



Public Health
England

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, Food Standards Scotland,
the Food Standards Agency in Northern Ireland and the Food Standards Agency in
Wales

Revised November 2017

NatCen
Social Research that works for society

MRC | Elsie Widdowson
Laboratory



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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 Years 2 to 5 (combined) report for Wales. Unweighted sample sizes are shown at the foot of each table.
2. The National Diet and Nutrition Survey Rolling Programme (NDNS RP) requires weights to adjust for differences in sample selection and response. The weights adjust for:
 - differential selection probabilities of addresses, households and individuals
 - non-response to the individual questionnaire
 - non-response to the nurse visit
 - non-response to providing a blood sample
3. The data were analysed with the 'survey'^{1,2} package in the statistical software R (version 3.3.2)
4. The following conventions have been used in tables:
 - – no observations (zero value)
 - 0 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 and 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.
8. The term folate 'deficiency' has been used when referring to clinical thresholds and 'insufficiency' when referring to biochemical thresholds.

¹ T. Lumley (2014) 'survey: analysis of complex survey samples'. R package version 3.0.2.

² T. Lumley (2004) Analysis of complex survey samples. Journal of Statistical Software 9(1): 1-19.

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Revision note: update and corrections to data and thresholds

This report (version 2.0, published November 2017) has been updated since first publication (in March 2015) in 3 ways: (i) changes to the criteria used to assess the extent of population folate deficiency in the light of publication of revised thresholds and uncertainty about the applicability of the biochemical threshold to the NDNS RP data, (ii) incorporation of the new World Health Organization (WHO)-recommended threshold (assay-specific) for folate concentration delineating relative risk of fetal folate-sensitive neural tube defects (NTDs), and (iii) correction of a calibration error which had led to a bias in the assay used in the NDNS RP for quantitation of serum unmetabolised (free) folic acid.

(i) Changes to criteria for biochemical folate insufficiency

Since 2005, the WHO have endorsed use of biochemical/metabolic thresholds for assessing population folate status, indicating the folate concentrations below which plasma homocysteine is likely to be elevated, therefore presenting a potential risk to health.¹ These thresholds were used to aid interpretation of the NDNS RP folate data when this report was first published in March 2015. Subsequently in September 2016, adjustment factors were published to take account of differences in assays used to derive red blood cell (RBC) and serum folate concentrations.² In the previous version of this report, folate status was interpreted using the WHO biochemical thresholds without awareness that they were based on data obtained with different assays from NDNS RP, and therefore that assay-specific adjustments to the thresholds would be appropriate. Following review and consideration of the published adjustment factors for these assays, there is some uncertainty regarding the applicability of the adjusted WHO biochemical thresholds² to the UK population as represented by the NDNS RP (see Chapter 1, section 1.9 and Appendix 4 for more information). Consequently, the assay-corrected thresholds as published² have not been used in this revised report; instead provisional ranges have been derived from statistical analysis of NDNS data, to give an indication of the percentage of the population at increased risk of biochemical folate insufficiency. Population distributions have been included to facilitate comparisons over time in the NDNS RP and to enable interpretation against new status threshold estimates should a consensus emerge in the future.

The results presented in this version of the report have been interpreted relative to the widely-accepted Institute of Medicine³ clinical thresholds, endorsed by WHO,¹ for indicating folate deficiency as shown by haematological change.

(ii) New threshold for prevention of folate-sensitive neural tube defects (NTDs)^{1,4}

In April 2015 WHO published thresholds for indication of increased risk of fetal folate-sensitive NTDs at population level. In this report, interpretation of RBC folate concentrations for women of childbearing age (16 to 49 years) has been updated to include examination of the data in relation to the recommended threshold. In line with WHO recommendations, this threshold has also been adjusted to be appropriate for the assay used for the NDNS RP samples.

(iii) Bias in the assay for unmetabolised (free) folic acid

Results in this report have been updated to take account of a correction in a calibration factor that had led to a bias in the assay for unmetabolised (free) folic acid in serum (see Appendix 1 for more information). This was identified following original publication of this report by the laboratory contractor for folate analysis in the NDNS RP. Following correction of this bias, the results for unmetabolised folic acid reported in this revised report are on average 25 to 30% lower than published in 2015. The correction of this bias has resulted in slightly lower mean and narrower distribution of serum folate concentrations than originally published; the effect on calculated RBC folate concentrations is minimal.

Overall conclusions regarding folate status are unchanged by the correction of unmetabolised folic acid results. The dataset deposited in the UK Data Archive⁵ will include the adjusted results for RBC folate, serum folate and unmetabolised folic acid concentrations.

¹ WHO. Serum and red blood cell folate concentrations for assessing folate in populations. Vitamins and Mineral Nutrition Information System. 2015; 01.1-7.

² C. M. Pfeiffer, M. R. Sternberg, H. C. Hamner, *et al.* Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population. *Am J Clin Nutr* 2016;104:1607–15

³ Institute of Medicine. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: The National Academies Press.

<https://doi.org/10.17226/6015> (accessed 06/11/17).

⁴ Tinker SC, Hamner H, Qi YP, Crider KS. U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey, 2007–2010. *National Health and Nutrition Examination Survey 2007 to 2012. Birth Defects Research (Part A)* 103:517–526, 2015.

⁵ <http://data-archive.ac.uk/> (accessed 06/11/17).

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 National Diet and Nutrition Survey Rolling Programme (NDNS RP) and Years 2 to 5 for Wales. This was originally published in March 2015 as a supplementary report to the UK NDNS RP Years 1 to 4 report.¹

This revised version of the report (published November 2017) primarily uses clinical thresholds indicating risk of haematological change rather than the biochemical thresholds used when first published. Population distributions have been included to facilitate comparison with evolving estimates of the biochemical thresholds, with reference to provisional estimates of these thresholds derived from statistical analysis of NDNS RP data. Since publication of the original report, the World Health Organization (WHO) has published thresholds for indication at population level of increased risk of fetal folate-sensitive neural tube defects (NTDs). These have also been incorporated into the revised report. Additionally the data include correction of a calibration issue which had led to a bias in the assay for unmetabolised folic acid used in the NDNS RP, detected following initial publication. This version of the report therefore supersedes the original version published in March 2015. See section 1.9 and appendices 1 and 4 for further detail about these changes.

Folate in the diet comes from naturally occurring folates in foods and from folic acid added to fortified foods such as some breakfast cereals and dietary supplements. Folate is involved in single carbon transfer (methylation) reactions, including those necessary for the synthesis of purines, pyrimidines, glycine and methionine.² It is needed for DNA synthesis and thus for the production and maintenance of new cells. Shortage of folate compromises the formation and maturation of red blood cells and leads to macrocytic anaemia.

Results are reported for red blood cell (RBC) folate, serum folate and unmetabolised folic acid for the standard NDNS age/sex groups and for women of childbearing age. Due to small cell sizes, especially in children under 11 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 RBC and serum 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 1,769 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 3 days. Of this total:

- in Scotland: 440 adults and 216 children aged 4 to 18 years, representing 51% of adults and 21% of those aged 4 to 10 years and 38% of those aged 11 to 18 years who completed a diet 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 diet 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 diet diary

RBC and serum folate were measured using assay methods which provide the most accurate quantitation possible, as determined by an international expert review and workshop in 2008 on methods for assessing folate status.³ Laboratory analyses were provided by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA. Whole blood folate (from which RBC folate is calculated) was quantitated by the long-established microbiological assay method. Serum folate was quantitated 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 unmetabolised folic acid. Both methods are the same as those currently used for the US National Health and Nutrition Examination Survey (NHANES).⁴ These assays do not give the same results as the clinical assays used to quantitate folate status in previous NDNS. Therefore, comparisons of blood folate concentrations between the NDNS RP and previous NDNS have not been made.⁵

The following sets of thresholds are used in this report for assessment of folate status and risk of deficiency at the population level. All thresholds used in this report are adjusted² where necessary to be appropriate for the assays used, as advised by WHO.⁶

I. Clinical thresholds for folate deficiency:

- Less than 305nmol/L for RBC folate (using the appearance of hypersegmented neutrophils as a haematological indicator of suboptimal DNA synthesis in the bone marrow and therefore the potential for progression to megaloblastic anaemia)⁷
- Less than 7nmol/L for serum folate (6.8nmol/L quoted by WHO as upper limit of concentrations indicating folate deficiency)⁶
- Less than 13nmol/L for serum folate (13.4nmol/L quoted by WHO as the concentration below which folate deficiency is possible)⁶

- II. Threshold for women of reproductive age, for avoiding folate-sensitive neural tube defect (NTD)-affected pregnancies at the population level:
 - Greater than 748nmol/L for RBC folate (quoted by WHO as optimal to minimise folate-sensitive NTD risk)^{6,8}
 - There is no recommended serum folate threshold for minimising NTDs

- III. Risk of biochemical folate insufficiency^{6,9} (see section 1.9 and Appendix 4 for derivation of these estimated assay-appropriate thresholds¹⁰ for the UK population using the NDNS RP dataset) denoted by:
 - RBC folate concentration which probably lies between 450nmol/L and 550nmol/L
 - Serum folate concentration which probably lies between 10nmol/L and 15nmol/L
 - Because these are preliminary estimates of the biochemical thresholds, population distributions are included in the main Tables 1 to 4 to facilitate comparisons with evolving estimates in future

Red blood cell (RBC) folate

RBC folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L).⁷ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

Overall UK population (Table 1)

The percentage of girls aged 11 to 18 years with RBC folate concentration below the clinical threshold indicating risk of anaemia was 13%; it was 5% or less in the other age/sex groups. The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 39% and 60% of children aged 11 to 18 years, 25% and 46% of adults aged 19 to 64 years, 18% and 36% of adults aged 65 years and over and 14% and 28% of children aged 4 to 10 years.

Women of childbearing age 16 to 49 years (Table 3)

Among women of childbearing age, 75% of participants had a RBC folate concentration lower than the threshold for optimal avoidance of folate-sensitive fetal NTDs (748nmol/L).^{6,8} Mean RBC folate was significantly lower for women aged 16 to 24 years than for those aged 35 to 49 years.

The overall proportion of women of childbearing age (16 to 49 years) with a RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was 7%, with the highest proportion being those aged 16 to 24 years (12%).

The proportion of women of childbearing age with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 32% and 52%.

Devolved countries (Tables 2.1 to 2.3 and Table 4)¹¹

In Scotland, mean RBC folate concentrations 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 11% of women aged 19 to 64 years and 13% of girls aged 11 to 18 years were below the clinical threshold indicating risk of anaemia (305nmol/L);⁷ these proportions are higher than in the UK as a whole for women aged 19 to 64 years (5%) and equal for girls aged 11 to 18 years (13%). The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 42% and 64% of children aged 11 to 18 years, 29% and 50% of adults aged 19 to 64 years, 28% and 39% of adults aged 65 years and over and 8% and 18% of children aged 4 to 10 years.

For women of childbearing age (16 to 49 years), the proportion in Scotland with a RBC folate concentration below the clinical threshold⁷ indicating risk of anaemia was 11%; higher than in the UK as a whole (7%). In Scotland, 81% of women of childbearing age had a RBC folate concentration lower than the threshold for optimal avoidance of folate-sensitive fetal NTDs (748nmol/L),^{6,8} this proportion is higher than in the UK as a whole (75%).

In Northern Ireland, mean RBC folate concentrations 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. Eleven percent of girls aged 11 to 18 years and women aged 19 to 64 years had concentrations below the clinical threshold indicating risk of anaemia (305nmol/L), compared with 13% and 5% respectively in the UK as a whole.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 50% and 68% of children aged 11 to 18 years and 30% and 53% of adults aged 19 to 64 years.

For women of childbearing age the proportion with an RBC folate concentration below the clinical threshold⁷ indicating risk of anaemia was 15% in Northern Ireland; higher than in the UK as a whole (7%). In Northern Ireland, 83% of women of childbearing age had a RBC folate concentration lower than the threshold for optimal avoidance of folate-sensitive fetal NTDs (748nmol/L).^{6,8}

In Wales, mean RBC folate concentration was significantly higher than in the UK as a whole for men aged 19 to 64 years, however, there were no other significant differences in mean RBC folate levels between Wales and the UK as a whole for any age group. Four percent of children aged 11 to 18 years and women aged 19 to 64 years had concentrations below the clinical threshold indicating risk of anaemia (305nmol/L), compared with 9% and 5% respectively in the UK as a whole.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 44% and 57% of children aged 11 to 18 years and 18% and 39% of adults aged 19 to 64 years.

For women of childbearing age in Wales the proportion with an RBC folate concentration below the clinical threshold⁷ indicating risk of anaemia was 6% (UK 7%). In Wales, 79% of women of childbearing age had a RBC folate concentration lower than the threshold for optimal avoidance of folate-sensitive fetal NTDs (748nmol/L), similar to the UK as a whole (75%).^{6,8}

Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L).⁶ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO clinical threshold for possible folate deficiency.

Overall UK population (Table 1)

The percentage of adults and children with serum folate below the WHO clinical threshold for folate deficiency (7nmol/L) was no more than 5% in any age group. The percentage with serum folate concentration below the WHO threshold for possible deficiency (13nmol/L) was 31% for adults aged 19 to 64 years, 27% for adults aged 65 years and over, 41% for older children and 6% for children aged 4 to 10 years. The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 21% and 50% of children aged 11 to 18 years, 15% and 42% of adults aged 19 to 64 years, 11% and 34% of adults aged 65 years and over and 3% and 13% of children aged 4 to 10 years.

Women of childbearing age 16 to 49 years (Table 3)

There is no recommended serum folate threshold for minimising folate-sensitive NTDs.

For women of childbearing age in the UK as a whole, the proportion with a serum folate concentration below the WHO clinical threshold for folate deficiency (7nmol/L) was 3%.

The proportion of women of childbearing age with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 17% and 44%.

Devolved Countries (Tables 2.1 to 2.3 and Table 4)

In Scotland and Northern Ireland mean serum folate levels were significantly lower than in the UK as a whole for adults aged 19 to 64 years (both for men and women combined and separately for women) and for women of childbearing age (16 to 49 years).

In Wales, mean serum folate for adults aged 65 years and over was significantly lower than for the same age group in the UK as a whole. There was no significant difference in mean serum folate between Wales and the UK as a whole for women of childbearing age.

In Scotland, 7% of women aged 19 to 64 years had serum folate below the WHO clinical threshold indicating folate deficiency (7nmol/L). The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 24% and 54% of children aged 11 to 18 years, 19% and 49%

of adults aged 19 to 64 years, 16% and 40% of adults aged 65 years and over and 1% and 4% of children aged 4 to 10 years.

In Northern Ireland, 5% of children aged 11 to 18 years and 6% of women aged 19 to 64 years had serum folate below the WHO clinical threshold indicating folate deficiency (7nmol/L). The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 19% and 49% of children aged 11 to 18 years and 19% and 54% of adults aged 19 to 64 years.

In Wales, 5% of children aged 11 to 18 years had serum folate below the WHO clinical threshold indicating folate deficiency (7nmol/L). The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 20% and 48% of children aged 11 to 18 years, 15% and 51% of adults aged 19 to 64 years and 12% and 53% of adults aged 65 years.

In Scotland 7% and in Northern Ireland 8% of women of childbearing age had serum folate levels below the WHO clinical threshold for folate deficiency (7nmol/L). These are higher proportions than in the same group in the UK as a whole (3%).

Unmetabolised folic acid

Descriptive statistics for unmetabolised (free) folic acid concentrations for each age/sex group are presented in Tables 1 to 4 (see Chapter 1 for more details). Folic acid is the synthetic form most often used for fortification and supplements because of its stability.

In the UK population in Years 1 to 4 of the NDNS RP unmetabolised folic acid was undetectable in almost a third of adults aged 19 to 64 years (Appendix 2 Table F).

Comparisons between NDNS RP and NHANES folate concentrations

Mean RBC and serum folate concentrations in the US (2005 to 2010; post-fortification)¹² as assessed in NHANES⁴ are approximately twice the concentrations in the UK using the same assay as NDNS RP, indicating higher medium term population folate sufficiency. This is likely to be due to mandatory fortification of bread flour with folic acid in the US (see section 2.3 for more details).

¹ National Diet and Nutrition Survey: results from Years 1 to 4 (combined) of the rolling programme for 2008/09-2011/12 <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012> (accessed 06/11/17).

² Report on Health and Social Subjects 41 *Dietary Reference Values (DRVs) for Food Energy and Nutrients for the UK*, Report of the Panel on DRVs of the Committee on Medical Aspects of Food Policy (COMA) 1991. The Stationery Office. London

³ Duthie S, Bird S, Mayer C, MacDonald H (2009) Informed Systematic Review and Critical Comparison of Analytical Methods for the Quantification of Blood Folate Status in the Population.

⁴ <http://www.cdc.gov/nchs/nhanes.htm> (accessed 06/11/17).

⁵ 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 quantitation 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).

⁶ WHO. Serum and red blood cell folate concentrations for assessing folate in populations. Vitamins and Mineral Nutrition Information System. 2015; 01.1-7

⁷ Institute of Medicine. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: The National Academies Press.

<https://doi.org/10.17226/6015> (accessed 06/11/17).

⁸ Tinker SC, Hamner H, Qi YP, Crider KS. U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey, 2007–2010. National Health and Nutrition Examination Survey 2007 to 2012. Birth Defects Research (Part A) 103:517–526, 2015

⁹ WHO. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. Food and Nutrition Bulletin. 2008; 29. S238–S244

¹⁰ C. M. Pfeiffer, M. R. Sternberg, H. C. Hamner, *et al.* Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population. Am J Clin Nutr 2016;104:1607–15.

¹¹ It should be noted that cell sizes are small for some age/sex groups in Scotland, Northern Ireland and Wales therefore caution should be taken when interpreting the data.

¹² Pfeiffer CM, Hughes JP, Lacher DA, Bailey RL, Berry RJ, Zhang M, Yetley EA, Rader JI, Sempos CT, Johnson CL. Estimation of trends in serum and RBC folate in the U.S. population from pre- to post-fortification using assay-adjusted data from the NHANES 1988-2010. J Nutr. 2012; 142:886–93.

Introduction

Revision note: Update and correction to data and thresholds

This report (version 2.0, published November 2017) provides an update to the original publication (in March 2015) in 3 ways: (i) changes to criteria for biochemical folate insufficiency, (ii) new thresholds for prevention of folate-sensitive neural tube defects, and (iii) correction of bias in the assay for unmetabolised folic acid. For information about these amendments please refer to section 1.9 and the full revision note [here](#).

1.1 Background

This report presents the results for RBC folate, serum folate and unmetabolised (free) folic acid in blood samples taken for the NDNS RP during the nurse visit for participants aged:

- 1.5 years and over in the UK as a whole
- aged 4 years and over in Scotland¹
- aged 11 years and over in Northern Ireland and Wales¹

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).

RBC and serum folate were measured by assay methods which provide the most accurate quantitation possible, as determined by an international expert review and workshop in 2008 on methods for assessing folate status.² 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 RBC folate. The LC-MS/MS method used to quantitate serum 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).³ LC-MS/MS captures the individual forms of folate in serum including unmetabolised folic acid; serum folate as reported is calculated as the sum of all forms including unmetabolised 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. Therefore the microbiological assay, long established as the 'gold standard' for folate quantitation is used in NDNS RP for quantitation of whole blood folate.

Methods used for folate quantitation in previous NDNS and the current RP are tabulated in table A. The methods used for the quantitation 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 quantitate blood folate in NDNS RP and the previous NDNS

Survey	Red blood cell (RBC) folate	Serum/plasma folate	Unmetabolised (free) folic acid
Previous NDNS of children aged 1.5 to 4.5 years (1992/93) ⁴	Biorad Quantaphase I (radio-protein-binding method)	Biorad Quantaphase I (radio-protein-binding method)	Not quantitated separately
Previous NDNS of adults aged 65 years and over (1994/95) ⁵	Biorad Quantaphase II (radio-protein-binding method)	Biorad Quantaphase II (radio-protein-binding method)	Not quantitated separately
Previous NDNS of children aged 4 to 18 years (1997) ⁶	Abbott IMx (microparticle enzyme immunoassay)	Abbott IMx (microparticle enzyme immunoassay)	Not quantitated separately
Previous NDNS of adults aged 19 to 64 years (2000/01) ⁷	Abbott IMx (microparticle enzyme immunoassay)	Abbott (microparticle enzyme immunoassay)	Not quantitated separately
NDNS RP Years 1 to 4; UK, Scotland and Northern Ireland ^{8,9,10}	Calculated from microbiological assay for whole blood (WB) folate and LC-MS/MS for serum folate	LC-MS/MS	LC-MS/MS
NDNS RP Years 2 to 5; Wales ¹¹	Calculated from microbiological assay for WB folate and LC-MS/MS for serum folate	LC-MS/MS	LC-MS/MS

1.2 Folate function, dietary sources, and recommendations

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. Foliates 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 (for example, in breakfast cereals) and in dietary supplements.

Dietary intake of folate is presented in the main NDNS RP UK⁸ and devolved country reports^{9,10,11} based on assessment of food consumption over 4 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.

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 NTDs if taken in the periconceptual period.¹² 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 NTDs. 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)¹³ 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 B.¹⁴ Comprehensive results for overall dietary intake and the range of other nutritional status indicators obtained from blood and urine samples collected in NDNS RP are presented in the main UK Years 1 to 4 combined report⁸ and respective devolved country reports,^{9,10,11} along with other survey results. Appendix W of each report indicates which results are presented in the respective reports and which results are included in the datasets on the UK Data Archive.¹⁵

Table B. Reference nutrient intakes (RNI) and lower reference nutrient intakes (LRNI) for folate

Age group (years)	RNI for folate ($\mu\text{g}/\text{d}$) ¹⁴	LRNI for folate ($\mu\text{g}/\text{d}$) ¹⁴
1.5 to 3 years	70	35
4 to 6 years	100	50
7 to 10 years	150	75
11 to 18 years	200	100
19 to 64 years	200	100
65 years and over	200	100

1.3 Survey methods

An overview of the purpose, documents, methodologies, participant consent and procedures for quality control are provided in the main NDNS RP reports.^{8,9,10,11} 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 participants¹⁶ 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 4 years and over. Participants with diabetes who were not willing or not able to fast and those aged 1.5 to 3 years were invited to provide a non-fasting blood sample.

1.4 Blood sampling response

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

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

	UK as a wholeⁱ (Years 1 to 4)	Scotland (Years 1 to 4)	Northern Ireland (Years 1 to 4)	Wales (Years 2 to 5)
Adults (aged 19+ years)				
Number providing a blood sample	1,769	440	264	228
% of fully productive participants providing a blood sample	51	51	56	49
Children (aged 1.5 to 18 years)ⁱⁱ				
Number providing a blood sample	902	216	96	60
% of fully productive participants providing a blood sample	27	27	41	34

ⁱ These 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).

ⁱⁱ Blood 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 4 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 (as red blood cell folate and serum folate) 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 4 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 4 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}

1.5 Blood sampling

Blood samples were collected as described in Appendix O of the main reports.^{8,9,10,11} An EDTA monovette tube from each participant's sample set was sent by post to the Immunology and Biochemistry Laboratory at Addenbrooke's Hospital in Cambridge (Addenbrooke's) for prompt processing. At the laboratory 100µL of EDTA-anticoagulated 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. Any deterioration in whole blood folate concentration during overnight posting is likely to be less than 10% of total folate concentration.¹⁷ The remainder of the whole blood was used for full blood

count (including haematocrit) and quantitation of HbA1c. Serum was prepared at the field laboratories from freshly-clotted blood as detailed in Appendix O of the main reports and was stored frozen at -80°C pending analysis.

1.6 Determination of folate status

Folate status is monitored by the quantitation of folates in red blood cells and in serum. RBC folate is usually a better indicator of long-term status than plasma or serum 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 folate concentrations respond rapidly to change in dietary intake. The assay for serum 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 as the concentrations of circulating folate forms in various states of methylation.

Whole blood folate, serum folate and serum unmetabolised folic acid concentrations were quantitated at CDC, USA (see Appendix 1). Haematocrit (Hct) was measured at Addenbrooke's using a Coulter Counter-based assay.

Whole blood folate concentration was quantitated by CDC by an automated microbiological assay using *Lactobacillus rhamnosus* and calibrated with methyltetrahydrofolate (5-methylTHF). RBC folate was calculated from whole blood folate, serum folate and Hct. Details of the method are given in Appendix 1 of this report.

Serum folate concentrations were quantitated by the CDC LC-MS/MS. This method is regarded internationally as the 'gold standard'. It quantitates 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 results for total serum folate and unmetabolised folic acid concentrations. Concentrations of individual folate forms are not reported here but will be deposited in the UK Data Archive.¹⁵ Details of the method are given in Appendix 1 of this report.

1.7 Unmetabolised (free) folic acid

Unmetabolised (free) folic acid in serum is quantitated as one of the components of serum folate as assayed by LC-MS/MS. It is the synthetic form most often used for fortification and supplementation because of its stability. The long-term biological effects of exposure to unmetabolised (free) folic acid in humans are unknown (SACN 2017).¹⁸

Unmetabolised (free) folic acid results that have been adjusted to correct for a calibration issue which had led to bias, 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.

For details of the correction made to unmetabolised folic acid concentrations please see Appendix 1, section A.1.5.

Almost a third of participants had unmetabolised 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 NHANES³ and has been described by Hornung and Reed (1990).¹⁹ The proportion of participants with a notional value for unmetabolised folic acid is provided in Table F of Appendix 2 of this report.

There are no established thresholds for unmetabolised folic acid and currently no other published population data in unfortified populations.

1.8 Analysis of the results data

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.^{8,9,10,11} The number of RBC and serum 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).^{8,9,10,11} Results are presented where cell sizes are sufficient (30 or greater) for the age groups 1.5 to 3 years, 4 to 10 years, 11 to 18 years, 19 to 64 years and 65 years and over and are further split by sex for those aged 4 years and over.

Four individuals had plausible but unusually high blood folate concentrations which were highly influential on the mean and standard deviation (sd) estimation of the subgroups which contained them, causing the estimates to be inflated. All 4 individuals had taken a 5mg/day folic acid supplement daily during the dietary recording period which was approximately 8 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 4 outliers are provided in section 2.4 and Appendix 3 respectively. To ensure a robust interpretation, only the results of the statistical analyses excluding these observations are used in Tables 1 to 4; descriptive statistics of the full sample (including these data) are presented in Tables G.1 to G.4. With the exception of these four individuals, all other supplement takers are included in the statistical analyses.

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 3 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 statistical methods used for the mean comparisons of the UK data⁸ with Scotland, Northern Ireland or Wales data are provided in Appendix Y of the corresponding country report.^{9,10,11}

1.9 Thresholds indicating inadequate folate nutritional status

Multiple criteria are used to determine the extent of folate adequacy in populations, referring to different levels of health concern ranging from clinical deficiency to alterations in biochemistry. Interpretation of thresholds developed to indicate inadequate folate status is complicated by significant differences between the assays used to quantitate folate concentrations. The importance of assay adjustments in the interpretation of folate status has been brought to light in several recent publications^{20,21} and endorsed by WHO.²² This is discussed in more detail in Appendix 4. Folate deficiency thresholds are outlined in Table D.

- I. A clinical threshold of 305nmol/L²³ for RBC folate has been adopted in the tables and text of this report. This indicates folate concentrations in the bone marrow which are likely to be too low to support normal DNA synthesis and the generation of normal blood cells. In this situation hypersegmented neutrophils would be present in the circulation. This is an early indication of the development of folate-deficiency anaemia, that is, reduced and abnormal erythrocyte production and therefore a more stringent indication of poor folate status than the biochemical threshold used in the first publication of this report. Serum folate below 13nmol/L²² is proposed by WHO as indicating possible folate deficiency, below 7nmol/L²² as indicating actual folate deficiency. These clinical thresholds have been used in the text and tables of this report: the percentage with serum folate below 13nmol/L includes those with serum folate below 7nmol/L.
- II. RBC folate concentrations in women of childbearing age are also interpreted against the threshold (748nmol/L) for minimising risk of NTDs.^{20,21} This was initially derived as 906nmol/L²⁴ using the Molloy microbiological assay²⁵ and has been multiplied by a factor to make it applicable to NDNS RP data derived using the CDC microbiological assay.²¹ Use of this threshold is also endorsed by WHO.²²

- III. In the first version of this report folate status was interpreted against the WHO biochemical thresholds for monitoring population status on the basis of avoiding increased risk of raised homocysteine concentration, that is RBC folate 340nmol/L and serum folate 10nmol/L. These metabolic changes have been linked to increased incidence of chronic diseases including cancer, cardiovascular disease and neurological conditions.^{26,27,28} In September 2016 modifications were published to all folate thresholds to be appropriate for the assay used. For the CDC assay used to quantitate NDNS RP folate, the assay-appropriate biochemical threshold for RBC folate, derived from NHANES data, is 624nmol/L and for serum folate 14nmol/L.²⁰

This represents a considerable increase in estimated biochemical threshold concentrations and consequently in the proportion of the population falling below it. However, these thresholds were derived for the US (NHANES) population with considerably higher folate status resulting from mandatory fortification and therefore are not necessarily applicable to the UK. Therefore in order to validate the thresholds for use in this report, the same statistical analysis was performed using NDNS RP folate and homocysteine data. This data analysis has indicated that the assay-appropriate RBC folate biochemical threshold (624nmol/L) does not appear to be appropriate for the UK population as represented in NDNS RP (see Appendix 4). The analysis showed that the RBC folate concentration at which there is a risk of elevated plasma homocysteine in the UK population is likely to be lower than that calculated for the US population in NHANES.²⁹ The folate and homocysteine concentrations are poorly correlated, and therefore thresholds derived from them can only be approximate estimates (see Appendix 4). The indications are that the biochemical threshold for RBC folate probably lies between 450 to 550nmol/L (that is, lower than that calculated by Selhub *et al.*²⁹ using data from NHANES). In addition, indications suggest that the biochemical threshold for serum folate in the UK population probably lies between 10 to 15nmol/L (that is, similar to that calculated by Selhub *et al.*²⁹ using data from NHANES). Percentages of each subgroup of the population with folate concentrations falling below the upper and lower limits of these estimated ranges for RBC folate and serum folate are indicated in the text to provide an assessment of increased risk of folate insufficiency. Because of the uncertainty around the provisional biochemical thresholds derived for NDNS RP data, they should be used with caution. Population folate concentration distributions are included in the tables so that data can be interpreted against these concentrations if a consensus regarding new biochemical thresholds for the UK should emerge in the future.

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 4 years and over in Scotland and 11 years and over in Northern Ireland and Wales.

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Table D. Thresholds indicating folate deficiency

See Appendix 4 for more detail

Threshold as referred to in this report	Clinical (IoM)		NTD (WHO)		Biochemical (WHO)		
	Risk of anaemia		Reduced risk of folate-sensitive neural tube defect (NTD) affected pregnancies		Associated with raised homocysteine		
Interpretation	Threshold (concentration applicable to assay with which it was derived)	Concentration applicable to NDNS RP (MBA for whole blood folate, LC-MS/MS for serum folate)	WHO threshold (concentration applicable to assay with which it was derived)	Concentration applicable to NDNS RP (RBC folate derived from MBA for whole blood folate, LC-MS/MS for serum folate)	WHO threshold (concentration applicable to assay with which it was derived)	Concentration applicable to NHANES ²	Preliminary estimate of concentration applicable to NDNS RP
RBC concentration	305nmol/L (appearance of hypersegmented neutrophils)	305nmol/L (appearance of hypersegmented neutrophils)	906nmol/L	748nmol/L	340nmol/L	624nmol/L	450 to 550nmol/L
Serum folate concentration	7nmol/L (deficiency) 13nmol/L (possible deficiency)	7nmol/L (deficiency) 13nmol/L (possible deficiency)	n/a	n/a	10nmol/L	14nmol/L	10 to 15nmol/L
Assay with which the threshold was derived	Previous CDC microbiological assay (similar to current CDC MBA so no factor is applied) ²		Molloy microbiological assay (Univ of Cork) ²⁵		Biorad Quantaphase II immunoassay		

Chapter 2. Blood folate concentrations for the UK as a whole

Revision note: update and correction to data and thresholds

This report (version 2.0, published November 2017) provides an update to the original publication (in March 2015) in 3 ways: (i) changes to criteria for biochemical folate insufficiency, (ii) new thresholds for prevention of folate-sensitive neural tube defects, and (iii) correction of bias in the assay for unmetabolised folic acid. For information about these amendments please refer to the full revision note [here](#).

2.1 Red blood cell (RBC) folate

Red blood cell folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L).¹ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

RBC folate status for women of childbearing age in the UK as a whole is discussed in Chapter 4 of this report.

The mean RBC folate concentration in the UK as a whole 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.

The mean RBC folate concentration for boys aged 4 to 10 years in the UK as a whole was 727nmol/L, 584nmol/L for boys aged 11 to 18 years, 687nmol/L for girls aged 4 to 10 years and 500nmol/L for girls aged 11 to 18 years.

In the UK as a whole, RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was found in 13% of girls aged 11 to 18 years, 5% of boys

aged 11 to 18 years and adults aged 19 to 64 years, 4% of women aged 65 years and over and 3% or less of other age groups.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 49% and 67% of girls aged 11 to 18 years and between 30% and 53% of boys aged 11 to 18 years. The proportion was between 25% and 46% of adults aged 19 to 64 years, 18% and 36% of adults aged 65 years and over and 14% and 28% of children aged 4 to 10 years.

(Table 1)

2.2 Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds² indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L). In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion. For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO clinical threshold for possible folate deficiency.

Serum folate status for women of childbearing age in the UK as a whole is discussed in chapter 4 of this report.

The mean serum folate concentration in the UK as a whole for men aged 19 to 64 years was 18.0nmol/L, 21.2nmol/L for women aged 19 to 64 years, 22.6nmol/L for men aged 65 years and over and 27.4nmol/L for women aged 65 years and over.

The mean serum folate concentration for boys aged 4 to 10 years in the UK as a whole was 30.9nmol/L, 18.4nmol/L for boys aged 11 to 18 years, 27.6nmol/L for girls aged 4 to 10 years and 16.9nmol/L for girls aged 11 to 18 years.

In the UK as a whole, serum folate concentration below the WHO clinical threshold indicating folate deficiency (7nmol/L) was found in 5% of boys aged 11 to 18 years, 4% of men aged 19 to 64 years, and 3% or less of other age/sex groups.

Serum folate concentration below the WHO clinical threshold indicating possible folate deficiency (13nmol/L) was found in 43% of boys aged 11 to 18 years, 38% of girls aged 11 to 18 years, 33% of men aged 19 to 64 years, 29% of women aged 19 to 64 years, 33% of men aged 65 years and over, 22% of women aged 65 years and over, and 6% of children aged 4 to 10 years.

The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 21% and 50% of children aged 11 to 18 years, 15% and 42% of adults aged 19 to 64 years, 8% and 42% of men aged 65 years and over, 12% and 28% of women aged 65 years and over and 3% and 13% of children aged 4 to 10 years.

(Table 1)

2.3 Comparison between NDNS RP and NHANES folate concentrations

The methods used in NDNS RP for quantitation of whole blood and RBC folate are the same as those currently used for the US National Health and Nutrition Examination Survey (NHANES),³ 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.⁴

The comparison shows that median RBC and serum folate concentrations in the UK are about half the geometric mean concentrations in NHANES 2005 to 2010 (after the introduction of folic acid fortification of flour in the US) but are similar to concentrations found in NHANES 1998 to 1994 (before the introduction of fortification).

¹ Institute of Medicine. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: The National Academies Press. <https://www.nap.edu/catalog/6015/dietary-reference-intakes-for-thiamin-riboflavin-niacin-vitamin-b6-folate-vitamin-b12-pantothenic-acid-biotin-and-choline> (accessed 06/11/17).

² WHO. Serum and red blood cell folate concentrations for assessing folate in populations. Vitamins and Mineral Nutrition Information System. 2015; 01.1-7.

³ <http://www.cdc.gov/nchs/nhanes.htm> (accessed 06/11/17).

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 quantitation 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 3. Blood folate concentrations for Scotland, Northern Ireland and Wales and comparisons with results for the UK as a whole

Revision note: Update and Correction to data and thresholds

This report (version 2.0, published November 2017) provides an update to the original publication (in March 2015) in 3 ways: (i) changes to criteria for biochemical folate insufficiency, (ii) new thresholds for prevention of folate-sensitive neural tube defects, and (iii) correction of bias in the assay for unmetabolised folic acid. For information about these amendments please refer to the full revision note [here](#).

3.1 Statistical analysis

Statistical comparison of means has been carried out for this chapter to compare the mean UK RBC and serum folate results to the equivalent mean Scotland, Northern Ireland and Wales results.¹ The UK RBC and serum folate results have been used as the reference group (refer to appendix Y of the relevant NDNS RP reports^{2,3,4,5} 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.

3.2 Scotland

3.2.1 Red blood cell (RBC) folate

Red blood cell folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L).⁶ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the

threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

RBC folate status for women of childbearing age in Scotland is discussed in Chapter 4 of this report.

The mean RBC folate concentration for men aged 19 to 64 years was 648nmol/L, 589nmol/L for women aged 19 to 64 years, and 677nmol/L for adults aged 65 years and over. The mean RBC folate concentration for children in Scotland aged 4 to 10 years was 706nmol/L, 608nmol/L for boys aged 11 to 18 years and 498nmol/L for girls aged 11 to 18 years.

In Scotland, RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was found in 13% of girls aged 11 to 18 years, 8% of boys aged 11 to 18 years, 11% of women aged 19 to 64 years, 6% of women aged 65 years and over and 2% or less of other age groups.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 47% and 72% of girls aged 11 to 18 years and between 38% and 57% of boys aged 11 to 18 years. The proportion was between 34% and 57% of women aged 19 to 64 years, 25% and 43% of men aged 19 to 64 years, 28% and 39% of adults aged 65 years and over and 8% and 18% of children aged 4 to 10 years.

For women aged 19 to 64 years mean RBC folate concentration was significantly lower in Scotland than in the UK as a whole (589nmol/L compared to 652nmol/L respectively).

For adults aged 65 years and over mean RBC 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.

With the exception of men aged 19 to 64 years, for all age/sex groups over 11 years, the proportion of participants with a RBC folate concentration below the threshold of 305nmol/L for risk of clinical anaemia was the same or greater in Scotland than in the UK as a whole.

(Table 2.1)

3.2.2 Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L).⁷ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion. For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO threshold for possible folate deficiency.

Serum folate status for women of childbearing age in Scotland is discussed in Chapter 4 of this report.

The mean serum folate concentration in Scotland for men aged 19 to 64 years was 16.9nmol/L, 18.5nmol/L for women aged 19 to 64 years and 22.5nmol/L for adults aged 65 years and over.

The mean serum folate concentration for children aged 4 to 10 years in Scotland was 31.2nmol/L, 18.1nmol/L for boys aged 11 to 18 years and 15.8nmol/L for girls aged 11 to 18 years.

In Scotland, serum folate concentration below the WHO clinical threshold indicating folate deficiency (7nmol/L) was found in 4% of boys aged 11 to 18 years, 4% of men aged 19 to 64 years, 7% of women aged 19 to 64 years, 4% of adults aged 65 years and over and less than 0.5% in other age-sex groups.

Serum folate concentration below the WHO clinical threshold indicating possible folate deficiency (13nmol/L) was found in 48% of girls aged 11 to 18 years, 37% of boys aged 11 to 18 years, 34% of men aged 19 to 64 years, 41% of women aged 19 to 64 years, 32% of adults aged 65 years and over and 3% of children aged 4 to 10 years.

The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 25% and 61% of girls aged 11 to 18 years and between 23% and 47% of boys aged 11 to 18 years. The proportion was

between 19% and 49% of adults aged 19 to 64 years, 16% and 40% of adults aged 65 years and over and 1% and 4% of children aged 4 to 10 years.

For women aged 19 to 64 years, mean serum folate concentration was significantly lower in Scotland than in the UK as a whole (18.5nmol/L compared to 21.2nmol/L respectively). Lower mean serum folate was therefore also seen for all adults aged 19 to 64 years (17.7nmol/L compared to 19.7nmol/L respectively).

With the exception of children aged 11 to 18 years (sex-combined and sex-split) the proportion of participants with a serum folate concentration below the WHO clinical threshold for folate deficiency (7nmol/L) was the same or greater in Scotland than in the UK as a whole for all age/sex groups. With the exception of children aged 4 to 18 years (sex-combined and sex-split) the proportion of participants with a serum folate concentration below the WHO threshold for possible folate deficiency (13nmol/L) was the same or greater in Scotland than in the UK as a whole for all age/sex groups.

(Table 2.1)

3.3 Northern Ireland

3.3.1 Red blood cell (RBC) folate

Red blood cell folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L).⁶ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

RBC folate status for women of childbearing age in Northern Ireland is discussed in Chapter 4 of this report.

The mean RBC folate concentration for men aged 19 to 64 years was 614nmol/L, 565nmol/L for women aged 19 to 64 years and 500nmol/L for children aged 11 to 18 years.

In Northern Ireland, RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was found in 11% of girls aged 11 to 18 years, 11% of women aged 19 to 64 years and 3% or less of other age groups.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 50% and 68% of children aged 11 to 18 years and between 30% and 53% of adults aged 19 to 64 years.

For women aged 19 to 64 years mean RBC folate concentration was significantly lower in Northern Ireland than in the UK as a whole (565nmol/L compared to 652nmol/L respectively). Mean RBC 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 RBC folate concentration below the clinical threshold (305nmol/L) indicating risk of anaemia was higher in Northern Ireland than in the UK as a whole for women aged 19 to 64 years.

(Table 2.2)

3.3.2 Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L).⁷ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion. For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO threshold for possible folate deficiency.

Serum folate status for women of childbearing age in Northern Ireland is discussed in Chapter 4 of this report.

In Northern Ireland the mean serum folate concentration was 16.8nmol/L for children aged 11 to 18 years. The mean serum folate concentration for men aged 19 to 64 years was 17.7nmol/L and 17.8nmol/L for women aged 19 to 64 years.

In Northern Ireland, serum folate concentration below the WHO clinical threshold indicating folate deficiency (7nmol/L) was found in 5% of children aged 11 to 18 years, 3% of men aged 19 to 64 years and 6% of women aged 19 to 64 years.

Serum folate concentration below the WHO clinical threshold indicating possible folate deficiency (13nmol/L) was found in 44% of children aged 11 to 18 years and 46% of adults aged 19 to 64 years.

The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 19% and 49% of children aged 11 to 18 years and between 19% and 54% of adults aged 19 to 64 years.

For women aged 19 to 64 years mean serum folate concentration was significantly lower in Northern Ireland than in the UK as a whole (17.8nmol/L compared to 21.2nmol/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 folate concentration below the clinical threshold of 7nmol/L was greater in Northern Ireland (6%) than in the UK as a whole (3%). The proportion of girls aged 11 to 18 years with a serum folate below the clinical threshold of 7nmol/L was also greater in Northern Ireland (5%) than in the UK as a whole (3%).

The proportion of participants with a serum folate below the WHO threshold for possible folate deficiency was higher in Northern Ireland than in the UK as a whole for men aged 19 to 64 years (44% and 33% respectively), for girls aged 11 to 18 years (52% and 38% respectively) and for women aged 19 to 64 years (48% and 29% respectively).

(Table 2.2)

3.4 Wales

3.4.1 Red blood cell (RBC) folate

Red blood cell folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L).⁶ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The

percentages below the lower and upper limits of the estimated range within which the threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

RBC folate status for women of childbearing age in Wales is discussed in Chapter 4 of this report.

The mean RBC folate concentration for men in Wales aged 19 to 64 years was 679nmol/L, 646nmol/L for women aged 19 to 64 years and 563nmol/L for children aged 11 to 18 years.

In Wales, RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was found in 4% of children aged 11 to 18 years, 4% of women aged 19 to 64 years, and less than 0.5% of men aged 19 to 64 years.

The proportion of the population with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L), was between 44% and 57% of children aged 11 to 18 years, 21% and 46% of women aged 19 to 64 years and 14% and 31% of men aged 19 to 64 years.

With the exception of men aged 19 to 64 years (Wales 679nmol/L; UK as a whole 618nmol/L) there were no significant differences in mean RBC folate concentrations between Wales and the UK as a whole. The proportions with concentrations below the clinical threshold tended to be lower in Wales than in the UK for most age/sex groups but the cell sizes are small, so results should be interpreted with caution.

(Table 2.3)

3.4.2 Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L).⁷ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO threshold for possible folate deficiency.

Serum folate status for women of childbearing age in Wales is discussed in Chapter 4 of this report.

The mean serum folate concentration for men in Wales aged 19 to 64 years was 17.4nmol/L, 20.8nmol/L for women aged 19 to 64 years, 18.7nmol/L for adults aged 65 years and over and 17.2nmol/L for children aged 11 to 18 years.

In Wales, serum folate concentration below the WHO clinical threshold indicating folate deficiency (7nmol/L) was found in 5% of children aged 11 to 18 years, 3% of adults aged 19 to 64 years and 2% of adults aged 65 years and over.

Serum folate concentration below the WHO clinical threshold indicating possible folate deficiency (13nmol/L) was found in 37% of children aged 11 to 18 years, 39% of adults aged 19 to 64 years and 41% of adults aged 65 years and over.

The proportion of the population with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L), was between 20% and 48% of children aged 11 to 18 years, 15% and 51% of adults aged 19 to 64 years and 12% and 53% of adults aged 65 years and over.

For adults aged 65 years and over mean serum folate concentration was significantly lower in Wales than in the UK as a whole (18.7nmol/L compared to 25.5nmol/L respectively). There were no significant differences for other age/sex groups.

The proportion of participants in Wales with serum folate concentrations below the clinical threshold indicating folate deficiency (7nmol/L) was similar to the proportion in the UK as whole for all age/sex groups. The proportion of participants in Wales with serum folate below the threshold for possible folate deficiency (13nmol/L) was higher than in the UK as a whole for all age/sex groups except children aged 11 to 18 years.

(Table 2.3)

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.

² National Diet and Nutrition Survey: results from Years 1 to 4 (combined) of the rolling programme for 2008 and 2009 to 2011 and 2012 <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012> (accessed 06/11/17).

³ National Diet and Nutrition Survey Rolling Programme (NDNS RP): results from Years 1- 4 (combined) for Scotland (2008/09-2011/12) <http://www.food.gov.uk/sites/default/files/ndns-scotland-full-report.pdf> (accessed 06/11/17).

⁴ National Diet and Nutrition Survey Rolling Programme (NDNS RP): Results from Years 1-4 (combined) for Northern Ireland (2008/09-2011/12) <http://www.food.gov.uk/northern-ireland/researchni/ndns-ni> (accessed 06/11/17).

⁵ National Diet and Nutrition Survey Rolling Programme (NDNS RP): Results from Years 2-5 (combined) for Wales (2009/10-2012/13) <http://gov.wales/docs/caecd/research/2015/151209-national-diet-nutrition-survey-rolling-programme-years-2-5-en.pdf> (accessed 06/11/17).

⁶ Institute of Medicine. 1998. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: The National Academies Press. <https://doi.org/10.17226/6015> (accessed 06/11/17).

⁷ WHO. Serum and red blood cell folate concentrations for assessing folate in populations. Vitamins and Mineral Nutrition Information System. 2015; 01.1-7.

Chapter 4. Blood folate concentrations of women of childbearing age (16 to 49 years)

Revision note: update and correction to data and thresholds

This report (version 2.0, published November 2017) provides an update to the original publication (in March 2015) in 3 ways: (i) changes to criteria for biochemical folate insufficiency, (ii) new thresholds for prevention of folate-sensitive neural tube defects, and (iii) correction of bias in the assay for unmetabolised folic acid. For information about these amendments please refer to the full revision note [here](#).

New thresholds for prevention of folate-sensitive neural tube defects (NTDs)

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 (NTDs) if taken in the periconceptual period.¹ Since this report was first published in March 2015 WHO has produced guidance regarding optimal RBC folate concentrations in women of reproductive age for prevention of folate-sensitive fetal NTDs.² In accordance with the WHO guidance, thresholds should also be adjusted where necessary to take into account specific assay methods used for analysis.^{2,3}

The RBC folate concentrations associated with population risks of folate-sensitive NTDs applicable to the CDC microbiological assay used for NDNS RP folate quantitation, are tabulated by Tinker *et al.*⁴ as below:

High risk	$\leq 585\text{nmol/L}$
Elevated risk	586 to 747nmol/L
Optimal concentration	748 to 1215nmol/L
No additional benefit	$\geq 1216\text{nmol/L}$

No serum folate thresholds have been defined for minimizing the risk of folate-sensitive NTDs.

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

RBC and serum folate results are presented for women of childbearing age as a whole (16 to 49 years) and split into 3 age groups: 16 to 24 years, 25 to 34 years and 35 to 49 years. All statistically significant differences between the 16 to 24 years or 25 to 34 years age group folate results and the 35 to 49 years age group (reference group) are highlighted in the tables. No statistical comparison has been carried out for a group that has less than 50 individuals.

4.1.1 Red blood cell (RBC) folate

Red blood cell folate reflects folate status over the lifetime of the erythrocytes in the circulation and is thus a good indication of medium-term folate status.

Interpretation is against the clinical threshold indicating suboptimal DNA synthesis in the bone marrow and therefore risk of anaemia (305nmol/L)⁵ and the threshold indicating elevated risk of NTDs (748nmol/L).⁴ In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of RBC folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (450nmol/L and 550nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion.

The mean RBC folate concentration for women of childbearing age (16 to 49 years) in the UK as a whole was 614nmol/L. Mean RBC folate 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 RBC folate for women aged 16 to 24 years was significantly lower than the RBC folate for women aged 35 to 49 years. There was no significant difference between the RBC 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 RBC folate concentration below the threshold indicating elevated risk of NTDs (748nmol/L) was 75% for the age group as a whole: 81% for those aged 16 to 24 years, 75% for those aged 25 to 34 years and 72% for those aged 35 to 49 years.

The proportion of women of childbearing age (16 to 49 years) who had a RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was 7%, 12% for those aged 16 to 24 years, 7% for those aged 25 to 34 years and 5% for those aged 35 to 49 years.

The proportion of women of childbearing age (16 to 49 years) with RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450-550nmol/L), was between 32% and 52%. This was between 47% and 63% for those aged 16 to 24 years, 32% and 54% for those aged 25 to 34 years and 25% and 46% for those aged 35 to 49 years.

(Table 3)

4.1.2 Serum folate

Serum folate concentration indicates short-term folate status and is affected by recent folate intake.

Interpretations are against the WHO clinical thresholds² indicating folate deficiency (7nmol/L) and possible folate deficiency (13nmol/L). In addition an estimate of biochemical folate insufficiency is made, using an estimated threshold range, based on the correlation of serum folate concentration with homocysteine concentration in the UK population from NDNS RP data. This is provided to facilitate comparison with evolving estimates of the biochemical threshold (see section 1.9 and Appendix 4). The percentages below the lower and upper limits of the estimated range within which the threshold lies (10nmol/L and 15nmol/L) indicate the proportions of each age/sex group which might be at increased risk of biochemical folate insufficiency using this criterion. For serum folate, which changes rapidly in response to folate intake, this range overlaps the WHO clinical threshold for possible folate deficiency.

The mean serum folate concentration for women of childbearing age in the UK as a whole was 19.0nmol/L for those aged 16 to 24 years, 20.2nmol/L for those aged 25 to 34 years and 20.1nmol/L for 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 folate concentration below the WHO clinical threshold indicating folate deficiency (7nmol/L) was 2% for those aged 16 to 24 years, 3% for those aged 25 to 34 years and 3% for those aged 35 to 49 years. The proportion below the WHO threshold for possible folate deficiency (13nmol/L) was 41% for those aged 16 to 24 years, 31% for those aged 25 to 34 years and 30% for those aged 35 to 49 years.

The proportion of women of childbearing age (16 to 49 years) with serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10-15nmol/L), was between 17% and 44%. This was between 23% and 50% for those aged 16 to 24 years, 18% and 45% for those aged 25 to 34 years and 14% and 41% for those aged 35 to 49 years.

(Table 3)

4.2 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 RBC and serum folate results in women of childbearing 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^{6,7,8,9} 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.

4.2.1 Scotland

4.2.1.1 Red blood cell (RBC) folate

The mean RBC folate concentration for women aged 16 to 49 years in Scotland was 564nmol/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 RBC folate concentration below the threshold indicating elevated risk of NTDs (748nmol/L)⁴ was 81% in Scotland, which was greater than in the UK as a whole (75%).

The proportion of women aged 16 to 49 years who had a RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was 11% in Scotland, which was greater than in the UK as a whole (7%). In Scotland, between 35% and 60% had RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L).

(Table 4)

4.2.1.2 Serum folate

The mean serum folate concentration for women aged 16 to 49 years in Scotland was 17.2nmol/L, which was significantly lower than in the UK as a whole (19.9nmol/L).

The proportion of women of childbearing age who had a serum folate concentration below the WHO threshold indicating folate deficiency (7nmol/L) was 7% for those aged 16 to 49 years in Scotland, which was greater than in the UK as a whole (3%). The proportion below the WHO threshold for possible folate deficiency (13nmol/L) was 46% for women aged 16 to 49 years in Scotland, which was greater than that for the UK as a whole (33%).

In women aged 16 to 49 years in Scotland, between 25% and 53% had a serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L).

(Table 4)

4.2.2 Northern Ireland

4.2.2.1 Red blood cell (RBC) folate

The mean RBC folate concentration for women in Northern Ireland aged 16 to 49 years was 512nmol/L, which was significantly lower than in the UK as a whole (614nmol/L).

The proportion of women aged 16 to 49 years who had a RBC folate concentration below the threshold indicating elevated risk of NTDs (748nmol/L)⁴ was 83% in Northern Ireland, which was greater than in the UK as a whole (75%).

The proportion of women aged 16 to 49 years in Northern Ireland who had a RBC folate concentration below clinical threshold indicating risk of anaemia (305nmol/L) was 15%, which was greater than in the UK as a whole (7%).

In Northern Ireland, between 46% and 66% had RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450-550nmol/L).

(Table 4)

4.2.2.2 Serum folate

The mean serum folate concentration for women in Northern Ireland aged 16 to 49 years was 16.1nmol/L, which was significantly lower than in the UK as a whole (19.9nmol/L).

The proportion of women in Northern Ireland who had a serum folate concentration below the WHO clinical threshold for folate deficiency (7nmol/L) was 8%, which was greater than in the UK as a whole (3%). The proportion of women aged 16 to 49 years with serum folate below the WHO threshold for possible folate deficiency (13nmol/L) was 58% in Northern Ireland, which was greater than that for the UK as a whole (33%).

In women aged 16 to 49 years in Northern Ireland, between 31% and 62% had a serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L).

(Table 4)

4.2.3 Wales

4.2.3.1 Red blood cell (RBC) folate

The mean RBC folate concentration for women in Wales aged 16 to 49 years was 608nmol/L, which was similar to that in the UK as a whole (614nmol/L).

The proportion of women aged 16 to 49 years who had a RBC folate concentration below the threshold indicating elevated risk of NTDs (748nmol/L)⁴ was 79% in Wales, which was higher than in the UK as a whole (75%).

The proportion of women aged 16 to 49 years in Wales who had a RBC folate concentration below the clinical threshold indicating risk of anaemia (305nmol/L) was 6%, which was similar to the UK as a whole (7%).

In Wales, between 24% and 48% had RBC folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (450 to 550nmol/L).

(Table 4)

4.2.3.2 Serum folate

The mean serum folate concentration for women in Wales aged 16 to 49 years was 18.7nmol/L, which was similar to that in the UK as a whole (19.9nmol/L).

The proportion of women in Wales who had a serum folate concentration below the WHO threshold indicating folate deficiency (7nmol/L) was 3%, which was the same as that in the UK as a whole (3%). The proportion of women aged 16 to 49 years with serum folate below the WHO threshold for possible folate deficiency (13nmol/L) was 45% in Wales, which was greater than that for the UK as a whole (33%).

In women aged 16 to 49 years in Wales, between 14% and 56% had a serum folate concentration indicating increased risk of biochemical folate insufficiency, as defined by the estimated range within which the threshold lies (10 to 15nmol/L).

(Table 4)

¹ Medical Research Council Vitamin Study Research Group (1991). Prevention of neural tube defects: Results of the Medical Research Council Vitamin Study. *Lancet*: 338, 131-137.

² WHO. Serum and red blood cell folate concentrations for assessing folate in populations. *Vitamins and Mineral Nutrition Information System*. 2015; 01.1-7.

³ C. M. Pfeiffer, M. R. Sternberg, H. C. Hamner, *et al.* Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population. *American Journal of Clinical Nutrition*. 2016.

<http://ajcn.nutrition.org/content/104/6/1607.full.pdf> (accessed 06/11/17).

⁴ Tinker SC, Hamner H, Qi YP, Crider KS. U.S. Women of Childbearing Age Who Are at Possible Increased Risk of a Neural Tube Defect-Affected Pregnancy Due to Suboptimal Red Blood Cell Folate Concentrations, National Health and Nutrition Examination Survey, 2007–2010. *National Health and Nutrition Examination Survey 2007 to 2012. Birth Defects Research (Part A)* 103:517–526, 2015.

⁵ Institute of Medicine. 1998. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline*. Washington, DC: The National Academies Press.

<https://doi.org/10.17226/6015> (accessed 06/11/17).

⁶ National Diet and Nutrition Survey: results from Years 1 to 4 (combined) of the rolling programme for 2008/09-2011/12 <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012> (accessed 06/11/17).

⁷ National Diet and Nutrition Survey Rolling Programme (NDNS RP): results from Years 1- 4 (combined) for Scotland (2008/09-2011/12) <http://www.food.gov.uk/sites/default/files/ndns-scotland-full-report.pdf> (accessed 06/11/17).

⁸ National Diet and Nutrition Survey Rolling Programme (NDNS RP): Results from Years 1-4 (combined) for Northern Ireland (2008/09-2011/12) <http://www.food.gov.uk/northern-ireland/researchni/ndns-ni> (accessed 06/11/17).

⁹ National Diet and Nutrition Survey Rolling Programme (NDNS RP): Results from Years 2-5 (combined) for Wales (2009/10-2012/13) <http://gov.wales/docs/caecd/research/2015/151209-national-diet-nutrition-survey-rolling-programme-years-2-5-en.pdf> (accessed 06/11/17).

Appendix 1. Folate assay methods and quality control

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 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 folate concentrations respond rapidly to change in dietary intake.

Both serum folate and whole blood folate were quantitated by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA. Haematocrit (Hct) was quantitated 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 folate concentration.

A.1.1 Red blood cell (RBC) folate quantitation

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

$$\text{RBC folate} = (\text{whole blood folate} - (\text{serum folate} \times (1 - \text{Hct}))) / \text{Hct}$$

Where a serum 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.1.2 Whole blood folate – analytical method

Whole blood haemolysate specimens (whole blood diluted and stabilized with ascorbic acid) were analysed for total folate using the *Lactobacillus rhamnosus* microbiologic growth assay by an adaptation of O'Broin *et al.*¹ and Molloy *et al.*,² as described by Pfeiffer *et al.*³ 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 to 1.0 nmol/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 haemolysate 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 $\leq 15\%$ ($\leq 10\%$ if only three replicates were used). No result was reported from less than three replicates. Assays were repeated where necessary.

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

A similar number of samples from each NDNS RP year (Years 1 to 4) were included within each assay run. Year 5 samples from Wales were run with the last of these.

All blood samples were posted at ambient temperature to the laboratory at Addenbrooke's where aliquots of the blood were preserved with 1% ascorbic acid. Any possible deterioration in folate concentration during overnight postage is likely to be less than 10%.⁴

A.1.2.1 Internal QC

Three whole blood bench QC pools were analysed 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 (nmol/L)

QC pool	LB11530a	MB11531a	HB11532a
Mean	284	442	697
SD	24	25	38
CV %	8.4%	5.7%	5.5%
Target	280	432	692

Four additional whole blood QC pools were analysed "blind" (that is, 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 (nmol/L)

QC pool	884	891	892	893
Mean	557	293	453	718
SD	31	20	42	39
CV%	5.6%	6.8%	9.3%	5.4%
<i>n</i>	41	31	27	31
Target	519	280	432	692

Accuracy has been established by spiking recovery, by periodic assaying of the 1st International Standard for Whole Blood Folate 95/528, and by successful participation in UK National External Quality Assessment Scheme (UK NEQAS) Haematinics programme (<http://www.ukneqas-haematinics.org.uk>).

A.1.3 Serum folate assay method

An isotope-dilution tandem mass spectrometry method coupled to liquid chromatography (LC-MS/MS) was used to quantitate all major folate forms in serum.^{5,6,7,8} The method performance has been described in detail in a recent paper by Fazili *et al.*⁵ The method quantitates five folate forms (5-methyltetrahydrofolate, folic acid (referred to as ‘unmetabolised 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 folate samples with 5-methylTHF results <7nmol/L were re-analysed to ensure that the estimate of low status was not attributable to analytical error.

Serum folate as reported 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).^{9,10} 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), 0.20 (unmetabolised folic acid) for years 1 and 2 and 0.09 (unmetabolised folic acid) for

years 3, 4 and 5, 0.20 (THF), 0.20 (5-formylTHF), 0.31 (5,10-methenylTHF), and 0.08 (MeFox) nmol/L.

A.1.4 Quality control (QC) for serum folate

Three serum bench QC pools were analysed in every run. The between-run imprecision for the three QCs and the target concentrations are shown in the following tables.

Year 1. Serum folate (nmol/L)

QC pool	LS11430d	MS11431d	HS11432d
Mean	20.6	44.4	73.5
SD	0.51	1.08	2.14
CV %	2.5%	2.4%	2.9%
Target	20.5	44.0	72.8

Year 2. Serum folate (nmol/L)

QC pool	LS11430d	MS11431d	HS11432d
Mean	20.8	44.4	73.0
SD	0.4	1.08	1.97
CV %	1.9%	2.4%	2.7%
Target	20.5	44.0	72.8

Year 3. Serum folate (nmol/L)

QC pool	LS11430f	MS11431f	HS11432f
Mean	21.5	45.4	74.6
SD	1.28	3.48	6.89
CV %	6.0%	7.7%	9.2%
Target	21.9	47.1	78.0

Year 4. Serum folate (nmol/L)

QC pool	LS11430f	MS11431f	HS11432f
Mean	21.3	45.3	75.2
SD	0.53	0.97	1.29
CV %	2.5%	2.1%	1.7%
Target	21.9	47.1	78.0

Year 1. Unmetabolised (free) folic acid (nmol/L)

QC sample	LS11430d	MS11431d	HS11432d
Mean	0.72	5.63	10.8
SD	0.12	0.62	1.1
CV %	17%	11%	10%
Target	0.68	5.45	10.5

Year 2. Unmetabolised (free) folic acid (nmol/L)

QC sample	LS11430d	MS11431d	HS11432d
Mean	0.74	5.43	10.3
SD	0.08	0.51	1
CV %	11.1%	9.4%	9.5%
Target	0.68	5.45	10.5

Year 3. Unmetabolised (free) folic acid (nmol/L)

QC sample	LS11430f	MS11431f	HS11432f
Mean	0.72	5.85	11.2
SD	0.08	0.4	0.81
CV %	11%	6.8%	7.2%
Target	0.67	5.81	11.1

Year 4. Unmetabolised (free) folic acid (nmol/L)

QC sample	LS11430f	MS11431f	HS11432f
Mean	0.71	5.76	11
SD	0.11	0.38	0.64
CV %	16%	6.7%	5.8%
Target	0.67	5.81	11.1

Accuracy has been established by spiking recovery, by periodic assaying 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 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 quantitates total folate, and by successful participation in the UK NEQAS Haematinics programme and other accredited external quality assessment schemes.

A.1.5 Correction of bias in folic acid concentrations

There is no external QA scheme to assess accuracy of unmetabolised folic acid concentrations. After the original publication of the NDNS RP folate results in March 2015 which included concentrations of unmetabolised folic acid, the CDC laboratory became aware that incomplete dissolution of the folic acid calibrator had led to overestimation of serum unmetabolised folic acid in these samples.

Therefore CDC corrected the assay and conducted a crossover study to adjust the previously incorrect unmetabolised folic acid results, the weighted Deming regression applied to the original results was:

new unmetabolised folic acid (nmol/L) = 0.7586 * original unmetabolised folic acid (nmol/L) - 0.016 (nmol/L)

Because folic acid is part of the formula used to calculate serum folate, which in turn is used to calculate RBC folate, CDC also revised the results for serum folate and RBC folate. The revised results are presented in this report.

The revised serum folate results are on average 0.5% lower than the original results published in March 2015 and that the revised RBC folate results are on average ~0.1% higher than the original results published in March 2015.

¹ O'Broin S, Kelleher B. Microbiological assay on microtitre plates of folate in serum and red cells. *J Clin Pathol.* 1992;45:344–7.

² AM Molloy, JM Scott. Microbiological assay for serum, plasma, and red cell folate using cryopreserved, microtiter plate method. *Methods Enzymol.* 1997;281:43–53.

³ Pfeiffer CM, Zhang M, Lacher DA, Molloy AM, Tamura T, Yetley EA, Picciano M-F, Johnson CL. Comparison of serum and red blood cell folate microbiologic assays for national population surveys. *J Nutr.* 2011;141:1402–9.

⁴ Christine M. Pfeiffer, Zia Fazili and Mindy Zhang; in: "Folate in Health and Disease" ed Lynn B. Bailey, 2nd edition, CRC Press 2010, page 555-6

⁵ Fazili Z, Whitehead Jr. Ralph, Paladugula N, Pfeiffer CM. A high-throughput LC-MS/MS method suitable for population biomonitoring measures five serum folate vitamers and one oxidation product. *Anal Bioanal Chem.* 2013;405:4549–60.

⁶ Fazili Z, Pfeiffer CM. Accounting for an isobaric interference allows correct determination of folate vitamers in serum by isotope dilution-liquid chromatography-tandem mass spectrometry. *J Nutr.* 2012;143:108–13.

⁷ Fazili Z, Pfeiffer CM. Measurement of folates in serum and conventionally prepared whole blood lysates: application of an automated 96-well plate isotope-dilution tandem mass spectrometry method. *Clin Chem.* 2004;50:2378–81.

⁸ Pfeiffer CM, Fazili Z, McCoy L, Zhang M, Gunter EW. Determination of folate vitamers in human serum by stable-isotope-dilution tandem mass spectrometry and comparison with radioassay and microbiologic assay. *Clin Chem.* 2004;50:423–32.

⁹ <http://www.cdc.gov/nchs/nhanes.htm> (accessed 17/11/17).

¹⁰ Hornung, RW, Reed, LD. *Applied Occupational and Environmental Hygiene*, 1990, 5: 46-51.

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

Notional values were computed for participants with an unmetabolised folic acid concentration below the limit of detection (LoD; 0.09nmol/L in Years 1 and 2 and 0.20nmol/L in Years 3, 4 and 5), by dividing the LoD by the square root of 2 (that is, inserting a notional value of 0.06nmol/L in Years 1 and 2 and 0.14nmol/L in Years 3, 4 and 5). 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

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 unmetabolised folic acid.

The proportion of participants with a notional value for unmetabolised 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 unmetabolised folic acid, for example, from supplements or fortified breakfast cereals, and therefore may have no unmetabolised 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 unmetabolised folic acid in Chapters 2 to 4.

This version of the report includes a correction of the calibration bias in the folic acid assay. This correction affects the magnitude of the limit of detection but does not affect the proportion of participants with a notional value for unmetabolised folic acid.

Table F. The percentage of participants^{1,2} with a notional value for unmetabolised (free) folic acid

Age and sex group ^a	UK as a whole and devolved country datasets ^a			
	UK as a whole (Years 1 to 4)	Scotland (Years 1 to 4)	Northern Ireland (Years 1 to 4)	Wales (Years 2 to 5)
Boys aged 4 to 10 years	22.2	-	-	-
Boys aged 11 to 18 years	28.3	33.3	30.0	-
Men aged 19 to 64 years	32.6	29.5	34.0	50.8
Men aged 65 years and over	35.4	45.9		-
Girls aged 4 to 10 years	14.7	-	-	-
Girls aged 11 to 18 years	31.6	33.8	34.0	
Women aged 19 to 64 years	31.4	37.7	33.1	39.3
Women aged 65 years and over	22.8	28.4	-	-
Children aged 1.5 to 3 years	7.9	-	-	-
Children aged 4 to 10 years	18.7	14.3	-	-
Children aged 11 to 18 years	29.9	33.6	32.2	32.7
Adults aged 19 to 64 years	31.9	34.2	33.5	43.5
Adults aged 65 years and over	28.0	34.6	18.2	25.5

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

^a It should be noted that the dataset used to produce this Table includes the 4 outliers discussed in Appendix 3.

¹ <http://www.cdc.gov/nchs/nhanes.htm> (accessed 06/11/17).

² Hornung, RW, Reed, LD. Applied Occupational and Environmental Hygiene, 1990, 5: 46-51.

Appendix 3. Outliers

Supplement takers are included in the dataset used to produce Tables 1 to 4 and Tables G.1 to G.4. Four individuals had plausible but unusually high blood folate concentrations. Analysis revealed that these individuals were highly influential on the mean and standard deviation (sd) estimation of the subgroups which contained them, causing the estimates to be inflated. These data were therefore excluded from statistical analyses and from data included in Tables 1 to 4.

The characteristics of the 4 individuals whose biochemical folate results were excluded from the main Tables 1 to 4 (but included in Tables G1 to G4) along with their dietary folate intakes (from all sources including supplements reported in their 4-day estimated food diary) are below. It should be noted that there was a gap of around 8 weeks between the completion of the 4-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 200 μ g folic acid from a multivitamin/mineral tablet in addition to an average daily intake of 199 μ g 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 123 μ g 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 187 μ g from his 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 89 μ g folate from her diet

Appendix 4. Thresholds for assessment of population folate deficiency, including statistical analysis of relationship between folate and homocysteine concentrations

A.4.1 Thresholds for assessment of population folate status

Folate status can be interpreted in multiple ways and therefore over time different thresholds have been proposed for defining folate sufficiency. Interpretation of population folate status is further complicated by genetic differences in folate handling and quantitatively different results generated from the various assays used over time for measurement of folate in blood samples. Initially thresholds were proposed in terms of risk of clinical deficiency (that is, the low concentrations at which macrocytic anaemia is more likely to develop) based on folate-restriction interventions with small numbers of subjects. In 2005 additional thresholds were introduced¹ to reflect biochemical indications of folate insufficiency, namely risk of raised plasma homocysteine concentration in NHANES III data. In 2015, guidelines were also established¹ for the determination of optimal folate status among women of reproductive age to minimise the occurrence of folate-sensitive NTDs and WHO have advised that this threshold be used as an indicator of folate insufficiency in women in this age group at the population level. Assay-specific conversion factors have been published² to allow the use of these thresholds with a range of folate assays which have been used over time to assess population folate status.

In September 2016 modifications were published² for all folate thresholds to be appropriate for the particular assay used. For the assay used to quantitate the NDNS RP folate data, the assay-appropriate biochemical threshold for RBC folate, derived from NHANES data, is 624nmol/L and for serum folate 14nmol/L. This represents a considerable increase in the estimation of the biochemical thresholds from those used in the first version of this report and consequently in the proportion of the population falling below them. See also section 1.9 and Table D of Chapter 1 of this report. For this reason it was decided to investigate the NDNS RP data for the relationship between folate and homocysteine concentrations to determine whether the assay-appropriate thresholds derived for the US population were also applicable to NDNS RP.

A.4.2 Relationship between serum folate concentrations and plasma homocysteine – statistical analysis.

Note – This section has been independently reviewed at the request of PHE by Dr Darren Greenwood, Biostatistician, University of Leeds.

Folate and vitamin B12 are both necessary for metabolic transfer of methyl groups during DNA synthesis. Homocysteine is also involved in this pathway and its plasma concentration tends to be elevated when there is insufficient folate or vitamin B12 available.

To investigate the relationship between folate and homocysteine for the UK NDNS RP population and to gauge the appropriateness of the WHO-recommended threshold for indicating risk of biochemical deficiency (RBC folate 624nmol/L, serum folate 14nmol/L)² a 2-phase linear regression model was used in a similar way to that in which Selhub *et al.*³ assessed the NHANES data. It should be noted that homocysteine concentrations in the 2 surveys were measured in different laboratories and several years apart, and no inter-method comparisons have been performed.

Two algorithms (Segmented and Piecewise linear regression) were investigated to model this 2-phase relationship in the statistical package R (v3.3.2), both of which gave very similar conclusions. Homocysteine was transformed to the natural log scale (ln) to ensure that the 2-phase relationship was linear.

The following restrictions were applied to the variables before modelling:

- Serum folate 0 to 100nmol/L (excluding individuals whose serum folate concentration was raised because they had recently taken a large dose of folic acid)
- Creatinine < 125µmol/L (excluding individuals whose homocysteine is raised secondary to impaired renal function)
- Serum vitamin B₁₂ >= 150pmol/L (excluding individuals whose homocysteine is raised because of low vitamin B₁₂ status)

Covariates were investigated in the model to assess their effectiveness in reducing the residual variation. These included sex, age, creatinine, estimated glomerular filtration rate and smoking status. Of these only sex and age proved to be useful in reducing the residual variation. Separate regression analyses were performed for different age splits for males and females without including any other covariates in the model.

From these analyses it was possible to estimate the folate cut-point at which the relationship with homocysteine changes. Slight differences in this cut-point were observed between the sex and age splits, however the scatterplots shown in figures A.4.1 and A.4.2 for all participants clearly indicate a large amount of variation around the biphasic relationship so we provide a single range for the biochemical threshold

which should be used for all sex and age splits. The RBC folate concentration which is associated with an increased risk of an elevated plasma homocysteine concentration is estimated to be lower than the assay-adjusted WHO-recommended threshold for indicating risk of biochemical folate insufficiency (624nmol/L). For NDNS RP samples the folate concentration at the cut-point is 496nmol/L; our estimate that the threshold probably lies between 450nmol/L and 550nmol/L reflects uncertainty caused primarily by the poor correlation (wide scatter) shown in the graph (see figure A.4.1). The serum folate concentration at the cut-point associated with increasing risk of raised plasma homocysteine concentration for the UK population (13.5nmol/L) is similar to the threshold associated with increased risk of raised homocysteine endorsed by WHO (14nmol/L after adjustment to be suitable for the LC-MS/MS assay used for NDNS RP samples). Our estimate that the biochemical threshold probably lies between 10nmol/L and 15nmol/L reflects uncertainty caused primarily by the poor correlation (wide scatter) shown in the graph (see figure A.4.2).

Therefore the text of this report interprets folate concentrations primarily against clinical thresholds indicating folate deficiency likely to cause haematological change and, where appropriate, thresholds indicating increased risk of folate-sensitive NTDs. Interpretation against provisional estimates of thresholds indicating risk of biochemical folate deficiency is necessarily tentative, quoting the percentages with RBC folate below 450nmol/L and 550nmol/L and the percentages with serum folate below 10nmol/L and 15nmol/L. Cumulative population distributions are also presented in the tables alongside these percentages, to facilitate interpretation against any future estimates of the biochemical threshold.

Figure A.4.1 Scatterplot of In[homocysteine] vs [red blood cell folate] for NDNS RP Years 1 to 5 all participants. The red lines represent the biphasic line of best fit (Piecewise) and cut-point at red blood cell folate = 496nmol/L

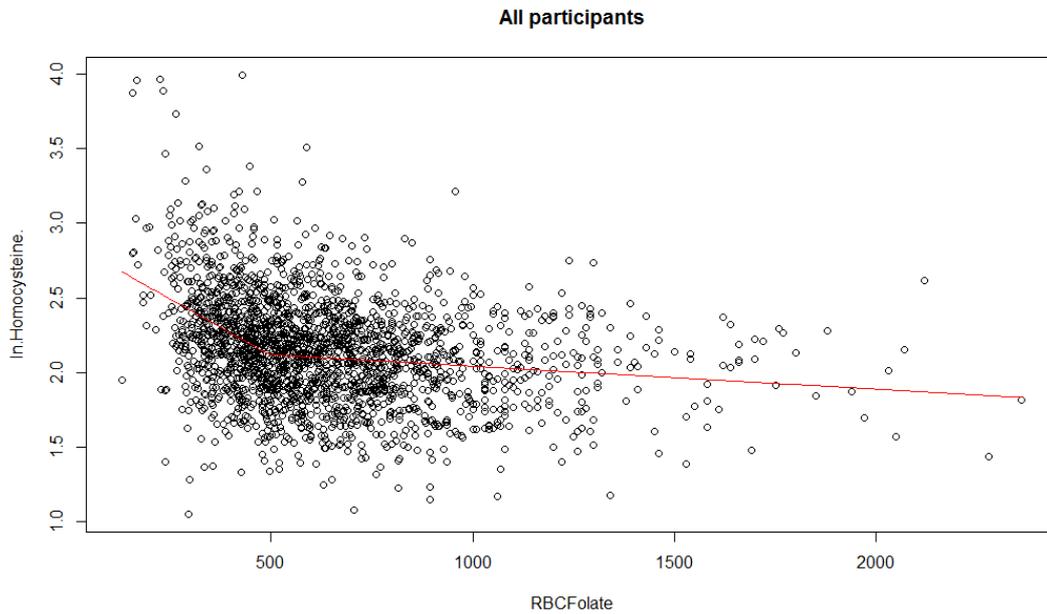
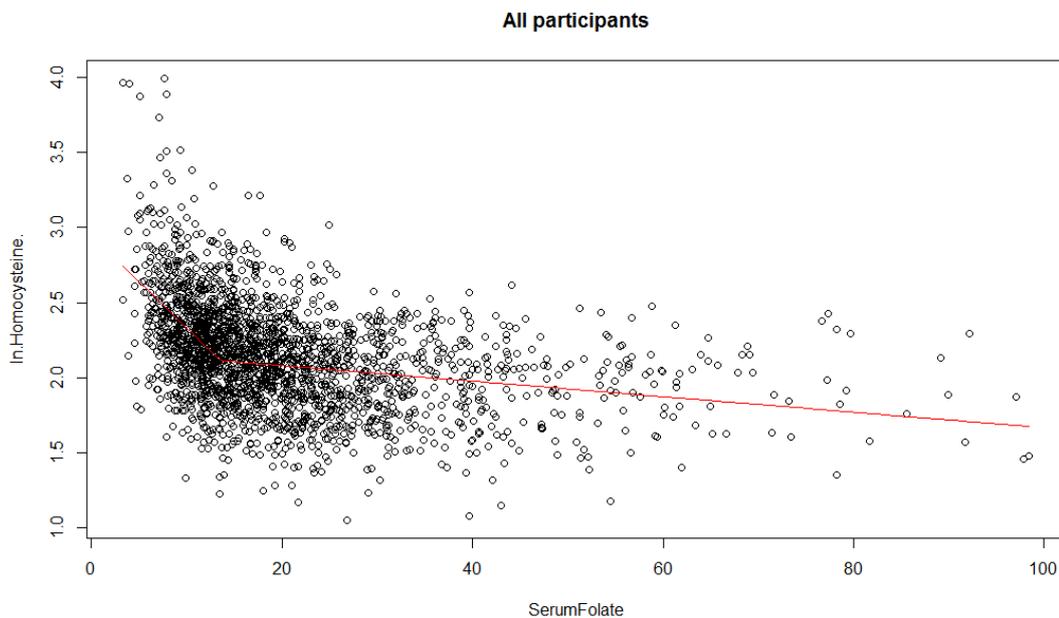


Figure A.4.2 Scatterplot of ln [homocysteine] vs [serum folate] for NDNS RP Years 1 to 5 all participants. The red lines represent the biphasic line of best fit (Piecewise) and cut-point at serum folate = 13.5nmol/L



¹ WHO. Serum and red blood cell folate concentrations for assessing folate in populations. Vitamins and Mineral Nutrition Information System. 2015; 01.1-7.

² C. M. Pfeiffer, M. R. Sternberg, H. C. Hamner, *et al.* Applying inappropriate cutoffs leads to misinterpretation of folate status in the US population. *American Journal of Clinical Nutrition*. 2016.

<http://ajcn.nutrition.org/content/104/6/1607.full.pdf> (accessed 06/11/17).

³ J. Selhub, P. F. Jacques, G. Dallal, S. Choumenkovitch, G. Rogers. The use of blood concentrations of vitamins and their respective functional indicators to define folate and vitamin B12 status. *Food Nutrition Bulletin*. 2008; 29 (2 suppl): S67-73.