Appendix A:

NHANES Reports Related to Nutritional Status

National Center for Health Statistics (NCHS) Series 11 Reports

http://www.cdc.gov/nchs/products/pubs/pubd/series/ser.htm#sr11

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/pubs/pubd/series/ser.htm#sr2

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.

National Center for Health Statistics (NCHS) Advance Data Reports

http://www.cdc.gov/nchs/about/major/nhanes/advancedatas.htm

Advance Data No. 349. Prevalence of leading types of dietary supplements used in the Third National Health and Nutrition Examination Survey, 1988–94.

Advance Data No. 341. Dietary intake of selected minerals for the United States population: 1999–2000.

Advance Data No. 339. Dietary intake of selected vitamins for the United States population: 1999–2000.

Advance Data No. 334. Dietary intake of ten key nutrients for public health, United States: 1999–2000.

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.

Appendix B:

References for Analytical Methods for Biochemical Indicators

Detailed Laboratory Procedure Manuals for Analytical Methods

- NHANES 1999–2000:
  http://www.cdc.gov/nchs/about/major/nhanes/lab_methods99_00.htm

- NHANES 2001–2002:
  http://www.cdc.gov/nchs/about/major/nhanes/lab_methods01_02.htm

Additional Useful Analytical Method References

Water-Soluble Vitamins & Related Biochemical Compounds

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.

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;42:1689-94.

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):152-3.

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):1261-8.

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;130:2850-4.

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;45:2236-42.

Fat-Soluble Vitamins & Micronutrients

Sowell AL, Huff DL, Yeager PR, Caudill SP, Gunter EW. Retinol, *alpha*-tocopherol, lutein/zeaxanthin, *beta*-cryptoxanthin, *trans*-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;40:411-6.

Trace Elements

Paschal DC, Kimberly MM. Automated direct determination of selenium in serum by electrothermal atomic absorption spectroscopy. At Spectrosc. 1986;7:75-8.

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;49:1019-21.

Iron-Status Indicators

Blanck HM, Pfeiffer CM, Caudill SP, Reyes R, Gunter EW, Imperatore G, et al. Serum iron and iron-binding capacity: a round-robin interlaboratory comparison study. Clin Chem. 2003;49:1672-5.

Looker AC, Gunter EW, Johnson CL. Methods to assess iron status in various NHANES surveys. Nutr Rev. 1995;53:246-54.

Isoflavones & 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.

Kuklenyik 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.

Appendix C:

Confidence Interval Estimation for Percentiles

A common practice to calculate confidence intervals from survey data is to use large-sample normal approximations. Ninety-five percent confidence intervals on point estimates of percentiles are often computed by adding and subtracting from the point estimate a quantity equal to twice its standard error. This normal approximation method may not be adequate, however, when estimating the proportion of subjects above or below a selected value (especially when the proportion is near 0.0 or 1.0 or when the effective sample size is small).

In addition, confidence intervals on proportions deviating from 0.5 are not theoretically expected to be symmetric around the point estimate. Further, adding and subtracting a multiple of the standard error to an estimate near 0.0 or 1.0 can lead to impossible confidence limits (i.e., proportion estimates below 0.0 or above 1.0).

We used the method of Korn and Graubard (1998) to compute Clopper-Pearson 95 percent confidence intervals about percentile estimates. We describe the method below, using SAS Proc Univariate and SUDAAN. SAS code for calculating these confidence intervals can be downloaded from http://www.cdc.gov/exposurereport.

Procedure to calculate confidence intervals about percentiles

Step 1: Use SAS (SAS Institute Inc., 1999) Proc Univariate to obtain a point estimate of the percentile of a chemical's results for the demographic group of interest (e.g., the 90th percentile of blood lead results for children aged 1–5 years). Use the Freq option to assign the correct sample weight for each chemical result.

Step 2: Use SUDAAN (SUDAAN Users Manual, 2001) Proc Descript with Taylor Linearization DESIGN = WR (i.e., sampling with replacement) and the proper sampling weight to estimate the proportion (p) of subjects with results below the percentile estimate obtained in Step 1 and to obtain the standard error (se_p) associated with this proportion estimate. Compute the degrees-of-freedom adjusted effective sample size

$$n_{df} = ((t_{num}/t_{denom})^2)p(1 - p)/(se_p^{\,2}) \qquad (1)$$

where t_{num} and t_{denom} are 0.975 critical values of the Student's t distribution with degrees of freedom equal to the sample size minus 1 and the number of PSUs minus the number of strata, respectively. Note: the degrees of freedom for t_{denom} can vary with the demographic subgroup of interest (e.g., males).

Step 3: After obtaining an estimate of p (i.e., the proportion obtained in Step 2), compute the Clopper-Pearson 95 percent confidence interval ($P_L(x,n_{df})$, $P_U(x,n_{df})$) as follows:

$$P_L(x,n_{df}) = v_1F_{v1,v2}(0.025)/(v_2 + v_1F_{v1,v2}(0.025)) \quad \& \quad P_U(x,n_{df}) = v_3F_{v3,v4}(0.975)/(v_4 + v_3F_{v3,v4}(0.975)) \qquad (2)$$

where x is equal to p times n_{df}, $v_1 = 2x$, $v_2 = 2(n_{df} - x + 1)$, $v_3 = 2(x + 1)$, $v_4 = 2(n_{df} - x)$, and $F_{d1,d2}(\beta)$ is the β quantile of an F distribution with d_1 and d_2 degrees of freedom. (Note: If n_{df} is greater than the actual sample size, or if p is equal to zero, then the actual sample size should be used.) This step will produce a lower and an upper limit for the estimated proportion obtained in Step 2.

Step 4: Use SAS Proc Univariate (again using the Freq option to assign weights) to determine the chemical values that correspond to the proportion obtained in Step 2 and the lower and upper limits on this proportion obtained in Step 3.

Example:

To estimate the 75th percentile, use SAS Proc Univariate with the Freq option to get a weighted point estimate of the chemical value that corresponds to the 75th percentile. Then use SUDAAN to estimate the weighted proportion of subjects with results below the 75th percentile (which should be very near 0.75). Next, obtain a confidence interval on this proportion by computing the weighted Clopper-Pearson 95 percent confidence limits using the degrees-of-freedom adjusted effective sample size. Suppose these confidence limits are 0.67 and 0.81, then use SAS Proc Univariate with the Freq option to determine the chemical values corresponding to the weighted 67th and 81st percentiles. These point estimates are the lower and upper confidence limits on the 75th percentile.

References

Korn EL, Graubard BI. Confidence intervals for proportions with small expected number of positive counts estimated from survey data. Survey Methodology. 1998;24:193-201.

Appendix D:

Limit of Detection Table

The table below presents the analytical limit of detection (LOD) for each of the different indicators. 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 urinary phytoestrogens. We used the higher of the two LOD values for the analysis of the combined four-year data.

Indicator	Units	1999–2000	2001–2002
Water-Soluble Vitamins & Related Biochemical Compounds			
Serum folate	ng/mL	0.1	0.1
Red blood cell (RBC) folate	ng/mL RBC	20	20
Serum vitamin B12	pg/mL	20	20
Plasma homocysteine	µmol/L	0.35	0.35
Plasma methylmalonic acid	µmol/L	0.05	0.05
Fat-Soluble Vitamins & Micronutrients			
Serum vitamin A	µg/dL	1.03	1.03
Serum vitamin E	µg/dL	40.7	40.7
Serum *gamma*-tocopherol	µg/dL	10.7	10.7
Serum *alpha*-carotene	µg/dL		0.7
Serum *trans*-beta-carotene	µg/dL		0.8
Serum *beta*-cryptoxanthin	µg/dL		0.9
Serum lutein/zeaxanthin	µg/dL		2.4
Serum *trans*-lycopene	µg/dL		0.8
Serum vitamin D, 25-hydroxy	ng/mL		1.5
Iron-Status Indicators			
Serum ferritin	ng/mL	1.1	1.1
Serum iron	µg/dL	2	
Serum total iron-binding capacity	µg/dL	6	
Serum transferrin saturation	%	n/a	
Erythrocyte protoporphyrin	µg/dL RBC	1	
Trace Elements			
Urinary iodine	ng/mL		1.0
Serum selenium	ng/mL	8	
Isoflavones & Lignans			
Urinary genistein	µg/L	0.3	0.8
Urinary daidzein	µg/L	0.5	1.6
Urinary equol	µg/L	3.0	3.3
Urinary O-desmethylangolensin	µg/L	0.2	0.4
Urinary enterodiol	µg/L	0.8	1.5
Urinary enterolactone	µg/L	0.6	1.9

References

Taylor JK. Quality assurance of chemical measurements. Chelsea (MI): Lewis Publishing; 1987.

Appendix E:

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

Water-Soluble Vitamins & Related Biochemical Compounds

Bentley TGK, Willett WC, Weinstein MC, Kuntz KM. Population-level changes in folate intake by age, gender, and race/ethnicity after folic acid fortification. Am J Public Health. 2006;96:2040-7.

Boulet SL, Yang Q, Mai C, Mulinare J, Pfeiffer CM. Folate status in women of childbearing age by race/ethnicity—United States 1999–2000, 2001–2002, and 2003–2004. Morb Mortal Wkl Rep. 2007;55(51):1377-80.

Dietrich M, Brown CJP, Block G. The effect of folate fortification of cereal-grain products on blood folate status, dietary folate intake and dietary folate sources among adult non-supplement users in the United States. J Am Coll Nutr. 2005;24:266-74.

Ganji V, Kafai MR. Trends in serum folate, RBC folate, and circulating total homocysteine concentrations in the United States: analysis of data from National Health and Nutrition Examination Surveys, 1988–1994, 1999–2000, and 2001–2002. J Nutr. 2006;136:153-8.

Ganji V, Kafai MR. Population reference values for plasma total homocysteine concentrations in US adults after the fortification of cereals with folic acid. Am J Clin Nutr. 2006;84:989-94.

Ganji V, Kafai MR. Population references for plasma total homocysteine concentrations for US children and adolescents in the post-folic acid fortification era. J Nutr. 2005;135:2253-6.

Ganji V, Kafai MR. Serum total homocysteine concentration determinants in non-Hispanic white, non-Hispanic black, and Mexican-American populations of the United States. Ethn Dis. 2004;14:476-82.

Ganji V, Kafai MR. Demographic, health, lifestyle, and blood vitamin determinants of serum total homocysteine concentrations in the Third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr. 2003;77:826-33.

Morris MS, Jacques PF, Rosenberg IH, Selhub J. Elevated serum methylmalonic acid concentrations are common among elderly Americans. J Nutr. 2002;132:2799-2803.

Must A, Jacques PF, Rogers G, Rosenberg IH, Selhub J. Serum total homocysteine concentrations in children and adolescents: results from the Third National Health and Nutrition Examination Survey (NHANES III). J Nutr. 2003;133:2643-9.

Pfeiffer CM, Osterloh JD, Kennedy-Stephenson J, Picciano MF, Yetley EA, Rader JI, Johnson CL. Trends in circulating concentrations of total homocysteine among US adolescents and adults: findings from the 1991–1994 and 1999–2004 National Health and Nutrition Examination Surveys. Clin Chem. 2008;54:801-13.

Pfeiffer CM, Johnson CL, Jain RB, Yetley EA, Picciano MF, Rader JI, Fisher KD, et al. Trends in blood folate and vitamin B12 concentrations in the United States, 1988–2004. Am J Clin Nutr. 2007;86:718-27.

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

Selhub J, Jacques PF, Rosenberg IH, Rogers G, Bowman BA, Gunter EW. Serum total homocysteine concentrations in the Third National Health and Nutrition Examination Survey (1991–1994): population reference ranges and contribution of vitamin status to high serum concentrations. Ann Intern Med. 1999;131:331-9.

U.S. Centers for Disease Control and Prevention. Folate status in women of childbearing age, by race/ethnicity — United States, 1999–2000. Morb Mortal Wkl Rep. 2002;51:808-10.

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.

Yang Q-H, Carter HK, Mulinare J, Berry RJ, Friedman JM, Erickson JD. Race-ethnicity differences in folic acid intake in women of childbearing age in the United States after folic acid fortification: findings from the National Health and Nutrition Examination Survey, 2001–2002. Am J Clin Nutr. 2007;85:1409-16.

Fat-Soluble Vitamins & Micronutrients

Ford ES, Schleicher RL, Mokdad AH, Ajani UA, Liu S. Distribution of serum concentrations of α-tocopherol and γ-tocopherol in the U.S. population. Am J Clin Nutr. 2006;84:375-83.

Ford ES, Ajani UA, Mokdad AH. Brief communication: the prevalence of high intake of vitamin E from the use of supplements among U.S. adults. Ann Intern Med. 2005;143:143-5.

Ford ES, Liu S, Mannino DM, Giles WH, Smith SJ. C-reactive protein concentration and concentrations of blood vitamins, carotenoids, and selenium among United States adults. Eur J Clin Nutr. 2003;57:1157-63.

Gillespie C, Ballew C, Bowman BA, Donehoo R, Serdula MK. Intraindividual variation in serum retinol concentrations among participants in the Third National Health and Nutrition Examination Survey, 1988–1994. Am J Clin Nutr. 2004;79:625-32.

Gruber M, Chappell R, Millen A, LaRowe T, Moeller SM, Iannaccone A, et al. Correlates of serum lutein + zeaxanthin: findings from the Third National Health and Nutrition Examination Survey. J Nutr. 2004;134:2387-94.

Knutsen SF, Fraser GE, Linsted KD, Beeson WL, Shavlik DJ. Comparing biological measurements of vitamin C, folate, *alpha*-tocopherol and carotene with 24-hour dietary recall information in nonhispanic blacks and whites. Ann Epidemiol. 2001;11:406-16.

Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. Bone. 2002;30:771-7.

Nesby-O'Dell S, Scanlon KS, Cogswell ME, Gillespie C, Hollis BW, Looker AC, et al. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: third National Health and Nutrition Examination Survey, 1988-1994. Am J Clin Nutr. 2002;76:187-92.

Stimpson JP, Urrutia-Rojas X. Acculturation in the United States is associated with lower serum carotenoid levels: third National Health and Nutrition Examination Survey. J Am Diet Assoc. 2007;107:1218-23.

Zadshir A, Tareen N, Pan D, Norris K, Martins D. The prevalence of hypovitaminosis D among US adults: data from the NHANES III. Ethn Dis. 2005;15(4 Suppl 5):S5-97-101.

Iron-Status Indicators

Brotanek JM, Gosz J, Weitzman M, Flores G. Iron deficiency in early childhood in the United States: risk factors and racial/ethnic disparities. Pediatrics. 2007;120:568-75.

Looker AC, Cogswell ME, Gunter EW. Iron deficiency—United States, 1999–2000. Morb Mortal Wkly Rep. 2002;51:897-9.

Looker AC, Dallman PR, Carroll M, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. JAMA. 1997;277:973-5.

Looker AC, Gunter EW, Johnson CL. Methods to assess iron status in various NHANES surveys. Nutr Rev. 1995;53:246-54.

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.

Michels Blanck H, Cogswell ME, Gillespie C, Reyes M. Iron supplement use and iron status among US adults: results from the Third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2005;82:1024-31.

Ramakrishnan U, Frith-Terhune A, Cogswell M, Kettel Khan L. Dietary intake does not account for differences in low iron stores among Mexican American and non-Hispanic white women: third National Health and Nutrition Examination Survey, 1988–1994. J Nutr. 2002;132:996-1001.

Trace Elements

Caldwell KL, Jones R, Hollowell JG. Urinary iodine concentration: United States National Health and Nutrition Examination Survey 2001–2002. Thyroid. 2005;15:692-9.

Hollowell JG, Staehling NW, Hannon WH, Flanders DW, Gunter EW, Maberly GF, et al. Iodine nutrition in the United States. Trends and public health implications: iodine excretion data from National Health and Nutrition Examination Surveys I and III (1971–1974 and 1988–1994). J Clin Endocrinol Metab. 1998;83:3401-8.

Semba RD, Ricks MO, Ferrucci L, Xue QL, Guralnik JM, Fried LP. Low serum selenium is associated with anemia among older adults in the United States. Eur J Clin Nutr. In press 2008.

Vogt TM, Ziegler RG, Patterson BH, Graubard BI. Racial differences in serum selenium concentrations: analysis of US population data from the Third National Health and Nutrition Examination Survey. Am J Epidemiol. 2007;166:280-8.

Isoflavones & Lignans

U.S. Centers for Disease Control and Prevention. Third National Report on Human Exposure to Environmental Chemicals. Atlanta (GA): CDC; 2005.

Valentin-Blasini L, Sadowski MA, Walden D, Caltabiano L, Needham LL, Barr DB. Urinary phytoestrogen concentrations in the U.S. population (1999–2000). J Expo Anal Environ Epidemiol. 2005;15:509-23.

Valentin-Blasini L, Blount BC, Caudill SP, Needham LL. Urinary and serum concentrations of seven phytoestrogens in a human reference population subset. J Expo Anal Environ Epidemiol. 2003;13:276-82.