuperscript{8} and devolved country reports\textsuperscript{9,10,11} based on assessment of food consumption over four days. Analysis of blood samples provides an indication of the folate status of the population usually over a longer period; that is, the level of nutrients available to the body (after absorption) for use in metabolic processes.

Red blood cell folate is usually a better measure of long-term status than plasma or serum total folate because it reflects body stores at the time of red blood cell synthesis and is indicative of folate status over the 120-day lifespan of the red blood cells, whereas serum total folate concentrations respond rapidly to change in dietary intake.

Comprehensive results for overall dietary intake and the range of other nutritional status measures obtained from blood and urine samples collected in NDNS RP are presented in the main UK Years 1 to 4 combined report\textsuperscript{8} and respective devolved country reports,\textsuperscript{9,10,11} along with other survey results. Appendix W of each report indicates which results are presented in these reports and which results are included in the datasets on the UK Data Archive.\textsuperscript{12}
1.2 Survey methods

An overview of the purpose, documents, methodologies, participant consent and procedures for quality control are provided in the main reports. Each report also contains technical appendices which detail procedures associated with obtaining, transporting and processing blood samples from participants, along with priority order for analytes and overall response rates achieved (appendices N to Q).

Blood samples were requested from all fully productive participants aged 1.5 years and over who were visited by a nurse. Where participant consent was obtained, fasted blood samples were collected by venepuncture for those aged four years and over. Participants with diabetes who were not willing or not able to fast and those aged 1.5 to three years were invited to provide a non-fasting blood sample.
1.3 Blood sampling response

The numbers and proportion of participants providing a blood sample is shown in Table B below.

### Table B. Number and proportion of blood samples collected for age groups included in this report

<table>
<thead>
<tr>
<th></th>
<th>UK as a whole¹ (Years 1-4)</th>
<th>Scotland (Years 1-4)</th>
<th>Northern Ireland (Years 1-4)</th>
<th>Wales (Years 2-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adults (aged 19+ years)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number providing a blood sample</td>
<td>1,769</td>
<td>440</td>
<td>264</td>
<td>228</td>
</tr>
<tr>
<td>% of fully productive participants providing a blood sample</td>
<td>51</td>
<td>51</td>
<td>56</td>
<td>49</td>
</tr>
<tr>
<td><strong>Children (aged 1.5-18 years)²</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number providing a blood sample</td>
<td>902</td>
<td>216</td>
<td>96</td>
<td>60</td>
</tr>
<tr>
<td>% of fully productive participants providing a blood sample</td>
<td>27</td>
<td>27</td>
<td>41</td>
<td>34</td>
</tr>
</tbody>
</table>

¹These are total numbers for Years 1-4 core and boost UK participants and include the numbers for Scotland, Northern Ireland and Wales. Additional recruitment was undertaken in Scotland, Northern Ireland and Wales in order to achieve large enough samples in these countries to enable cross-country comparisons to be made. Boosted samples in Scotland and Northern Ireland were included from Year 1. A boosted sample in Wales was included from Year 2 (starting April 2009).

²Blood samples were requested from participants aged 1.5 years and over. However, due to the small numbers of children aged 1.5 to 3 years and 4 to 10 years, who provided a blood sample, folate results have only been reported for those aged four years and over in Scotland and 11 years and over in Northern Ireland and Wales.

This report presents laboratory results of serum and whole blood analysis, and where appropriate statistical analysis of these data for children aged 1.5 years and over in the UK as a whole (902), children aged four years and over in Scotland and children aged 11 years and over in Northern Ireland and Wales (216, 96 and 60 children respectively). Due to the small number of children under 11 years who provided a blood sample, results are not presented for children under four years in Scotland nor children aged under 11 years in Northern Ireland and Wales. Further details are provided in chapter 2 and appendix O of the main reports. ⁸,⁹,¹⁰,¹¹

1.4 Analysis of the blood samples and results data

Details of analytical methods as performed by CDC are given in appendix 1 of this supplementary report. Serum total folate concentrations were measured by LC-MS/MS which quantifies each folate form separately; serum total folate concentration is the sum of all folate forms, including free (unmetabolised) folic acid. Whole blood folate was measured by an automated microbiological assay using *Lactobacillus rhamnosus*. Red blood cell folate was calculated from whole blood folate concentration, serum total folate concentration and haematocrit.
The number of serum and red blood cell folate results within each age group is not necessarily the same because there was not always sufficient blood available to do both analyses (see appendices N and O in the main reports for more detail).\textsuperscript{8,9,10,11} Blood analyte data were weighted to account for differential non-response to providing a blood sample, in order to adjust for any bias arising from blood sampling refusals and/or failures. Details of the methodology used to weight the data are provided in chapter 2 and appendix B of the main reports.\textsuperscript{8,9,10,11} Results are presented where cell sizes are sufficient (30 or greater) for the age groups 1.5 to three years, four to ten years, 11 to 18 years, 19 to 64 years and 65 years and over and are further split by sex for those aged four years and over. The percentages below the World Health Organization (WHO) thresholds\textsuperscript{14} indicating risk of biochemical folate deficiency for the general population have been included in tables 1 to 4.

It should be noted that three adults with unusually high blood folate levels have been excluded from the main tables as they caused the estimates of mean and standard deviation (sd) to be inflated. All three individuals had taken a 5mg/day folic acid supplement daily during the dietary recording period which was approximately eight weeks before blood samples were taken. Further details regarding the characteristics of these outliers and the descriptive statistics for all of the data including these three outliers are provided in section 2.4 and appendix 3 respectively. No other folic acid supplement users have been excluded from the dataset.

All the statistical analyses including descriptive statistics took into account the complex survey design. Statistical comparisons of means have been made where cell sizes are sufficient (50 or greater) between the devolved countries (Scotland, Northern Ireland and Wales) and the UK sample as a whole for standard NDNS age groups (4 to 10 years, 11 to 18 years, 19 to 64 years and 65 years and over) split by sex and for women of childbearing age (16 to 49 years). Statistical comparisons of means have also been made between the three sub-age groups of women of childbearing age; 16 to 24 years, 25 to 34 years and 35 to 49 years for the UK as a whole. The current NDNS RP data have not been compared against previous NDNS data because the analytical methods used are different; crossover studies to facilitate comparisons were not possible because of the time elapsed between the end of previous NDNS and the start of the RP. Details of the methods used for the mean comparisons of the UK data\textsuperscript{8} with Scotland, Northern Ireland or Wales data are provided in Appendix Y of the corresponding country report.\textsuperscript{9,10,11}

1.5 Folate function, dietary sources, recommendations and thresholds of folate concentrations indicating biochemical folate deficiencies

Folate is required for methylation (1-carbon transfer) which is essential for synthesis of DNA, and therefore for cell proliferation. Shortage of folate compromises the formation and maturation of red blood cells in the bone marrow and leads to anaemia. Folates are
found in a wide variety of foods. Rich food sources of folate include liver, yeast extract and green leafy vegetables such as spinach, kale and brussel sprouts. Folic acid (a synthetic form of folate) is used as a food fortificant (eg, in breakfast cereals) and in dietary supplements.

Low folate status of women of childbearing age (16 to 49 years) is a particular public health concern. Increased folic acid intake through supplementation has been shown to reduce the risk of neural tube defects if taken in the periconceptional period. The Committee on Medical Aspects of Food and Nutrition Policy (COMA) reviewed this evidence and concluded that folic acid supplementation reduces the risk of embryonic neural tube defects. Women planning pregnancy are therefore advised to take a 400µg folic acid supplement daily until the 12th week of pregnancy. This advice is government policy and was endorsed by the Scientific Advisory Committee on Nutrition (SACN) who also considered issues of fortification in relation to high and low folate intakes.

The Reference Nutrient Intakes (RNI) and Lower Reference Nutrient Intakes (LRNI) for folate are presented in table C.
Table C. Reference Nutrient Intake (RNI) and Lower Reference Nutrient Intakes (LRNI) for folate

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>RNI for folate (µg/d)</th>
<th>LRNI for folate (µg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5-3 years</td>
<td>70</td>
<td>35</td>
</tr>
<tr>
<td>4-6 years</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>7-10 years</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>11-18 years</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>19-64 years</td>
<td>200</td>
<td>100</td>
</tr>
<tr>
<td>65 years and over</td>
<td>200</td>
<td>100</td>
</tr>
</tbody>
</table>

A WHO consultation in 2008\(^1\) arrived at a consensus on the cut-offs that should be used at this time for assessing the nutritional status of populations for folate.\(^2\) These differ from and supersede the thresholds used in previous NDNS.

The WHO consensus (2008)\(^3\) concentrations indicating biochemical folate deficiencies, based on metabolic indicators (e.g. raised plasma homocysteine concentration), are:

- less than 10nmol/L (4ng/mL)\(^4\) for serum total folate or
- less than 340nmol/L (151ng/mL) for red blood cell folate

These thresholds were established using the microbiological method and therefore the threshold for red blood cell folate is directly applicable to this report. In NDNS RP serum total folate concentrations are measured by LC-MS/MS which are in close agreement to the microbiological serum total method. The threshold for serum total folate is therefore also applicable to the LC-MS/MS folate data reported here.

There are no established thresholds for free folic acid concentration.

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1. Blood samples were requested from participants aged 1.5 years and over. However, due to small cell sizes for those aged 1.5 to 3 years and 4 to 10 years, folate results have only been reported for those aged four years and over in Scotland and 11 years and over in Northern Ireland and Wales.
11 The NDNS RP report for Wales (Years 2 to 5 combined) is due for publication during 2015. http://data-archive.ac.uk/ (accessed 07/02/15).
12 Participants were classed as “fully productive” if they completed three or four days of the food and drink diary.
18 Thresholds are derived from the US NHANES III (National Health and Nutrition Examination Survey), based on the plasma vitamin concentrations below which plasma metabolites become elevated (total homocysteine concentration for folate). In addition, these cutoffs are consistent with data in the Institute of Medicine report on recommended intakes of folate, in which blood vitamin concentrations were used to determine Estimated Average Requirements (EAR).
19 Conversion factor used for folic acid: 2.265 (1\text{ng/mL} = 2.265\text{nmol/L}).
Chapter 2. Determination of folate status

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

2.1 Introduction

Folate status is monitored by the measurement of folates in serum and in red blood cells. Red blood cell folate is usually a better measure of long-term status than plasma or serum total folate because it reflects body stores at the time of red blood cell synthesis and is indicative of average folate status over the 120-day lifespan of the red blood cells, whereas serum total folate concentrations respond rapidly to change in dietary intake. The assay for serum total folate can differentiate between the various folate forms; it gives additional information on the concentration of folic acid, taken as supplements or present in fortified foods such as breakfast cereals, prior to its reduction and incorporation into the folate pool, as well the concentrations of circulating folate forms in various states of methylation. In this report, we include the serum total concentration of unmetabolised (free) folic acid and the total concentration of all folate forms, which includes free folic acid.

2.2 Blood sampling

Blood samples were collected as described in appendix O of the main reports. An EDTA and a serum gel monovette tube from each participant’s sample set were sent by post, to the Immunology and Biochemistry Laboratory at Addenbrooke’s Hospital in Cambridge (Addenbrooke’s) for prompt analysis. At the laboratory 100µL of whole blood was transferred to a freshly-thawed tube containing 1.0mL of 1% ascorbic acid, which acts as a preservative for folate. After mixing, the aliquot was frozen at -80°C and stored at this temperature pending analysis. Serum was prepared as detailed in appendix O of the main reports and was stored frozen at -80°C pending analysis.

2.3 Analytical methods

Serum total folate, whole blood folate and serum free folic acid concentrations were measured in NDNS RP samples at CDC, US (see section 2.3 and appendix 1). Haematocrit was measured at Addenbrooke’s using a Coulter Counter-based assay.

Serum total folate was measured by LC-MS/MS at CDC. This method is regarded internationally as the “gold standard” for serum total folate. It measures each bioactive folate form separately; these concentrations can be summed to derive the total concentration of all folate forms in a sample. This report includes serum total folate and free folic acid concentrations. Concentrations of individual folate forms are not reported here. Details of the method are given in appendix 1 of this report.
Whole blood folate concentration was measured by a long-established microbiological method regarded as “gold standard” for whole blood folate. Red blood cell folate was calculated from whole blood folate, serum total folate and haematocrit (Hct). Details of the method are given in appendix 1 of this report.

These assays do not give the same results as the Biorad Quantaphase and Abbott IMx assays which were used for previous NDNS; therefore comparisons of concentrations between the RP and previous NDNS should not be made.

2.4 Outliers

Three individuals had plausible but unusually high blood folate concentrations, these were:

- a female in Scotland in the 65 years and over age group who reported taking a 5mg folic acid supplement and 200μg folic acid from a multivitamin/mineral tablet on each of the four diary days in addition to an average daily intake of 199μg folate from her diet
- a female in Wales aged 19 to 64 years who reported taking a 5mg folic acid supplement on each of the four diary days, taken in addition to an average daily intake of 123μg folate from her diet
- a male in Wales aged 19 to 64 years who reported taking a 5mg folic acid supplement on each of the four diary days, in addition to an average daily intake of 187μg folate from his diet

The statistical analyses were conducted with and without these folate concentrations and this revealed that they were highly influential on the mean and standard deviation (sd) estimation of the subgroups which contained them, causing the estimates to be inflated. To ensure a robust interpretation, only the results of the statistical analyses excluding these observations are used in tables 1 to 4 and descriptive statistics of the full sample are presented in appendix 3 (tables G.1 to G.4). With the exception of these three individuals, all other supplement takers are included in the dataset.

2.5 Free (unmetabolised) folic acid

Free (unmetabolised) folic acid is measured as one of the components of serum total folate as assayed by LC-MS/MS. It is present in serum only as a result of ingestion of folic acid added to foods or taken as a supplement. The long-term biological effects of exposure to free folic acid in humans are unknown (SACN 2006).5

Free folic acid results are presented in tables 1 to 2.3 for the standard NDNS age/sex groups for the UK as a whole and split by country and are also presented in tables 3 and 4 for women of childbearing age (16 to 49 years) for the UK as a whole and split by country.
A proportion of participants had free folic acid concentrations below the limit of detection (LoD), therefore these participants were assigned a notional value calculated by dividing the LoD by the square root of 2. This approach is consistent with that used for NHANES\(^6\) and has been described by Hornung and Reed (1990).\(^7\) The proportion of participants with a notional value for free folic acid is provided in table F of appendix 2 of this report.

There are no established thresholds for free folic acid and currently no other published population data.

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4. The NDNS RP report for Wales (Years 2 to 5 combined) is due for publication during 2015.
Chapter 3. Blood folate concentrations for the UK as a whole

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

It should be noted that the cell size for children aged 1.5 to three years is small (less than 50), therefore results for this age group should be interpreted with caution.

3.1 Serum total folate

The mean serum total folate concentration for boys and girls aged 1.5 to three years was 39.6nmol/L. The mean serum total folate concentration for boys aged four to 10 years was 31.0nmol/L and 27.7nmol/L for girls aged 4 to 10 years, 18.5nmol/L for boys aged 11 to 18 years and 17.1nmol/L for girls aged 11 to 18 years.

The mean serum total folate concentration for men aged 19 to 64 years was 18.1nmol/L, 21.4nmol/L for women aged 19 to 64 years, 22.7nmol/L for men aged 65 years and over and 27.6nmol/L for women aged 65 years and over.

In children, 2.6% of those aged four to 10 years, 16.9% of boys aged 11 to 18 years and 21.8% of girls aged 11 to 18 years had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L).\(^1\)

The proportion of adults who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L)\(^1\) was 15.5% for men aged 19 to 64 years, 13.9% for women aged 19 to 64 years, 8.5% for men aged 65 years and over and 12.4% for women aged 65 years and over.

3.2 Red blood cell folate

The mean red blood cell folate concentration for children aged 1.5 to three years was 793nmol/L. The mean red blood cell folate concentration for children aged four to ten years was 727nmol/L for boys and 687nmol/L for girls. The mean red blood cell folate concentration for children aged 11 to 18 years was 583nmol/L for boys and 499nmol/L for girls.

The mean red blood cell folate concentration for men aged 19 to 64 years was 621nmol/L, 652nmol/L for women aged 19 to 64 years, 729nmol/L for men aged 65 years and over and 787nmol/L for women aged 65 years and over.
In the four to ten years age group, 1.6% of boys and 5.9% of girls had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L). The same was true for 9.3% of boys aged 11 to 18 years and 19.7% of girls aged 11 to 18 years.

The proportion of adults who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L) was 6.8% for men aged 19 to 64 years, 8.6% for women aged 19 to 64 years, 7.3% for men aged 65 years and over and 10.8% for women aged 65 years and over.

(Table 1)

3.3 Comparison between NDNS and NHANES folate concentrations

The methods used in NDNS RP for measurement of whole blood and red blood cell folate are the same as those currently used for the US National Health and Nutrition Examination Survey (NHANES), performed by the same laboratory, CDC. Within NHANES there have been a number of methodological changes over time and CDC have performed cross-over comparison studies to enable combination and comparison of NHANES folate data across the data series, and pre- and post- fortification in the US. As methods in NDNS RP are the same as in NHANES currently, it is possible to compare between current NDNS RP data and the NHANES data series, pre- and post-fortification.

The comparison shows that median serum total folate and red blood cell folate concentrations in the UK are about half those in NHANES 2005-2010 (after the introduction of folic acid fortification in the US but are similar to concentrations found in NHANES 1998-1994 (before the introduction of fortification).

\[\text{\textsuperscript{1}}\text{ WHO. Conclusions of a WHO technical consultation on folate and vitamin B}\text{\textsubscript{12}}\text{ deficiencies. Food and Nutrition Bulletin. 2008; 29. S238–S244.}\]
\[\text{\textsuperscript{2}}\text{http://www.cdc.gov/nchs/nhanes.htm (accessed 15/01/15).}\]
\[\text{\textsuperscript{3}}\text{Cross-over comparison studies have not been conducted for the LC-MS/MS assay used for the current RP and those assays used previously to measure folate in the past NDNS. Direct comparisons cannot therefore be made between these current and past NDNS datasets. CDC have conducted comparison studies for different assays used in NHANES for folate measurement to enable comparison over time. NDNS RP methods are the same used in NHANES currently. The results of NDNS RP therefore can be compared with NHANES folate data pre- and post-fortification.}\]
Chapter 4. Blood folate concentrations for Scotland, Northern Ireland and Wales and comparisons with results for the UK as a whole

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

4.1 Statistical analysis

Statistical comparison of means has been carried out for this chapter to compare the mean UK serum total folate and red blood cell folate results to the equivalent mean Scotland, Northern Ireland and Wales results.¹ The UK serum total folate and red blood cell folate results have been used as the reference group (refer to appendix Y of the relevant NDNS RP reports²,³,⁴,⁵ for a more detailed explanation of the statistical analysis). All statistically significant differences between the UK mean results (reference group) and the equivalent mean Scotland, Northern Ireland or Wales folate results are highlighted in the tables. No statistical comparisons have been carried out for an age group that has less than 50 individuals. Numbers are low in some age/sex groups in Scotland, Northern Ireland and Wales, particularly the 65 years and over group, therefore caution should be exercised when interpreting findings.

4.2 Scotland

4.2.1 Serum total folate

The mean serum total folate concentration for children aged four to ten years in Scotland was 31.3nmol/L, 18.2nmol/L for boys aged 11 to 18 years and 15.9nmol/L for girls aged 11 to 18 years.

The mean serum total folate concentration in Scotland for men aged 19 to 64 years was 17.0nmol/L, 18.6nmol/L for women aged 19 to 64 years, 20.0nmol/L for men aged 65 years and over and 24.8nmol/L for women aged 65 years and over.

In the 11 to 18 years age group, 23.0% of boys and 23.6% of girls in Scotland had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L).⁶ There were very few cases below the threshold for children aged four to ten years (0.5%).
The proportion of adults who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L) was 16.0% for men aged 19 to 64 years, 21.7% for women aged 19 to 64 years, 13.5% for men aged 65 years and over and 17.7% for women aged 65 years and over.

For adults aged 19 to 64 years mean serum total folate concentration was significantly lower in Scotland than in the UK as a whole for women (18.6nmol/L compared to 21.4nmol/L respectively) and for men and women combined (17.8nmol/L compared to 19.8nmol/L respectively). Mean concentrations in Scotland were also slightly lower than the UK in other age groups, except for children aged four to ten years, but the differences were not statistically significant.

With the exception of children aged four to ten years, the proportion of participants with a serum total folate concentration below the threshold of 10nmol/L was greater in Scotland than in the UK as a whole for all age/sex groups. These differences were not statistically tested. (Table 2.1)

4.2.2 Red blood cell folate

The mean red blood cell folate concentration for children in Scotland aged four to ten years was 706nmol/L, 607nmol/L for boys aged 11 to 18 years and 498nmol/L for girls aged 11 to 18 years.

The mean red blood cell folate concentration for men aged 19 to 64 years was 648nmol/L, 588nmol/L for women aged 19 to 64 years, 651nmol/L for men aged 65 years and over and 697nmol/L for women aged 65 years and over.

For children in Scotland, 2.0% of children aged four to ten years, 7.5% of boys aged 11 to 18 years and 23.3% of girls aged 11 to 18 years had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L).

The proportion of adults in Scotland who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L) was 6.0% for men aged 19 to 64 years, 13.9% for women aged 19 to 64 years, 11.6% for men aged 65 years and over and 20.1% for women aged 65 years and over.

For women aged 19 to 64 years mean red blood cell folate concentration was significantly lower in Scotland than in the UK as a whole (588nmol/L compared to 652nmol/L respectively). For adults aged 65 years and over mean red blood cell folate concentration was also significantly lower in Scotland than in the UK as a whole.
(677nmol/L compared to 762nmol/L respectively). There were no statistically significant differences for other age/sex groups.

For girls aged 11 to 18 years, women aged 19 to 64 years and women aged 65 years and over, the proportion of participants with a red blood cell folate concentration below the threshold of 340nmol/L was greater in Scotland than in the UK as a whole.\(^7\) (Table 2.1)

### 4.3 Northern Ireland

#### 4.3.1 Serum total folate

In Northern Ireland the mean serum total folate concentration was 18.1nmol/L for boys aged 11 to 18 years and 16.1nmol/L for girls aged 11 to 18 years. The mean serum total folate concentration for men aged 19 to 64 years was 17.8nmol/L, 17.9nmol/L for women aged 19 to 64 years and 26.2nmol/L for adults aged 65 years and over.

In the 11 to 18 years age group, 17.4% of boys and 19.6% of girls had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L).\(^6\)

The proportion of adults who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L)\(^6\) was 15.7% for men aged 19 to 64 years, 23.0% for women aged 19 to 64 years and 7.5% for adults aged 65 years and over.

For women aged 19 to 64 years mean serum total folate concentration was significantly lower in Northern Ireland than in the UK as a whole (17.9nmol/L compared to 21.4nmol/L respectively). Mean serum total folate concentration was also significantly lower in Northern Ireland than in the UK as a whole for adults aged 19 to 64 years (17.9nmol/L compared to 19.8nmol/L respectively). There were no statistically significant differences in other age groups.

For women aged 19 to 64 years, the proportion of participants with a serum total folate concentration below the threshold of 10nmol/L was greater in Northern Ireland (23%) than in the UK as a whole (13.9%).\(^7\) (Table 2.2)

#### 4.3.2 Red blood cell folate

The mean red blood cell folate concentration in Northern Ireland was 553nmol/L for boys aged 11 to 18 years and 457nmol/L for girls aged 11 to 18 years.
The mean red blood cell folate concentration for men aged 19 to 64 years was 613nmol/L, 565nmol/L for women aged 19 to 64 years and 776nmol/L for adults aged 65 years and over.

In the 11 to 18 years age group, 14.7% of boys and 25.4% of girls had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L).\(^6\)

The proportion of adults in Northern Ireland who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L)\(^6\) was 7.7% for men aged 19 to 64 years, 14.8% for women aged 19 to 64 years and 2.0% for those aged 65 years and over.

For women aged 19 to 64 years mean red blood cell folate concentration was significantly lower in Northern Ireland than in the UK as a whole (565nmol/L compared to 652nmol/L respectively). Mean red blood cell folate concentration was also significantly lower in Northern Ireland than in the UK as a whole for adults aged 19 to 64 years (593nmol/L compared to 637nmol/L respectively). There were no significant differences in other age groups.

The proportion of participants with a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L)\(^6\) was higher in Northern Ireland than in the UK as a whole for women aged 19 to 64 years and children aged 11 to 18 years.\(^7\) (Table 2.2)

4.4 Wales

4.4.1 Serum total folate

The mean serum total folate concentration was 17.3nmol/L for children aged 11 to 18 years in Wales.

The mean serum total folate concentration for men aged 19 to 64 years was 17.6nmol/L, 20.9nmol/L for women aged 19 to 64 years and 18.8nmol/L for adults aged 65 years and over.

In the 11 to 18 years age group, 20.3% in Wales had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L).\(^6\)

The proportion of adults in Wales who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L)\(^6\) was 14.4% for men aged 19 to 64 years, 15.9% for women aged 19 to 64 years and 12.4% for those aged 65 years and over.
For adults aged 65 years and over mean serum total folate concentration was significantly lower in Wales than in the UK as a whole (18.8nmol/L compared to 25.6nmol/L respectively). There were no significant differences for other age/sex groups although mean intakes tended to be slightly lower in Wales.

For women aged 19 to 64 years and adults aged 65 years and over, the proportion of participants with a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L) was slightly greater in Wales than in the UK as a whole.\(^7\) (Table 2.3)

### 4.4.2 Red blood cell folate

The mean red blood cell folate concentration was 563nmol/L for children aged 11 to 18 years in Wales.

The mean red blood cell folate concentration for men aged 19 to 64 years was 679nmol/L, 647nmol/L for women aged 19 to 64 years and 598nmol/L for adults aged 65 years and over.

In the 11 to 18 years age group, 7.6% had a red blood cell folate concentration below the lower threshold of the normal range.\(^6\)

The proportion of adults in Wales who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L) was 1.2% for men aged 19 to 64 years, 8.3% for women aged 19 to 64 years and 9.8% for adults aged 65 years and over.

With the exception of men aged 19 to 64 years there were no significant differences in mean red blood cell folate concentrations between Wales and the UK as a whole. The proportions with concentrations below the WHO threshold tended to be slightly lower in Wales but the cell sizes are small, so results should be interpreted with caution. (Table 2.3)

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\(^1\) No comparisons are presented between population folate status in England and that in the UK as a whole; the great majority of the UK participants (84%) were resident in England and therefore results for the UK as a whole broadly indicate population folate status in England.


5 The NDNS RP report for Wales (Years 2 to 5 combined) is due for publication during 2015.


7 However statistical significance testing of these differences was not performed due to some cell sizes being small.
Chapter 5. Blood folate concentrations of women of childbearing age (16 to 49 years)

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

Low folate status of women of childbearing age (16 to 49 years) is a particular public health concern. Increased folic acid intake through supplementation has been shown to reduce the risk of neural tube defects if taken in the periconceptional period. At the time of drafting this report, WHO were in consultation regarding the proposal of thresholds for folate status with regards to risk of neural tube defects for women of childbearing age.

5.1 Folate status of women of childbearing age in the UK as a whole

5.1.1 Serum total folate

The mean serum total folate concentration for women of childbearing age in the UK as a whole was 19.2nmol/L for those aged 16 to 24 years and 20.3nmol/L for those aged 25 to 34 years and those aged 35 to 49 years. There were no statistically significant differences in the mean concentrations between age groups.

The proportion of women of childbearing age who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L) was 22.1% for those aged 16 to 24 years, 17.7% for those aged 25 to 34 years and 13.1% for those aged 35 to 49 years. (Table 3)

5.1.2 Red blood cell folate

The mean red blood cell folate concentration for women of childbearing age in the UK as a whole was 552nmol/L for those aged 16 to 24 years, 611nmol/L for those aged 25 to 34 years and 647nmol/L for those aged 35 to 49 years. The mean red blood cell folate for women aged 16 to 24 years was significantly lower than the red blood cell folate for women aged 35 to 49 years. However, there was no significant difference between the red blood cell folate concentration for women aged 25 to 34 years and that for women aged 35 to 49 years.

The proportion of women who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L) was 15.6% for those
aged 16 to 24 years, 9.5% for those aged 25 to 34 years and 10.1% for those aged 35 to 49 years.\(^3\)

(Table 3)

5.2 Folate status of women of childbearing age in Scotland, Northern Ireland and Wales and comparisons to the UK as a whole

Statistical comparison of means has been carried out for this chapter to compare the mean Scotland, Northern Ireland or Wales serum total folate and red blood cell folate results in women of child bearing age (16 to 49 years) to the equivalent mean results for the UK as a whole. All statistically significant differences between the Scotland, Northern Ireland or Wales folate results and the equivalent results for the UK as whole (reference group, refer to appendix Y of the relevant NDNS RP country reports\(^4,5,6,7\) for a more detailed explanation of the statistical analysis) are highlighted in the tables. No statistical comparison has been carried out for a group that has less than 50 individuals. Cell sizes in the devolved countries are low, particularly in Wales, so caution should be exercised when interpreting the findings.

5.2.1 Scotland

5.2.1.1 Serum total folate

The mean serum total folate concentration for women aged 16 to 49 years in Scotland was 17.4nmol/L, which was significantly lower than in the UK as a whole (20.0nmol/L). The proportion of women of childbearing age who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10nmol/L)\(^2\) was 24.4% for those aged 16 to 49 years in Scotland, which was greater than in the UK as a whole (16.5%).\(^3\)

(Table 4)

5.2.1.2 Red blood cell folate

The mean red blood cell folate concentration for women aged 16 to 49 years in Scotland was 563nmol/L, which was lower than in the UK as a whole (614nmol/L); however this difference did not reach statistical significance. The proportion of women aged 16 to 49 years who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340nmol/L)\(^2\) was 14.8% in Scotland, which was greater than in the UK as a whole (11.3%).\(^3\)

(Table 4)
5.3.2 Northern Ireland

5.3.2.1 Serum total folate

The mean serum total folate concentration for women in Northern Ireland aged 16 to 49 years was 16.3 nmol/L, which was significantly lower than in the UK as a whole (20.0 nmol/L). The proportion of women in Northern Ireland who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10 nmol/L)^2 was 30.6%, which was greater than in the UK as a whole (16.5%).^3 (Table 4)

5.3.2.2 Red blood cell folate

The mean red blood cell folate concentration for women in Northern Ireland aged 16 to 49 years was 512 nmol/L, which was significantly lower than in the UK as a whole (614 nmol/L). The proportion of women in Northern Ireland who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340 nmol/L)^2 was 20.2%, which was greater than in the UK as a whole (11.3%).^3 (Table 4)

5.4.3 Wales

5.4.3.1 Serum total folate

The mean serum total folate concentration for women in Wales aged 16 to 49 years was 18.8 nmol/L, which was slightly lower than in the UK as a whole (20.0 nmol/L); however this difference did not reach statistical significance. The proportion of women in Wales who had a serum total folate concentration below the WHO threshold indicating biochemical folate deficiency (10 nmol/L)^2 was 14.5%, which was similar to that in the UK as a whole (16.5%).^3 (Table 4)

5.4.3.2 Red blood cell folate

The mean red blood cell folate concentration for women in Wales aged 16 to 49 years was 611 nmol/L, which was similar to that in the UK as a whole (614 nmol/L). The proportion of women in Wales who had a red blood cell folate concentration below the WHO threshold indicating biochemical folate deficiency (340 nmol/L)^2 was 10.3%, which was similar to that in the UK as a whole (11.3%).^3 (Table 4)


However, statistical significance testing of these differences was not performed due to some cell sizes being small.


The NDNS RP report for Wales (Years 2 to 5 combined) is due for publication during 2015.
Appendix 1. Folate assay methods and quality control

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

The term “folate” includes several derivatives of the parent molecule folic acid (pteroyl monoglutamic acid) exhibiting equivalent biological activity, namely methylation (one-carbon transfer) reactions in many metabolic pathways. Red blood cell (RBC) folate is usually a better measure of long-term status than plasma or serum total folate because it reflects body stores at the time of red blood cell synthesis and is indicative of average folate status over the 120-day lifespan of the red blood cells, whereas serum total folate concentrations respond rapidly to change in dietary intake.

Both serum total folate and whole blood folate were measured by the Centres for Disease Control and Prevention (CDC), Atlanta, Georgia, US. Haematocrit was measured by the Immunology and Biochemistry Laboratory at Addenbrooke’s Hospital in Cambridge, UK (Addenbrooke’s).

RBC folate was calculated from whole blood folate concentration, haematocrit and serum total folate concentration.

A.1.1 Serum total folate assay method

An isotope-dilution tandem mass spectrometry method coupled to liquid chromatography (LC-MS/MS) was used to measure all major folate forms in serum.\textsuperscript{1,2,3,4} The method performance has been described in detail in a recent paper by Fazili \textit{et al.}\textsuperscript{1} The method quantifies five folate forms (5-methyltetrahydrofolate, folic acid (referred to as ‘free folic acid’ throughout this report), tetrahydrofolate, 5-formyltetrahydrofolate and 5,10-methenyltetrahydrofolate) and an oxidation product of 5-methyltetrahydrofolate known as MeFox.

The method uses 13C-labelled folate forms as internal standards. Solid-phase extraction and elution with an organic solvent is followed by LC-MS/MS in positive ion mode using electrospray ionization on a Sciex API 5500 triple-quadrupole MS system (Applied Biosystems) coupled to a HP1200C LC system (Agilent Technologies). Chromatographic separation was achieved using a Luna C-8 analytical column (Phenomenex) with an isocratic mobile phase. Quantitation was performed by peak area ratio (analyte to internal standard) and based on a six-point aqueous calibration curve that was carried through all sample preparation steps. The results are reported in nmol/L.
Serum total folate samples with 5-methylTHF results <7nmol/L were re-analyzed to ensure that the estimate of low status was not attributable to analytical error.

Serum total folate was calculated as the sum of the individual folate forms (using an imputed value of limit of detection (LOD) divided by the square root of 2 for results that were <LOD). If no result was obtained for one of the folate forms no results were reported. The LODs for this method have been determined to be 0.06 (5-methylTHF), 0.28 (free folic acid), 0.20 (THF), 0.20 (5-formylTHF), 0.31 (5,10-methenylTHF), and 0.08 (MeFox) nmol/L.

A.1.2 Quality control (QC) for serum total folate

Three serum bench QC pools were analyzed in every run. The between-run imprecision for three QC and the target concentration are shown in the tables below.

### Year 1. Serum total folate

<table>
<thead>
<tr>
<th>QC pool</th>
<th>LS11430d</th>
<th>MS11431d</th>
<th>HS11432d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>20.6</td>
<td>44.4</td>
<td>73.5</td>
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<tr>
<td>SD, nmol/L</td>
<td>0.51</td>
<td>1.08</td>
<td>2.14</td>
</tr>
<tr>
<td>CV %</td>
<td>2.5%</td>
<td>2.4%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td>20.5</td>
<td>44</td>
<td>72.8</td>
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</tbody>
</table>

### Year 2. Serum total folate

<table>
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<th>MS11431d</th>
<th>HS11432d</th>
</tr>
</thead>
<tbody>
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<td>44.4</td>
<td>73</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td>0.4</td>
<td>1.08</td>
<td>1.97</td>
</tr>
<tr>
<td>CV %</td>
<td>1.9%</td>
<td>2.4%</td>
<td>2.7%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td>20.5</td>
<td>44</td>
<td>72.8</td>
</tr>
</tbody>
</table>

### Year 3. Serum total folate

<table>
<thead>
<tr>
<th>QC pool</th>
<th>LS11430f</th>
<th>MS11431f</th>
<th>HS11432f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>21.5</td>
<td>45.4</td>
<td>74.6</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td>1.28</td>
<td>3.48</td>
<td>6.89</td>
</tr>
<tr>
<td>CV %</td>
<td>6.0%</td>
<td>7.7%</td>
<td>9.2%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td>21.9</td>
<td>47.1</td>
<td>78</td>
</tr>
</tbody>
</table>

### Year 4. Serum total folate

<table>
<thead>
<tr>
<th>QC pool</th>
<th>LS11430f</th>
<th>MS11431f</th>
<th>HS11432f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>21.3</td>
<td>45.3</td>
<td>75.2</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td>0.53</td>
<td>0.97</td>
<td>1.29</td>
</tr>
<tr>
<td>CV %</td>
<td>2.5%</td>
<td>2.1%</td>
<td>1.7%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td>21.9</td>
<td>47.1</td>
<td>78</td>
</tr>
</tbody>
</table>

### Year 1. Free folic acid

<table>
<thead>
<tr>
<th>QC sample</th>
<th>LS11430d</th>
<th>MS11431d</th>
<th>HS11432d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>0.72</td>
<td>5.63</td>
<td>10.8</td>
</tr>
</tbody>
</table>
National Diet and Nutrition Survey: Blood folate results for the UK, Scotland, Northern Ireland (Years 1 to 4 combined) and Wales (Years 2 to 5 combined)

<table>
<thead>
<tr>
<th>Year 2. Free folic acid</th>
<th>QC sample</th>
<th>LS11430d</th>
<th>MS11431d</th>
<th>HS11432d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td></td>
<td>0.74</td>
<td>5.43</td>
<td>10.3</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td></td>
<td>0.08</td>
<td>0.51</td>
<td>1</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>11.1%</td>
<td>9.4%</td>
<td>9.5%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td></td>
<td>0.68</td>
<td>5.45</td>
<td>10.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year 3. Free folic acid</th>
<th>QC sample</th>
<th>LS11430f</th>
<th>MS11431f</th>
<th>HS11432f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td></td>
<td>0.72</td>
<td>5.85</td>
<td>11.2</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td></td>
<td>0.08</td>
<td>0.4</td>
<td>0.81</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>11%</td>
<td>6.8%</td>
<td>7.2%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td></td>
<td>0.67</td>
<td>5.81</td>
<td>11.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year 4. Free folic acid</th>
<th>QC sample</th>
<th>LS11430f</th>
<th>MS11431f</th>
<th>HS11432f</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td></td>
<td>0.71</td>
<td>5.76</td>
<td>11</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td></td>
<td>0.11</td>
<td>0.38</td>
<td>0.64</td>
</tr>
<tr>
<td>CV %</td>
<td></td>
<td>16%</td>
<td>6.7%</td>
<td>5.8%</td>
</tr>
<tr>
<td>Target (nmol/L)</td>
<td></td>
<td>0.67</td>
<td>5.81</td>
<td>11.1</td>
</tr>
</tbody>
</table>

Accuracy has been established by spiking recovery, by periodic measurements of National Institute of Science and Technology standard reference material (NIST SRM) 1955 (Homocysteine and Folate in Frozen Human Serum; levels 1, 2 and 3) and 1st International Standard for Vitamin B12 and serum total folate 03/178 supplied by the National Institute of biological Standards and Control (NIBSC), by verification of agreement between the LC-MS/MS method and the microbiologic assay that measures total folate, and by successful participation in the UK National External Quality Assessment Scheme (UK NEQAS) Haematinics programme and other accredited external quality assessment schemes.

A.1.3 Red blood cell (RBC) folate quantitation

RBC folate is calculated from whole blood folate concentration see below, serum total folate concentration (see section 1.2) and haematocrit (Hct) (as measured as part of the full blood count) using the equation:

\[
\text{RBC folate} = \frac{\text{whole blood folate} - (\text{serum total folate} \times (1-\text{Hct}))}{\text{Hct}}
\]

Where a serum total folate concentration was not available (n = 158; 6.4% of participants), a surrogate of 18nmol/L was used in the calculation. Where Hct was not available (n = 39; 1.6% of participants), a surrogate of 0.4L/L was used.
A.1.4 Whole blood folate – analytical method

Whole blood hemolysate specimens (whole blood diluted and stabilized with ascorbic acid) were analyzed for total folate using the *Lactobacillus rhamnosus* microbiologic growth assay by an adaptation of O’Broin *et al.*\(^5\) and Molloy *et al.*,\(^6\) as described by Pfeiffer *et al.*\(^7\) Diluted specimen (four replicates at two dilutions) was added to an assay medium containing the microorganism and all of the nutrients necessary for the growth of the microorganism except for folate. Since the growth of *L. rhamnosus* is proportional to the amount of total folate present in the specimen, the total folate level was assessed by measuring the turbidity of the inoculated medium at 590 nm in a microplate reader. The assay was calibrated with 5-methyl-tetrahydrofolate (5-methylTHF), using an 11-point calibration curve (0–1.0nmol/L; 8 replicates/point) with a third degree polynomial curve fit.

Sample dilutions with a concentration below the lowest calibrator or above the highest calibrator were repeated for confirmation, at lower or higher dilution. The standard dilution used for whole blood hemolysate specimens in this study was 1/94.

Results from four replicates at two different dilutions were averaged to generate the final result and the CV from the four replicates had to be ≤15% (≤10% if only three replicates were used). No result was reported from less than three replicates.

Samples with a whole blood folate concentration <127nmol/L (corresponding to a RBC folate concentration of <317nmol/L RBC if a Hct of 0.4L/L is assumed) were considered to represent potential folate deficiency and were repeated for confirmation.

Samples from Years 1 to 4 were mixed so that each assay run included a similar number of samples from each NDNS RP year. Year 5 samples from Wales were run with the last of these.
A.1.4.1 Internal QC

Three whole blood bench QC pools were analyzed in duplicate in every run, bracketing the unknown samples ($n = 32$ runs). The between-run imprecision for whole blood folate and the target concentration are shown in the table below.

### Whole blood total folate concentration

<table>
<thead>
<tr>
<th>QC pool</th>
<th>LB11530a</th>
<th>MB11531a</th>
<th>HB11532a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>284</td>
<td>442</td>
<td>697</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td>24</td>
<td>25</td>
<td>38</td>
</tr>
<tr>
<td>CV %</td>
<td>8.4%</td>
<td>5.7%</td>
<td>5.5%</td>
</tr>
<tr>
<td>Target nmol/L</td>
<td>280</td>
<td>432</td>
<td>692</td>
</tr>
</tbody>
</table>

Four additional whole blood QC pools were analyzed “blind” (i.e. target concentration unknown to analyst) as part of this study at a rate of one blind QC sample in every 20 unknown samples. The between-run imprecision and target concentration are shown in the table below.

### Whole blood total folate concentration

<table>
<thead>
<tr>
<th>QC pool</th>
<th>884</th>
<th>891</th>
<th>892</th>
<th>893</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean, nmol/L</td>
<td>557</td>
<td>293</td>
<td>453</td>
<td>718</td>
</tr>
<tr>
<td>SD, nmol/L</td>
<td>31</td>
<td>20</td>
<td>42</td>
<td>39</td>
</tr>
<tr>
<td>CV%</td>
<td>5.6%</td>
<td>6.8%</td>
<td>9.3%</td>
<td>5.4%</td>
</tr>
<tr>
<td>$n$</td>
<td>41</td>
<td>31</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Target nmol/L</td>
<td>519</td>
<td>280</td>
<td>432</td>
<td>692</td>
</tr>
</tbody>
</table>

Accuracy has been established by spiking recovery, by periodic measurement of the 1st International Standard for Whole Blood Folate 95/528, and by successful participation in UK NEQAS haematins programme (http://www.ukneqas-haematins.org.uk).

---


Appendix 2. Proportion of participants with a notional value for free folic acid

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

Notional values were computed for participants with a free folic acid concentration below the limit of detection (LoD; 0.28nmol/L), by dividing the LoD by the square root of 2 (i.e. inserting a notional value of 0.20nmol/L). This method is consistent with that used in the National Health and Nutrition Examination Survey (NHANES)\(^1\) and has been described by Hornung and Reed (1990).\(^2\)

It should be noted that the LoD for the assay may vary over time. Small changes in LOD have a major effect on the proportion of participants ascribed a notional value. This information is therefore included only as context for the statistics and should not be used as a basis for estimation of population exposure to free folic acid.

The proportion of participants with a notional value for free folic acid is provided in table F for the UK as a whole and devolved country datasets. As some of these participants may not have consumed any free folic acid, e.g. from supplements or fortified breakfast cereals, and therefore may have no free folic acid in their blood but have been assigned a notional value which is greater than zero, this should be taken into account when interpreting the findings for free folic acid in chapters 3 to 5.
Table F. The percentage of participants with a notional value for free folic acid

<table>
<thead>
<tr>
<th>Age and sex group</th>
<th>UK as a whole and devolved country datasets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UK as a whole (Years 1-4)</td>
</tr>
<tr>
<td>Boys aged 4-10 years</td>
<td>21.9</td>
</tr>
<tr>
<td>Boys aged 11-18 years</td>
<td>28.8</td>
</tr>
<tr>
<td>Men aged 19-64 years</td>
<td>33.1</td>
</tr>
<tr>
<td>Men aged 65 years and over</td>
<td>35.8</td>
</tr>
<tr>
<td>Girls aged 4-10 years</td>
<td>16.1</td>
</tr>
<tr>
<td>Girls aged 11-18 years</td>
<td>31.5</td>
</tr>
<tr>
<td>Women aged 19-64 years</td>
<td>32.2</td>
</tr>
<tr>
<td>Women aged 65 years and over</td>
<td>23.2</td>
</tr>
<tr>
<td>Children aged 1.5-3 years</td>
<td>7.5</td>
</tr>
<tr>
<td>Children aged 4-10 years</td>
<td>19.2</td>
</tr>
<tr>
<td>Children aged 11-18 years</td>
<td>30.1</td>
</tr>
<tr>
<td>Adults aged 19-64 years</td>
<td>32.6</td>
</tr>
<tr>
<td>Adults aged 65 years and over</td>
<td>28.5</td>
</tr>
</tbody>
</table>

“-” indicates where results for an age/sex group have not been presented in this report.

Appendix 3. Outliers

Sonja Nicholson, Lorna Cox, Nida Ziauddeen, Polly Page, Chris Bates and Ann Prentice

Although supplement takers are included in the dataset used to produce tables 1-4 and tables G.1-G.4, three individuals had plausible but unusually high blood folate concentrations. The statistical analyses were conducted including and excluding these folate concentrations which revealed that these folate concentrations were highly influential on the mean and standard deviation (sd) estimates of the subgroups into which they were grouped, causing inflated estimates. To ensure a robust interpretation the results of the statistical analyses excluding these folate concentrations have been used for the main tables (tables 1 to 4) in this report and descriptive statistics of the full sample are presented in this appendix (tables G.1 to G.4).

The characteristics of the three individuals whose biochemical folate results were excluded from the main tables (but included in this appendix) along with their dietary folate intakes (from all sources including supplements reported in their four-day estimated food diary) are below. However, it should be noted that there was a gap of around eight weeks between the completion of the four-day estimated food diary and venepuncture:

- female in Scotland in the 65 years and over age group who reported taking a daily 5mg folic acid supplement and 200μg folic acid from a multivitamin/mineral tablet in addition to an average daily intake of 199μg folate from her diet
- female in Wales aged 19 to 64 years who reported taking a daily 5mg folic acid supplement taken in addition to an average daily intake of 123μg folate from her diet
- male in Wales aged 19 to 64 years who reported taking a daily 5mg folic acid supplement in addition to an average daily intake of 187μg from his diet