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Appendix A

NHANES Reports Related to Nutritional Status

National Center for Health Statistics (NCHS) Data Briefs

<http://www.cdc.gov/nchs/products/databriefs.htm>

Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS Data Brief, No 59. Hyattsville, MD: National Center for Health Statistics. 2011.

McDowell MA, Lacher DA, Pfeiffer CM, Mulinare J, Picciano MF, Rader JI, et al. Blood Folate Levels: The Latest NHANES Results. NCHS Data Brief, No 6. Hyattsville, MD: National Center for Health Statistics. 2008.

National Center for Health Statistics (NCHS) Advance Data Reports

<http://www.cdc.gov/nchs/products/ad.htm>

Advance Data No. 349. Prevalence of leading types of dietary supplements used in the Third National Health and Nutrition Examination Survey, 1988–94. 8 pp. (PHS) 2005–1250.

Advance Data No. 348. Dietary intake of fats and fatty acids for the United States population: 1999–2000. 7 pp. (PHS) 2005–1250.

Advance Data No. 341. Dietary intake of selected minerals for the United States population: 1999–2000. 6 pp. (PHS) 2004–1250.

Advance Data No. 339. Dietary intake of selected vitamins for the United States population: 1999–2000. 5 pp. (PHS) 2004–1250.

Advance Data No. 334. Dietary intake of ten key nutrients for public health, United States: 1999–2000. 4 pp. (PHS) 2003–1250.

National Center for Health Statistics (NCHS) Series 11 Reports

<http://www.cdc.gov/nchs/products/series/series11.htm>

Hollowell JG, van Assendelft OW, Gunter EW, Lewis BG, Najjar M, Pfeiffer C. Hematological and iron-related analytes—Reference data for persons aged 1 year and over: United States, 1988–1994. National Center for Health Statistics. Vital Health Stat Series No. 11(247), 2005.

Bialostosky K, Wright JD, Kennedy-Stephenson J, McDowell M, Johnson CL. Dietary intake of macronutrients, micronutrients, and other dietary constituents: United States 1988–1994. National Center for Health Statistics. Vital Health Stat Series No. 11(245), 2002.

Ervin RB, Wright JD, Kennedy-Stephenson J. Use of dietary supplements in the United States, 1988–1994. National Center for Health Statistics. Vital Health Stat Series No. 11(244), 1999.

Wright JD, Bialostosky K, Gunter EW, Carroll MD, Najjar MF, Bowman BA, Johnson CL. Blood folate and vitamin B12: United States, 1988–1994. National Center for Health Statistics. Vital Health Stat Series No. 11(243), 1998.

Fulwood R, Johnson CL, Bryner JD, Gunter EW, McGrath CR. Hematological and nutritional biochemistry reference data for persons 6 months–74 years of age: United States, 1976–1980. National Center for Health Statistics. Vital Health Stat Series No. 11(232), 1982.

National Center for Health Statistics (NCHS) Series 2 Reports

<http://www.cdc.gov/nchs/products/series/series02.htm>

Looker AC, Gunter EW, Cook JD, Green R, Harris JW. Comparing serum ferritin values from different population surveys. National Center for Health Statistics. Vital Health Stat Series No. 2(111), 1991.

Life Sciences Research Office (LSRO) Reports

Pilch SM. Assessment of the vitamin A nutritional status of the U.S. population based on data collected in the Health and Nutrition Examination Surveys. Bethesda (MD): Federation of American Societies for Experimental Biology; 1985.

Senti FR, Pilch SM. Analysis of the folate nutritional status of the U.S. population based on data collected in the Second National Health and Nutrition Examination Survey, 1976–1980. Bethesda (MD): Federation of American Societies for Experimental Biology; 1984.

Pilch SM, Senti FR. Assessment of iron nutritional status of the U.S. population based on data collected in the Second National Health and Nutrition Examination Survey, 1976–1980. Bethesda (MD): Federation of American Societies for Experimental Biology; 1984.

Pilch SM, Senti FR. Assessment of zinc nutritional status of the U.S. population based on data collected in the Second National Health and Nutrition Examination Survey, 1976–1980. Bethesda (MD): Federation of American Societies for Experimental Biology; 1984.

Life Sciences Research Office. 1989. An update report on nutrition monitoring in the United States. Prepared for the U.S. Department of Agriculture and the U.S. Department of Health and Human Services. DHHS Publication No. (PHS) 89–1255. Available from U.S. Government Printing Office, Washington, D.C.

Life Sciences Research Office. 1994. Assessment of folate methodology used in the Third National Health and Nutrition Survey (NHANES 1988-1994). Prepared for the Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services. Washington, D.C.

Appendix B

Information Presented in the Report

The table below provides information on the type of data included for each indicator and the years of NHANES covered.

| Indicator | Table: Concentrations | Figure: Concentrations by age group | Tables: Concentrations by race/ethnic group | Table and Figure: Concentrations by survey cycle | Table(s): Prevalence (Deficiency/Excess) | Table(s): Prevalence by survey cycle (Deficiency/Excess) |
|---|--------------------------|---|--|--|--|---|
| Water-Soluble Vitamins | | | | | | |
| B Vitamins and Related Biochemical Compounds | | | | | | |
| Serum folate | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | not shown*/none | not shown*/none |
| Red blood cell folate | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | 2003-2006/none | 1999-2006/none |
| Serum pyridoxal-5'-phosphate | 2005-2006 | 2005-2006 | 2005-2006 | none | 2005-2006/none | none/none |
| Serum 4-pyridoxic acid | 2005-2006 | 2005-2006 | 2005-2006 | none | none/none | none/none |
| Serum vitamin B12 | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | 2003-2006/none | 1999-2006/none |
| Plasma homocysteine | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | 2003-2006/none | 1999-2006/none |
| Plasma methylmalonic acid | 2003-2004 | 2003-2004 | 2003-2004 | 1999-2004 | 2003-2004/none | 1999-2004/none |
| Serum vitamin C | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006/none | 2003-2006/none |
| Fat-Soluble Vitamins and Nutrients | | | | | | |
| Vitamins A and E and Carotenoids | | | | | | |
| Serum vitamin A | 2005-2006 | 2005-2006 | 2005-2006 | 1999-2002; 2005-2006 | 2005-2006/2005-2006 | 1999-2002; 2005-2006/ 1999-2002; 2005-2006 |
| Serum retinyl palmitate | 2005-2006 | 2005-2006 | 2005-2006 | none | none/none | none/none |
| Serum retinyl stearate | 2005-2006 | not shown* | 2005-2006 | none | none/none | none/none |
| Serum vitamin E | 2005-2006 | 2005-2006 | 2005-2006 | 1999-2002; 2005-2006 | 2005-2006/not shown* | 1999-2002; 2005-2006/ not shown* |
| Serum <i>gamma</i> -tocopherol | 2005-2006 | 2005-2006 | 2005-2006 | 1999-2002; 2005-2006 | none/none | none/none |
| Serum <i>alpha</i> -carotene | 2005-2006 | 2005-2006 | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum <i>trans-beta</i> -carotene | 2005-2006 | 2005-2006 | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum <i>cis-beta</i> -carotene | 2005-2006 | not shown* | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum beta-cryptoxanthin | 2005-2006 | 2005-2006 | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum lutein and zeaxanthin | 2005-2006 | 2005-2006 | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum <i>trans</i> -lycopene | 2005-2006 | 2005-2006 | 2005-2006 | 2001-2002; 2005-2006 | none/none | none/none |
| Serum total lycopene | 2005-2006 | 2005-2006 | 2005-2006 | none | none/none | none/none |
| Serum 25-hydroxyvitamin D | 2003-2006 | 2003-2006 | 2003-2006 | 2001-2006 | 2003-2006/2003-2006 | 2001-2006/2001-2006 |
| Fatty Acids - Saturated | | | | | | |
| Plasma myristic acid (14:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma palmitic acid (16:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma stearic acid (18:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma arachidic acid (20:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosanoic acid (22:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma lignoceric acid (24:0) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |

| Indicator | Table: Concentrations | Figure: Concentrations by age group | Tables: Concentrations by race/ethnic group | Table and Figure: Concentrations by survey cycle | Table(s): Prevalence (Deficiency/Excess) | Table(s): Prevalence by survey cycle (Deficiency/Excess) |
|--|--------------------------|---|--|--|--|---|
| Fatty Acids - Monounsaturated | | | | | | |
| Plasma myristoleic acid (14:1n-5) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma palmitoleic acid (16:1n-7) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma <i>cis</i> -vaccenic acid (18:1n-7) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma oleic acid (18:1n-9) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma eicosenoic acid (20:1n-9) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosenoic acid (22:1n-9) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma nervonic acid (24:1n-9) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Fatty Acids - Polyunsaturated | | | | | | |
| Plasma linoleic acid (18:2n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma <i>alpha</i> -linolenic acid (18:3n-3) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma <i>gamma</i> -linolenic acid (18:3n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma eicosadienoic acid (20:2n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma <i>homo-gamma</i> -linolenic acid (20:3n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma arachidonic acid (20:4n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma eicosapentaenoic acid (20:5n-3) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosatetraenoic acid (22:4n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosapentaenoic acid (22:5n-3) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosapentaenoic acid (22:5n-6) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Plasma docosahexaenoic acid (22:6n-3) | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Trace Elements | | | | | | |
| Iron-Status Indicators | | | | | | |
| Serum ferritin | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | 2003-2006/2003-2006 | 1999-2006/1999-2006 |
| Serum soluble transferrin receptor | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006/none | 2003-2006/none |
| Body iron | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006 | 2003-2006/none | 2003-2006/none |
| Urinary iodine | 2003-2006 | 2003-2006 | 2003-2006 | 2001-2006 | none/none | none/none |
| Isoflavones & Lignans | | | | | | |
| Urinary genistein | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Urinary daidzein | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Urinary equol | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Urinary O-desmethylangolensin | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Urinary enterodiol | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Urinary enterolactone | 2003-2006 | 2003-2006 | 2003-2006 | 1999-2006 | none/none | none/none |
| Acrylamide Hemoglobin Adducts | | | | | | |
| Acrylamide hemoglobin adduct | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Glycidamide hemoglobin adduct | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |
| Glycidamide-to-acrylamide hemoglobin adduct ratio | 2003-2004 | 2003-2004 | 2003-2004 | none | none/none | none/none |

* Prevalence table is not shown if most or all estimates have been suppressed because of the RSE being $\geq 40\%$.

Appendix C

Cutoff Points used to Generate Prevalence Estimates

The table below presents the cutoff values used to calculate prevalence estimates of low or high concentrations of biochemical indicators for various population groups and the years for which NHANES data were available. The clinical interpretation of the cutoff values is described in the text that accompanies each chapter.

| Indicator | Units | Cutoff value | Population described | NHANES years available | |
|------------------------------------|--------|--------------|------------------------|------------------------|-----------|
| Water-Soluble Vitamins | | | | | |
| Serum folate | ng/mL | < 2 | ≥ 3 years | 1999–2002 | |
| | | | ≥ 1 years | 2003–2006 | |
| Red blood cell folate | ng/mL | < 95 | ≥ 3 years | 1999–2002 | |
| | | | ≥ 1 years | 2003–2006 | |
| Serum pyridoxal-5'-phosphate | nmol/L | < 20 | ≥ 1 years | 2005–2006 | |
| Serum vitamin B12 | pg/mL | < 200 | ≥ 3 years | 1999–2002 | |
| | | | ≥ 1 years | 2003–2006 | |
| Plasma homocysteine | μmol/L | > 13 | ≥ 3 years | 1999–2004 | |
| | | | ≥ 20 years | 2005–2006 | |
| Plasma methylmalonic acid | nmol/L | > 271 | ≥ 3 years | 1999–2004 | |
| Serum vitamin C | μmol/L | < 11.4 | ≥ 6 years | 2003–2006 | |
| Fat-Soluble Vitamins | | | | | |
| Serum vitamin A | μg/dL | < 20 | ≥ 6 years | 1999–2002; 2005–2006 | |
| | | | > 100 | 1999–2002; 2005–2006 | |
| Serum vitamin E | μg/dL | < 500 | ≥ 6 years | 1999–2002; 2005–2006 | |
| | | | > 20,000 | 1999–2002; 2005–2006 | |
| Serum 25-hydroxyvitamin D | nmol/L | < 30 | ≥ 6 years or ≥ 1 year* | 2001–2006 | |
| | | | 30–< 50 | ≥ 6 years or ≥ 1 year* | 2001–2006 |
| | | | < 40 | ≥ 6 years or ≥ 1 year* | 2001–2006 |
| | | | > 125 | ≥ 6 years or ≥ 1 year* | 2001–2006 |
| Iron-Status Indicators | | | | | |
| Serum ferritin | ng/mL | < 12 | 1-5 years | 1999–2006 | |
| | | | < 15 | Females 12-49 years | 1999–2006 |
| | | | < 15 | Males ≥ 6 years | 1999–2002 |
| | | | > 150 | Females 12-49 years | 1999–2006 |
| | | | > 200 | Males ≥ 12 years | 1999–2002 |
| Serum soluble transferrin receptor | mg/L | > 4.4 | Females 12-49 years | 2003–2006 | |
| Body iron | mg/kg | < 0 | 1-5 years | 2003–2006 | |
| | | | < 0 | Females 12-49 years | 2003–2006 |

*2001–2002: ≥ 6 years; 2003–2006: ≥ 1 year

Appendix D

References for Analytical Methods for Biochemical Indicators

Detailed Laboratory Procedure Manuals for Each Analytical Method:

- NHANES 2003–2004: http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab_methods_03_04.htm.
- NHANES 2005–2006: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab_methods_05_06.htm.

Additional Useful Analytical Method References:

Water-Soluble Vitamins

Gunter EW, Bowman BA, Caudill SP, Twite DB, Adams MJ, Sampson EJ. Results of an international round robin for serum folate and whole-blood folate. *Clin Chem.* 1996;42:1689–1694.

Life Sciences Research Office. Assessment of folate methodology used in the Third National Health and Nutrition Survey (NHANES 1988-1994). Washington, D.C.: Center for Food Safety and Applied Nutrition, Food and Drug Administration, Department of Health and Human Services; 1994.

McCoy LF, Bowen MB, Xu M, Chen H, Schleicher RL. Improved HPLC assay for measuring serum vitamin C with 1-methyluric acid used as an electrochemically active internal standard. *Clin Chem.* 2005;51:1062–1064.

Pfeiffer CM, Twite D, Shih J, Holets-McCormack SR, Gunter EW. Method comparison for total plasma homocysteine between the Abbott IMx analyzer and an HPLC assay with internal standardization. *Clin Chem.* 1999;45(1):152–153.

Pfeiffer CM, Huff DL, Smith SJ, Miller DT, Gunter EW. Comparison of plasma total homocysteine measurements in 14 laboratories: an international study. *Clin Chem.* 1999;45(8):1261–1268.

Pfeiffer CM, Smith SJ, Miller DT, Gunter EW. Comparison of serum and plasma methylmalonic acid measurements in 13 laboratories: an international study. *Clin Chem.* 1999;45:2236–2242.

Pfeiffer CM, Caudill SP, Gunter EW, Bowman BA, Jacques PF, Selhub J, et al. Discussion of critical issues related to the comparison of homocysteine values between the Third National Health and Nutrition Examination Survey (NHANES) and NHANES 1999+. *J Nutr.* 2000;130:2850–2854.

Rybak ME, Pfeiffer CM. Clinical analysis of vitamin B₆: Determination of pyridoxal 5'-phosphate and 4-pyridoxic acid in human serum by reversed-phase high-performance liquid chromatography with chlorite postcolumn derivatization. *Anal Biochem.* 2004;333:336–344.

Rybak ME, Jain RB, Pfeiffer CM. Clinical vitamin B₆ analysis: an inter-laboratory comparison of pyridoxal 5'-phosphate measurements in serum. *Clin Chem.* 2005;51:1223–1231.

Rybak ME, Pfeiffer CM. A simplified protein precipitation and filtration procedure for determining serum vitamin B₆ by high-performance liquid chromatography. *Anal Biochem.* 2009;388:175–177.

Fat-Soluble Vitamins and Nutrients

Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, alpha-tocopherol, lutein/zeaxanthin, beta-cryptoxanthin, lycopene, alpha-carotene, trans-beta-carotene, and four retinyl esters in serum determined simultaneously by reversed-phase HPLC with multi-wavelength detection. *Clin Chem.* 1994;40:411–416.

Trace Elements

Looker AC, Gunter EW, Johnson CL. Methods to assess iron status in various NHANES surveys. *Nutr Rev.* 1995;53:246–254.

Pfeiffer CM, Cook JD, Mei Z, Cogswell ME, Looker AC, Lacher DA. Evaluation of an automated soluble transferrin receptor (sTfR) assay on the Roche Hitachi analyzer and its comparison to two ELISA assays. *Clin Chim Acta* 2007;382:112–116.

Caldwell KL, Maxwell CB, Makhmudov A, Pino S, Braverman LE, Jones RL, et al. Use of inductively coupled plasma mass spectrometry to measure urinary iodine in NHANES 2000: comparison with previous method. *Clin Chem.* 2003;49:1019–1021.

Isoflavones and Lignans

Valentin-Blasini L, Blount BC, Rogers HS, Needham LL. HPLC-MS/MS method for the measurement of seven phytoestrogens in human serum and urine. *J Expo Anal Environ Epidemiol.* 2000;10:799–807.

Kuklennyik Z, Ye X, Reich JA, Needham LL, Calafat AM. Automated on-line and off-line solid phase extraction methods for measuring isoflavones and lignans in urine. *J Chromatogr Sci.* 2004;42:495–500.

Rybak ME, Parker DL, Pfeiffer CM. Determination of urinary phytoestrogens by HPLC-MS/MS: a comparison of atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI). *J Chromatogr B.* 2008;861:145–150.

Parker DL, Rybak ME, Pfeiffer CM. Phytoestrogen biomonitoring: an extractionless LC-MS/MS method for measuring urinary isoflavones and lignans using atmospheric pressure photoionization (APPI). *Anal Bioanal Chem.* 2012;402:1123–1136.

Acrylamide Hemoglobin Adducts

Vesper HW, Ospina M, Meyers T, Ingham L, Smith A, Gray JG, Myers GL. Automated method for measuring globin adducts of acrylamide and glycidamide at optimized Edman reaction conditions. *Rapid Commun Mass Spectrom.* 2006;20:959–964.

Appendix E

Confidence Interval Estimation for Percentiles

A large body of literature describes various methods to estimate percentiles and to derive the variance and confidence intervals for complex survey data. Highlighted in the literature are the following methods: Woodruff method (Woodruff 1952), “test inversion” method (Francisco and Fuller 1991), the Normal transformation method (Korn and Graubard 1999), and Replication methods (Kovar 1988, Rogers 2003).

Confidence intervals for percentiles in this report were calculated with the Woodruff method. This method uses the standard error of the empirical distribution function at the selected percentile and constructs a 95% confidence interval, followed by back transformation using the inverse of the empirical distribution. The previous National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population, 1999–2002 used a variation of the Woodruff method by combining it with the method of Clopper and Pearson proposed by Korn and Graubard (1999) for complex surveys. This approach was used previously because large-sample normal approximations used to calculate confidence intervals for proportions close to zero or 1 can lead to confidence intervals with poor coverage properties. However, a paper by Sitter and Wu (2001) concluded that despite the fact that confidence intervals around the empirical distribution function at tail regions perform poorly, the Woodruff confidence intervals obtained by inverting these poorly behaved intervals perform very well for percentiles. Therefore, the confidence intervals presented in this report are based on the Woodruff approach with no further modifications, as described in the steps below.

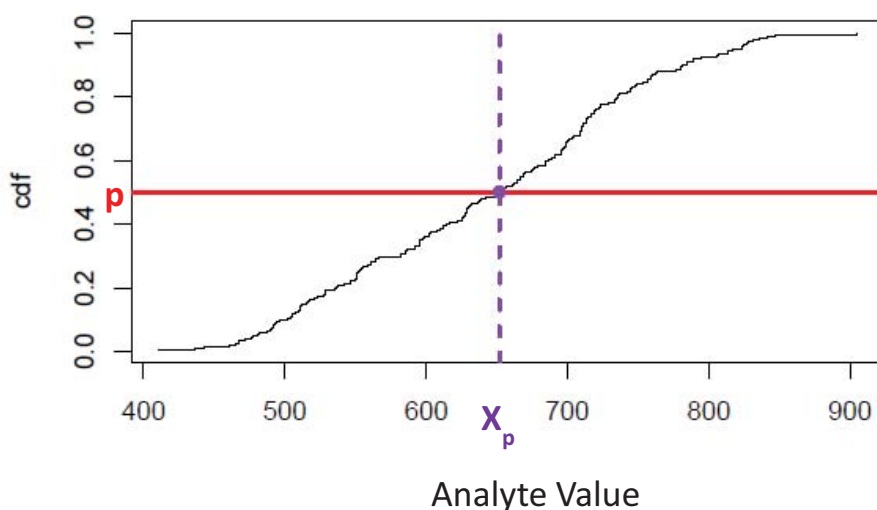
Background

Define an arbitrary percentile X_p , such that $F(X) = P(X \leq X_p) = p$. This is pictured in Figure 1, where the y-axis displays the empirical distribution function (cdf) over a set of hypothetical values. In this example, $p = 0.5$ and so $X_{0.5}$ is the median. Both SAS (version 9.2) and SUDAAN (version 10.0) find X_p through linear interpolation. Let $\hat{F}(x_j)$ be an estimate of the empirical distribution function at x and assume data x_1, x_2, \dots, x_n are a rank ordered listing of the sampled values, such that x_1 is the minimum value and x_n is the maximum value; then the estimated percentile by use of linear interpolation is calculated as:

$$\hat{X}_p = x_j + \frac{p - \hat{F}(x_j)}{\hat{F}(x_{j+1}) - \hat{F}(x_j)}(x_{j+1} - x_j) \quad \hat{F}(x_j) \leq p < \hat{F}(x_{j+1}).$$

To find the percentiles and confidence intervals in this report, we used results derived from SUDAAN's PROC DESCRIPT (DESIGN=WR) PERCENTILE statement and results from the Histogram output group.

Figure 1: Definition of a percentile



Step 1

Use SUDAAN (DESIGN=WR) to estimate the percentiles, the empirical distribution, and the standard error of the empirical distribution function at each point. SUDAAN uses a Horowitz-Thompson estimator of the empirical distribution function at each value. The estimated empirical distribution function can be outputted into a dataset using the HISTPCT statement in conjunction with an OUTPUT statement in PROC DESCRIPT. By default, SUDAAN estimates the empirical distribution function by using a maximum of 100 equally spaced percentages to divide the population into bins. We used the option /NPCT in the HISTPCT statement to change this default to allow a jump in the empirical distribution function at every unique data value, up to 2950 unique values. An output file is generated by SUDAAN to contain: the upper endpoint of the current bin in the histogram, the cumulative percent less than or equal to the upper endpoint of the current bin and the respective estimated standard error of the cumulative percent. This file is used to obtain 95% confidence intervals of the empirical distribution function at the selected percentile. In some rare cases, using the values of the upper endpoint available in this file may differ slightly from a rank order list of the weighted sampled values if there are more than 2950 distinct values. This difference may lead to very small differences when comparing the confidence limits to other software which uses Woodruff confidence limits based on the weighted sample values. Sample SUDAAN code for serum folate (FOL) is as follows:

```

PROC DESCRIPT DATA=NHANES03_06 FILETYPE=SAS DESIGN=WR ;
    NEST SDMVSTRA SDMVPSU/MISSUNIT;
    WEIGHT WTMEC4YR;
    VAR  LBXFOL;
    PERCENTILES 5 10 90 95 /MEDIAN ;
    HISPCT /NPCT=2950;
    OUTPUT QTILE /FILENAME=PCTILES
FILETYPE=SAS REPLACE ;
OUTPUT UPPEREND CUMPCT SECUMPCT  /FILENAME=HIST FILETYPE=SAS
REPLACE;

```

If you change the first OUTPUT statement in the above program to

```

OUTPUT QTILE LOWQTILE UPQTILE /FILENAME=PCTILES FILETYPE=SAS
REPLACE ;

```

SUDAAN will provide the upper and lower confidence limits based on the “test inversion” method of Francisco and Fuller. However, as mentioned earlier, this report does not use SUDAAN’s default method to generate confidence intervals for percentiles.

Step 2

Using SAS DATA steps to manipulate the output files from Step 1, find the value of the estimated cumulative distribution function that is less than or equal to p using the values of the cumulative percent produced by SUDAAN in the output file HIST:

Save $\hat{F}(x_j)$ and the corresponding standard error (SE) estimate at $\hat{F}(x_j)$ and proceed to step 3.

$$\hat{F}(x_j) \leq p < \hat{F}(x_{j+1})$$

Step 3

Using p (**the desired percentile**) and the standard error of the estimate of $\hat{F}(x_j)$, compute the 95% confidence interval for p : (p_L, p_U) as $p \pm t_{0.025, DF} SE$, where the degrees of freedom (DF) are the number of primary sampling units minus the number of strata and SE is the standard error from step 2. To get the appropriate degrees of freedom for each subgroup use the ATLEVEL1 and ATLEVEL2 options in SUDAAN’s PROC DESCRIPT to count up the number of strata and PSUs with valid data. This must be done in a separate call to PROC DESCRIPT than the one that calculates the percentiles because the HISTPCT statement is not available with ATLEVEL. Note: SAS (version 9.2) uses the empirical point estimate at the desired percentile, $\hat{p} = \hat{F}(x_j)$, in order to calculate the 95% confidence interval as $\hat{p} \pm t_{0.025, DF} SE$.

Step 4

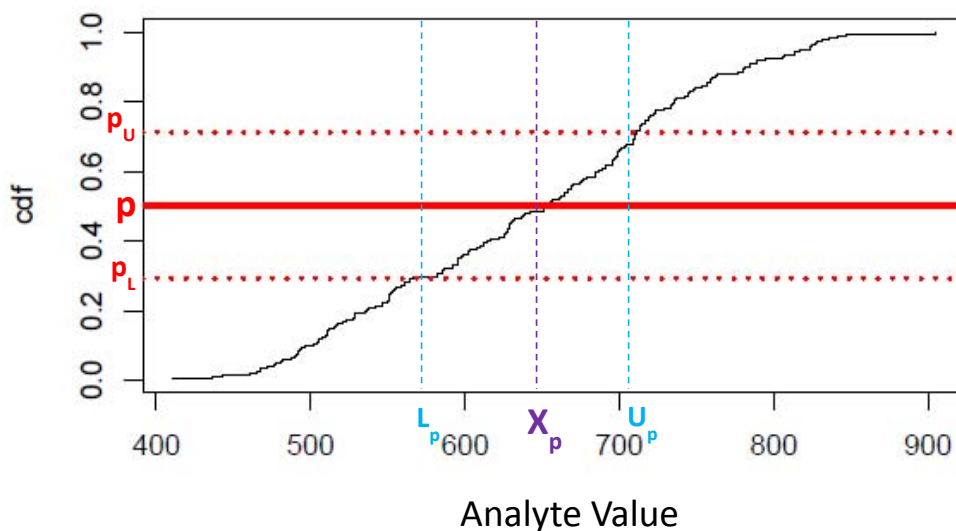
Map the lower (p_L) and upper (p_U) confidence intervals of the empirical distribution function at the desired percentile using the inverse of the empirical distribution function (see Figure 2) and linear interpolation to get the confidence interval for the percentile of interest.

Let \hat{L}_p be the lower confidence limit of the estimated percentile and \hat{U}_p be the upper confidence limit of the estimated percentile. x_1, x_2, \dots, x_n are a rank ordered listing of the values as produced by SUDAAN using HISTPCT, such that x_1 is the minimum value and x_n is the maximum value; then these can be found from the following expressions:

$$\hat{L}_p = \begin{cases} x_1 & p_L < \hat{F}(x_1) \\ x_j + \frac{p_L - \hat{F}(x_j)}{\hat{F}(x_{j+1}) - \hat{F}(x_j)} (x_{j+1} - x_j) & \hat{F}(x_j) \leq p_L < \hat{F}(x_{j+1}) \\ x_n & p_L = 1 \end{cases}$$

$$\hat{U}_p = \begin{cases} x_1 & p_U < \hat{F}(x_1) \\ x_j + \frac{p_U - \hat{F}(x_j)}{\hat{F}(x_{j+1}) - \hat{F}(x_j)} (x_{j+1} - x_j) & \hat{F}(x_j) \leq p_U < \hat{F}(x_{j+1}) \\ x_n & p_U = 1 \end{cases}$$

Figure 2



Commercial Software

PROC DESRIPT in SUDAAN (version 8.0 and higher) calculates confidence limits for the percentiles using the “test-inversion” method by Francisco and Fuller, as noted in Step 1.

PROC SURVEYMEANS (SAS version 9.1 and higher) can be used to obtain Woodruff like confidence intervals for percentiles. However, the SURVEYMEANS method differs slightly from the traditional Woodruff method as noted in Step 3.

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Appendix F

Limit of Detection Table

The table below presents the analytical limit of detection (LOD) for each indicator. The LOD is the level at which the measurement has a 95 percent probability of being greater than zero (Taylor 1987). For the same indicator, LOD values may change over time as a result of changes to analytical methods. This was the case for serum ferritin and urinary phytoestrogens. We used the highest LOD value when multi-year data were combined. The information provided in parentheses specifies what proportion of results was below the LOD for each indicator and survey cycle.

| Indicator | Units | 1999-2000 | 2001-2002 | 2003-2004 | 2005-2006 |
|--|--------|-------------|-------------|-------------|-------------|
| Water-Soluble Vitamins | | | | | |
| B Vitamins and Related Biochemical Compounds | | | | | |
| Serum folate | ng/mL | 0.1 (0%) | 0.1 (0%) | 0.1 (0%) | 0.1 (0%) |
| Red blood cell folate | ng/mL | 20 (0%) | 20 (0%) | 20 (< 1%) | 20 (0%) |
| Serum pyridoxal-5'-phosphate | nmol/L | no data | no data | no data | 0.3 (0%) |
| Serum 4-pyridoxic acid | nmol/L | no data | no data | no data | 0.3 (0%) |
| Serum vitamin B12 | pg/mL | 20 (0%) | 20 (0%) | 20 (0%) | 20 (0%) |
| Plasma homocysteine | µmol/L | 0.35 (0%) | 0.35 (0%) | 0.35 (0%) | 0.35 (0%) |
| Plasma methylmalonic acid | nmol/L | 50 (1%) | 50 (1%) | 50 (< 1%) | no data |
| Serum vitamin C | µmol/L | no data | no data | 0.68 (< 1%) | 0.68 (< 1%) |
| Fat-Soluble Vitamins and Nutrients | | | | | |
| Vitamins A and E and Carotenoids | | | | | |
| Serum vitamin A (retinol) | µg/dL | 1.03 (0%) | 1.03 (0%) | no data | 1.03 (< 1%) |
| Serum vitamin E (<i>alpha</i> -tocopherol) | µg/dL | 40.7 (0%) | 40.7 (0%) | no data | 40.7 (0%) |
| Serum <i>gamma</i> -tocopherol | µg/dL | 10.7 (< 1%) | 10.7 (< 1%) | no data | 10.7 (0%) |
| Serum <i>alpha</i> -carotene | µg/dL | no data | 0.7 (9%) | no data | 0.7 (7%) |
| Serum <i>trans-beta</i> -carotene | µg/dL | no data | 0.8 (< 1%) | no data | 0.8 (< 1%) |
| Serum <i>cis-beta</i> -carotene | µg/dL | no data | 0.7 (52%) | no data | 0.7 (51%) |
| Serum <i>beta</i> -cryptoxanthin | µg/dL | no data | 0.9 (< 1%) | no data | 0.9 (< 1%) |
| Serum lutein and zeaxanthin | µg/dL | no data | 2.4 (< 1%) | no data | 2.4 (< 1%) |
| Serum <i>trans</i> -lycopene | µg/dL | no data | 0.8 (< 1%) | no data | 0.8 (< 1%) |
| Serum total lycopene | µg/dL | no data | no data | no data | 1.0 (< 1%) |
| Serum retinyl palmitate | µg/dL | 0.2 (16%) | 0.2 (2%) | no data | 1.3 (22%) |
| Serum retinyl stearate | µg/dL | 0.5 (86%) | 0.5 (87%) | no data | 0.7 (88%) |
| Serum 25-hydroxyvitamin D | nmol/L | no data | 3.7 (0%) | 3.7 (0%) | 3.7 (0%) |
| Fatty Acids - Saturated | | | | | |
| Plasma myristic acid (14:0) | µmol/L | no data | no data | 1.6 (0%) | no data |
| Plasma palmitic acid (16:0) | µmol/L | no data | no data | 8.2 (0%) | no data |
| Plasma stearic acid (18:0) | µmol/L | no data | no data | 23.7 (0%) | no data |
| Plasma arachidic acid (20:0) | µmol/L | no data | no data | 0.6 (0%) | no data |
| Plasma docosanoic acid (22:0) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma lignoceric acid (24:0) | µmol/L | no data | no data | 0.1 (0%) | no data |

| Indicator | Units | 1999-2000 | 2001-2002 | 2003-2004 | 2005-2006 |
|--|-----------|-----------|------------|------------|-------------|
| Fatty Acids - Monounsaturated | | | | | |
| Plasma myristoleic acid (14:1n-5) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Plasma palmitoleic acid (16:1n-7) | µmol/L | no data | no data | 0.6 (0%) | no data |
| Plasma <i>cis</i> -vaccenic acid (18:1n-7) | µmol/L | no data | no data | 0.3 (0%) | no data |
| Plasma oleic acid (18:1n-9) | µmol/L | no data | no data | 5.2 (0%) | no data |
| Plasma eicosenoic acid (20:1n-9) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma docosenoic acid (22:1n-9) | µmol/L | no data | no data | 0.3 (3%) | no data |
| Plasma nervonic acid (24:1n-9) | µmol/L | no data | no data | 0.4 (0%) | no data |
| Fatty Acids - Polyunsaturated | | | | | |
| Plasma linoleic acid (18:2n-6) | µmol/L | no data | no data | 2.2 (0%) | no data |
| Plasma <i>alpha</i> -linolenic acid (18:3n-3) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma <i>gamma</i> -linolenic acid (18:3n-6) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Plasma eicosadienoic acid (20:2n-6) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Plasma <i>homo-gamma</i> -linolenic acid (20:3n-6) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma arachidonic acid (20:4n-6) | µmol/L | no data | no data | 0.3 (0%) | no data |
| Plasma eicosapentaenoic acid (20:5n-3) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Plasma docosatetraenoic acid (22:4n-6) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma docosapentaenoic acid (22:5n-3) | µmol/L | no data | no data | 0.2 (0%) | no data |
| Plasma docosapentaenoic acid (22:5n-6) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Plasma docosahexaenoic acid (22:6n-3) | µmol/L | no data | no data | 0.1 (0%) | no data |
| Trace Elements | | | | | |
| Iron-Status Indicators | | | | | |
| Serum ferritin | ng/mL | 1.1 (0%) | 1.1 (0%) | 3 (1%) | 3 (1%) |
| Serum soluble transferrin receptor | mg/L | no data | no data | 0.5 (0%) | 0.5 (0%) |
| Urinary iodine | ng/mL | no data | 1.0 (0%) | 1.0 (0%) | 1.0 (0%) |
| Isoflavones and Lignans | | | | | |
| Urinary genistein | µg/L | 0.3 (6%) | 0.8 (< 1%) | 0.3 (< 1%) | 1.0 (< 1%) |
| Urinary daidzein | µg/L | 0.5 (1%) | 1.6 (7%) | 0.3 (< 1%) | 0.4 (< 1%) |
| Urinary equol | µg/L | 3 (28%) | 3.3 (30%) | 0.3 (< 1%) | 0.06 (< 1%) |
| Urinary O-desmethylangolensin | µg/L | 0.2 (25%) | 0.4 (27%) | 0.2 (7%) | 0.2 (4%) |
| Urinary enterodiol | µg/L | 0.8 (8%) | 1.5 (4%) | 0.3 (1%) | 0.04 (< 1%) |
| Urinary enterolactone | µg/L | 0.6 (2%) | 1.9 (1%) | 0.3 (< 1%) | 0.1 (0%) |
| Acrylamide Hemoglobin Adducts | | | | | |
| Acrylamide hemoglobin adduct | pmol/g Hb | no data | no data | 3 (< 1%) | no data |
| Glycidamide hemoglobin adduct | pmol/g Hb | no data | no data | 4 (2%) | no data |

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Appendix G

Selected References of Descriptive NHANES Papers on Diet-and-Nutrition Biochemical Indicators

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