Published in final edited form as:

J Nutr. 2013 June ; 143(6): 1001S–1010S. doi:10.3945/jn.112.172882.

Selected physiologic variables are weakly to moderately associated with twenty-nine biomarkers of diet and nutrition, NHANES 2003–2006[1,,2,,3]

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Abstract

The physiological status of the individual may influence biomarkers of nutritional status. To help researchers with planning studies and interpreting data, we assessed the associations between common physiologic variables (fasting, inflammation, renal function, and pregnancy) and 29 biomarkers of diet and nutrition measured in blood or urine in a representative sample of the adult US population (≥20 y; pregnancy variable and iron indicators limited to women 20–49 y) participating in NHANES 2003–2006. We compared simple linear regression (model 1) to multiple linear regression (model 2, controlling for age, sex, race-ethnicity, smoking, supplement use, and the physiologic factors [and urine creatinine for urine biomarkers]) and report significant findings from model 2. Not being fasted was positively associated with most water-soluble vitamins (WSV) and related metabolites (RM). Some WSV, fat-soluble vitamin (FSV) and micronutrient (MN) and phytoestrogen concentrations were lower in the presence of inflammation (C-reactive protein ≥5 mg/L), while fatty acids and most iron indicators were higher. Most WSV&RM were higher when renal function was impaired (estimated glomerular filtration rate <60 mL/(min·1.73 m^2). Most WSV, FSV&MN, and fatty acid concentrations were higher in pregnant compared to non-pregnant women, but vitamins A and B-12 and most iron indicators were lower. The estimated changes in biomarker concentrations with different physiologic status were mostly small to moderate (≤|25%|) and generally similar between models; renal function, however, showed several large differences for WSV&RM. This descriptive analysis of associations between physiologic variables and a large number of nutritional biomarkers showed that controlling for demographic variables, smoking, and supplement use generally did not change the interpretation of bivariate results. The analysis serves as a useful basis for more complex future research.

[1]No specific sources of financial support. The findings and conclusions in this report are those of the authors and do not necessarily represent the official views or positions of the Centers for Disease Control and Prevention/Agency for Toxic Substances and Disease Registry or the Department of Health and Human Services.

[2]Author disclosures: B. M. H. Haynes, C. M. Pfeiffer, M. R. Sternberg, R. L. Schleicher, no conflicts of interest.

[3]Supplemental Methods 1–2, Supplemental Tables 1–3, and Supplemental Figure 1 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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INTRODUCTION

Biomarkers are used to assess nutritional status and associated health outcomes. Physiologic variables such as the fasting and health status of the individual can influence biomarkers and may confound study results (1, 2). While appropriate study design and data analysis may minimize the extent of confounding, practical considerations sometimes interfere with the research plan and investigators may ask whether they can meaningfully interpret data obtained from participants under less than ideal conditions.

Some biomarkers are short-term status indicators (e.g., serum folate [S-FOL][4] and 4-pyridoxic acid [4PA], the end product of vitamin B-6 catabolism) and may therefore be influenced by recent dietary intake and elevated in specimens from non-fasting participants (3). Several biomarkers are notably influenced by inflammation, being either positive (e.g., serum ferritin [FER]) or negative (e.g., retinol binding protein) acute phase proteins, or because the inflammatory response reduces circulating nutrient concentrations (e.g., iron) (4–7). The association between the systemic inflammatory response and biomarkers has been studied in detail for vitamins A (8, 9), B-6 (10–13), and iron status (14). The associations between impaired renal function and increased serum total homocysteine (tHcy; 15–18) and methylmalonic acid (MMA; 19–21) concentrations or altered iron status (22, 23) have also been documented. With chronic kidney disease (CKD) increasing in the United States (24) as rates of obesity (25) and diabetes (26) have been climbing over the last few decades, it is important to understand the effect of impaired renal function on nutritional biomarkers. Pregnancy generally increases micronutrient requirements because of the increased needs of the fetus, which in turn often depresses blood concentrations (3, 27).

In addition to collecting biological specimens for biomarker measurements to assess the nutritional status of the US population, the NHANES collects a wealth of information on the health status of the participants and records the fasting status. To provide a comprehensive picture of whether different classes of biomarkers of diet and nutrition are associated with common physiologic variables (fasting, inflammation, renal function, and pregnancy), we conducted a descriptive bivariate analysis using cross-sectional data from the adult US population participating in NHANES 2003–2006. We also compared simple and multiple linear regression after controlling for a standard set of covariates that emerged as important factors in class-specific analyses conducted as part of this supplement (see accompanying papers). The findings from this study will help researchers with planning studies involving nutritional biomarkers and interpreting data.

SUBJECTS AND METHODS

The NHANES collects cross-sectional data on the health and nutritional status of the civilian non-institutionalized US population (28). The 2003–2006 survey cycles provide a stratified,

[4]Abbreviations used: 25OHD, 25-hydroxyvitamin D; 4PA, 4-pyridoxic acid; B-12, total cobalamin; BI, body iron; CAR, carotenes; DAZ, daidzein; DMA, O-desmethylangolensin; EQU, equol; ETD, enterodiol; ETL, enterolactone; FER, ferritin; GEN, genistein; HbAA, acrylamide hemoglobin adduct; HbGA, glycidamide hemoglobin adduct; MMA, methylmalonic acid; MN, micronutrient; NCHS, National Center for Health Statistics; PLP, pyridoxal-5′-phosphate; RBC-FOL, RBC folate; RM, B-vitamin related metabolites; S-FOL, serum folate; sTfR, soluble transferrin receptor; tFA, total fatty acids; tHcy, total homocysteine; uI, urine iodine; VIA, retinol; VIC, ascorbic acid; VIE, *alpha*-tocopherol; XAN, xanthophylls.

multistage, probability sample designed to represent the American population on the basis of age, sex, and race-ethnicity. All respondents gave their informed consent, and the NHANES protocol was reviewed and approved by the NCHS Research Ethics Review Board.

Laboratory methods

Twenty-nine biomarkers of diet and nutritional status were analyzed by the CDC laboratory during all or part of NHANES 2003–2006 as part of 6 classes: water-soluble vitamins (WSV) and related metabolites (RM), fat-soluble vitamins (FSV) and micronutrients (MN), fatty acids, trace elements, phytoestrogens, and acrylamide hemoglobin adducts (Table 1). For additional information on how certain dependent variables were calculated, see Supplemental Methods 1. Laboratory method details are provided elsewhere (29, 30).

Study variables

Four independent variables were assessed: fasting, presence of inflammation, renal function, and pregnancy (Table 1). The definition of renal function is described in Supplemental Methods 1.

Analytic sample

All Mobile Examination Center-examined participants aged ≥20 y in the NHANES 2003–2004 and 2005–2006 with at least 1 biomarker of interest were eligible for inclusion in the study. We limited iron indicators (FER, soluble transferrin receptor [sTfR] and body iron [BI]) and the pregnancy variable to women 20–49 y (no data for iron indicators for other adults; no pregnant women older than 49 y). Depending on whether the biomarker was analyzed in both survey periods and on the full sample or a subsample, data were available for between ~1400 and nearly 9000 adult NHANES participants (Supplemental Table 1). We did not exclude participants based on any health conditions because our intent was to assess associations in the general population.

Statistical analyses

We assessed bivariate associations for categorical variables by calculating the geometric means (arithmetic means for ascorbic acid [VIC], 25-hydroxyvitamin D [25OHD], and BI) and 95% CI for each category and Spearman correlations for continuous variables (fasting, inflammation, and renal function). We used the Wald F test to compare means across categories and conducted pairwise comparisons if the overall Wald F test was significant and there were more than 2 categories. Linear regression was used to assess the association of the physiologic variables without (simple = model 1) and with (multiple = model 2) controlling for additional covariates that emerged as important factors in class-specific analyses conducted as part of this supplement: age, sex, race-ethnicity, smoking, and supplement use (and urine creatinine for urine biomarkers), in addition to the other physiologic variables. For a graphical representation of changes in *beta* coefficients from model 1 to model 2, see Supplemental Fig. 1. We assessed the magnitude of association by presenting the percent change in biomarker concentration with change in each covariate holding all remaining covariates constant (31). For biomarkers with accepted cutoff values for abnormal concentrations, we also calculated prevalence estimates by variable categories

(Supplemental Methods 2). Statistical analyses were carried out using SAS for Windows software version 9.2 (SAS Institute, Cary, NC) and SAS-callable SUDAAN (SUDAAN Release 10.0, 2008 RTI, Research Triangle Park, NC). Analyses using SUDAAN software accounted for the complex survey design by incorporating the survey weights to account for the unequal probabilities of selection and adjustment for non-response and by using a Taylor series linearization to calculate variance estimates. We flagged 2-sided *P*-values as statistically significant if <0.05.

RESULTS

Descriptive information for the civilian non-institutionalized US population in various subsamples of the NHANES 2003–2006 showed that approximately half of the sample was evaluated after ≥8 h of fasting, slightly more than 20% had elevated C-reactive protein (CRP) concentrations indicative of inflammation, slightly less than 20% had some form of CKD, and ~6% of women 20–49 y were pregnant (Supplemental Table 2). We observed correlations between the continuous independent variables and the biomarkers that were mostly weak ($|r_s|<0.2$) and inconsistent with regards to direction and significance (Supplemental Table 3). However, we observed moderate ($0.2\leq|r_s|<0.44$) and significant correlations in the following cases: between inflammation and pyridoxal-5′-phosphate (PLP), carotenes (CAR), and xanthophylls (XAN) (all negative); between inflammation and SFA (positive); and between renal function and B vitamins and RM (RBC-FOL, 4PA, tHcy and MMA), retinol (VIA), and *alpha*-tocopherol (VIE) (all negative).

Fasting

Fasting status was significantly associated with WSV & RM (except total cobalamin [B-12]), VIE, CAR, and with all phytoestrogens; however, it was not significantly associated with concentrations of most FSV & MN, iron and iodine status indicators, and acrylamide hemoglobin adducts (Table 2). Estimated changes in biomarker concentrations between non-fasting and fasting persons (<3 h vs. ≥8 h) were mostly similar before (model 1) and after controlling for covariates (model 2) (Table 5 and Supplemental Fig. 1, panel A). As derived from model 2, mean concentrations of most WSV and VIE were slightly but significantly higher (≤10%; 3.4 μmol/L for VIC) in non-fasting persons, but for MMA (21%) and 4PA (28%) we observed larger percent differences.

Inflammation

Inflammation was significantly associated with 19 out of 29 biomarkers (Table 3). Most FSV & MN (except VIE), fatty acids, and the iron and iodine status indicators were significantly associated with the inflammatory biomarker, CRP. Some WSV (S-FOL, RBC-FOL, PLP, and VIC), two phytoestrogens (O-desmethylangolensin [DMA] and enterolactone [ETL]), and hemoglobin glycidamide adduct (HbGA) were also significantly associated. Estimated changes in biomarker concentrations between persons with and without inflammation were mostly similar between models 1 and 2 (Table 5 and Supplemental Fig. 1, panel C). As derived from model 2, some analyte concentrations were significantly lower in the presence of inflammation (S-FOL, PLP, VIC, VIA, CAR, XAN, 25OHD, DMA, enterodiol [ETD], ETL, hemoglobin acrylamide adduct [HbAA]), while others were

significantly higher (RBC-FOL, SFA, MUFA, PUFA, total fatty acids [tFA], FER, BI, HbGA). Differences were small (≤10% for S-FOL, RBC-FOL, VIA, SFA, PUFA, tFA, HbAA, HbGA; 3.9 μmol/L for 25OHD), moderate (>10–25% for CAR, XAN, MUFA, FER, ETD; 8.2 μmol/L for VIC; 0.7 mg/kg for BI), or large (>25% for PLP, DMA, ETL).

Renal function

Impaired renal function was significantly associated with 20 out of 29 biomarkers (Table 3). Biomarkers not associated with renal function were XAN, PUFA, FER, BI, and all phytoestrogens except for ETL. Estimated changes in biomarker concentrations between persons with and without impaired renal function were less similar between models 1 and 2 than observed for fasting and inflammation (Table 5 and Supplemental Fig. 1, panel E). While we noted attenuation after controlling for covariates, mean concentrations of most WSV & RM (except PLP and VIC) were significantly higher in stage 3–5 CKD compared to normal renal function, with the largest percent differences observed for 4PA (66%), MMA (42%), and tHcy (32%). Whereas several FSV & MN (VIA, VIE, CAR) and fatty acids (SFA, MUFA, tFA) showed significant percent differences in model 1, only VIA (20%) and SFA (-5%) retained a small to moderate significant percent difference between persons with and without impaired renal function after controlling for covariates (model 2). We noted strong attenuation after controlling for covariates for urine iodine (uI) and the 2 acrylamide hemoglobin adducts; only HbGA retained a small (8%) significant percent difference between persons with and without impaired renal function.

Pregnancy

Pregnancy was significantly associated with 17 out of 29 biomarkers, most WSV & RM (except PLP and 4PA), most FSV & MN (except CAR), fatty acids, most iron indicators (except sTfR), and HbAA (Table 4). Estimated changes in biomarker concentrations between pregnant and non-pregnant women were generally similar between the 2 models (Table 5 and Supplemental Fig. 1, panel F). While we noted modest attenuation after controlling for covariates, folate (both serum [18%] and RBC [26%]), 4PA (34%) and VIC (3.8 μmol/L) concentrations were significantly higher, while B-12 (22%) and tHcy (30%) concentrations were significantly lower in pregnant women. FSV & MN and fatty acid concentrations were significantly higher (12–32%; 6.6 μmol/L for 25OHD) in pregnant women, except for VIA concentrations which were 22% lower. Iron status was lower in pregnant women, with significantly lower FER (33%) and BI (1.4 mg/kg) levels. Pregnant women had slightly but significantly lower HbAA levels but similar HbGA levels compared to non-pregnant women. We found no significant differences in phytoestrogen concentrations between pregnant and non-pregnant women.

DISCUSSION

In this descriptive analysis of cross-sectional data for 29 biomarkers of diet and nutrition from a nationally representative sample of American adults participating in NHANES 2003–2006 we observed the following: fasting and renal function were associated with most WSV & RM; inflammation was associated with most FSV & MN, fatty acids, most iron status indicators, and acrylamide hemoglobin adducts; and pregnancy was associated with most

WSV & RM, FSV & MN, fatty acids, and most iron status indicators. The estimated changes in biomarker concentrations with different physiologic status were generally small to moderate (≤|25%|) and for the most part similar before and after controlling for a standard set of covariates.

Fasting

Most WSV & RM were found at lower concentrations in fasting persons before and after controlling for covariates, most likely due to the rapid clearance of these nutrients from the plasma compartment in just a few hours after a meal (3). Of the FSV & MN, only VIE showed slightly higher (4%) concentrations in the fed state after controlling for covariates. VIE is closely correlated to its carrier protein, LDL-cholesterol, which also increases following meals. As expected, we did not find an association of fasting with VIA (dietary vitamin A is rapidly taken-up by the liver which closely regulates circulating VIA availability [27]), vitamin D (requirement is mostly met by exposure to sunlight [32]), iron status indicators (are weakly correlated to recent dietary intake of total iron [33]), and hemoglobin adducts of acrylamide (reflect time-weighted exposure to acrylamide over the past 4 mo [34]). Although we did not observe significant differences in phytoestrogen concentrations between fasting (≥8 h) and non-fasting (<3 h) persons, concentrations were consistently higher in fasting persons compared to those who had a shorter fast (3–<8 h). This may reflect the time needed for absorption, distribution and clearance, which has been reported as 5–10 h for GEN and DAZ (35), but longer for compounds (e.g., EQU) that are metabolized in the colon and recirculated before urine excretion (36). Finally, the significantly higher uI concentration in non-fasting compared to fasting persons after controlling for covariates, is most likely a result of the creatinine adjustment.

Inflammation

Of the 19 biomarkers of diet and nutrition that were associated with inflammation, 9 had lower and 10 had higher concentrations in persons with an inflammatory response. For example, PLP, CAR, and XAN concentrations were 29%, 18%, and 21% lower, respectively, when CRP was elevated after we controlled for covariates, which is consistent with data shown by others (5, 10–13, 37–39). Although the mechanism is not understood, we also found an inverse relationship between CRP and the isoflavone DMA (a metabolite of DAZ; 36% lower) and the lignans ETD and ETL (21% and 38% lower, respectively). Consistent with these observations, Chun *et al.* (40) showed that the dietary intake of some isoflavones (DAZ and GNS) was inversely associated with CRP and Pellegrini *et al.* (41) showed that lignan intake was inversely associated with other vascular inflammation biomarkers.

Perhaps the most well-known consequence of inflammation on a nutritional biomarker is its effect on the positive acute-phase iron storage protein ferritin. After controlling for covariates, we found 25% higher FER concentrations, no difference in sTfR concentrations, and 0.7 mg/kg higher BI levels in persons with compared to without inflammation. This confirms that in a generally healthy population, sTfR does not appear to be affected by inflammation (42). A novel observation in the present study was that fatty acid concentrations were slightly higher (3–11%) in those with elevated CRP. As triglyceride fatty acids consist of 80% SFA and MUFA (43), and triglycerides were shown to be

independently and positively associated with CRP in the NHANES III (44), our observation appears to support the connection between inflammation and triglycerides. Duncan *et al.* (5) aimed to examine the effect of inflammation on micronutrient concentrations with the intent of developing local hospital guidelines to aid in the interpretation of nutrition results. Similar to our study, they found lower PLP, VIC, VIA and 25OHD concentrations in patients who had elevated CRP concentrations leading the authors to suggest that interpretation of micronutrient concentrations requires knowledge of a patient's inflammatory status.

Renal function

Impaired renal function was associated with 20 out of 29 biomarkers included in our study, and the differences in biomarker concentrations between persons with stage 3–5 CKD compared to those with normal renal function were large (>25%) for several biomarkers (4PA, MMA, tHcy) after controlling for covariates. The strong association between renal insufficiency and elevated tHcy (16–19) or MMA (18–20) concentrations, independent of the B vitamin status, has been shown before and is likely because the reduced glomerular filtration rate can no longer efficiently clear these metabolites (45). The same probably applies to elevated 4PA concentrations, also found in older participants with impaired renal function in the British National Diet and Nutrition survey (13).

Pregnancy

Blood levels of WSV, VIA, and iron indicators are known to decrease during pregnancy (3, 27, 46, 47). Accordingly, after controlling for covariates, we found much reduced B-12 (22%), VIA (22%), FER (33%), and BI (1.4 mg/kg) levels in pregnant women. Despite the lower B-12 concentrations, MMA concentrations were not significantly different. Other studies reported decreasing B-12 concentrations throughout pregnancy, but only slightly fluctuating MMA concentrations within the normal range (48, 49). Contrary to textbook knowledge, we found higher S-FOL (18%), RBC-FOL (26%) and VIC (3.8 μmol/L) concentrations in pregnant women, even after controlling for supplement use to account for the higher proportion of pregnant women consuming dietary supplements (80% compared to only 55% of non-pregnant women). Other cross-sectional data from outpatient populations also showed an increase in folate reference ranges during pregnancy (50) and no difference in mean plasma VIC between pregnant and non-pregnant women (51). The much lower tHcy (30%) and slightly higher 25OHD (7%) concentrations in our study are consistent with previous observations (52–56). Also consistent with common knowledge that blood concentrations of VIE increase during pregnancy, parallel with an increase in total lipids (46), we observed higher VIE (22%), CAR (12%), XAN (32%), and higher fatty acid concentrations (19–29%) in pregnant women. The slightly lower (10%) HbAA levels in pregnant women may be a result of hemodilution.

Strengths and weaknesses

The present study is to our knowledge the first that has assessed the association of 4 common physiologic variables, typically considered when planning or evaluating data from nutrition studies, with a large number of biomarkers of diet and nutrition crossing several classes of nutrients. The large sample size of the NHANES in combination with the availability of objective biochemical data to study the effects of inflammation, renal function

and pregnancy made it possible to assess differences in biomarker concentrations even for rare conditions (~8% of the NHANES participants had stage 3–5 CKD and ~6% of women were pregnant). Furthermore, additional analyses for the same 29 biomarkers are presented as part of this journal supplement to assess their association with sociodemographic and lifestyle variables. While we controlled for a standard set of covariates that emerged as important factors in class-specific analyses conducted as part of this supplement, the interpretation of our findings may be limited by other confounding variables. Developing specific multi-variate models for each biomarker was beyond the scope of the current analysis. The benefit of this analysis lies in the wide spectrum covered and the consistent approach taken so that observations can be compared across biomarkers.

Conclusions

The selected physiologic variables studied in this analysis were generally weakly to moderately associated with 29 biomarkers of diet and nutrition. Controlling for demographic variables, smoking and supplement use did not change the interpretation of the bivariate analysis in most cases. These descriptive analyses will help researchers better plan for future studies and interpret resulting data.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge technical assistance from Yi Pan and contributions from the following laboratory members: William Brown, Kathleen Caldwell, Madhu Chaudhary-Webb, Huiping Chen, Monir Clark, Judith Heitz, Dana Henahan, Donna LaVoie, Marcela Muresan, Jenny Pao, Shahzad Momin, Daniel L. Parker, Elizabeth Pendergrast, Carissa Powers, Ashley Ribera, Antoinette Smith, Ashley Tippins, Cindy Tse, Christina Waters, Mary Xu and Mindy Zhang. C.M.P, M.R.S, and R.L.S designed the overall research project; B.M.H.H, C.M.P, M.R.S and R.L.S conducted most of the research; M.R.S analyzed most of the data; and B.M.H.H, C.M.P and R.L.S jointly wrote the initial draft, which was modified after feedback from all coauthors, and C.M.P had primary responsibility for content. All authors read and approved the final manuscript.

References

1. Pfeiffer, CM.; Schleicher, RL.; Caldwell, KL. Biochemical indices. In: Caballero, B., editor. Enzyclopedia of Human Nutrition. 3rd. Vol. 1. Waltham, MA: Academic Press; 2013. p. 156-74.
2. Blanck HM, Bowman BA, Cooper GR, Myers GL, Miller DT. Laboratory issues: use of nutritional biomarkers. J Nutr. 2003; 133:888S–94S. [PubMed: 12612175]
3. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes: Thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin, and choline. Washington, D.C.: National Academy Press; 998.
4. Tomkins A. Assessing micronutrient status in the presence of inflammation. J Nutr. 2003; 133:1649S–55S. [PubMed: 12730480]
5. Duncan A, Talwar D, McMillan DC, Stefanowica F, O'Reilly DStJ. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. Am J Clin Nutr. 2012; 95:64–71. [PubMed: 22158726]
6. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, Esposito K, Jönsson LS, Kolb H, Lansink M, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011; 106:S5–55. [PubMed: 22133051]

7. Louw JA, Webeck A, Louw MEJ, Kotze TJ, Cooper R, Labadarios D. Blood vitamin concentrations during the acute-phase response. Crit Care Med. 1992; 20:934–41. [PubMed: 1617986]
8. Thurnham DI, McCabe GP, Northrop-Clewes CA, Nestel P. Effects of subclinical infection on plasma retinol concentrations and assessment of prevalence of vitamin A deficiency: meta-analysis. Lancet. 2003; 362:2052–8. [PubMed: 14697804]
9. Stephensen CB, Gildengorin G. Serum retinol, the acute phase response, and the apparent misclassification of vitamin A status in the third National Health and Nutrition Examination Survey. Am J Clin Nutr. 2000; 72:1170–8. [PubMed: 11063445]
10. Sakakeeny L, Roubenoff R, Obin M, Fontes JD, Benjamin EJ, Bujanover Y, Jacques PF, Selhub J. Plasma pyridoxal-5-phosphate is inversely associated with systemic markers of inflammation in a population of U.S. adults. J Nutr. 2012; 142:1280–5. [PubMed: 22623384]
11. Morris MS, Sakakeeny L, Jacques PF, Picciano MF, Selhub J. Vitamin B-6 intake is inversely related to, and the requirement is affected by, inflammation status. J Nutr. 2010; 140:103–10. [PubMed: 19906811]
12. Friso S, Jacques PF, Wilson PWF, Rosenberg IH, Selhub J. Low circulating vitamin B6 is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. Circulation. 2001; 103:2788–91. [PubMed: 11401933]
13. Bates CJ, Pentieva KD, Prentice A, Mansoor MA, Finch S. Plasma pyridoxal phosphate and pyridoxic acid and their relationship to plasma homocysteine in a representative sample of British men and women aged 65 years and over. Br J Nutr. 1999; 81:191–201. [PubMed: 10434845]
14. Northrop-Clewes, CA. WHO/CDC. Assessing the iron status of populations: report of a joint World Health Organization/Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level. Geneva: World Health Organization; 2007. The interpretation of indicators of iron status during an acute phase response. [cited 2013 Feb 24]. Available from: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf
15. Francis ME, Eggers PW, Hostetter TH, Briggs JP. Association between serum homocysteine and markers of impaired kidney function in adults in the United States. Kidney Internat. 2004; 66:303–12.
16. Jacques PF, Bostom AG, Wilson PWF, Rich S, Rosenberg IH, Selhub J. Determinants of plasma total homocysteine concentration in the Framingham Offspring cohort. Am J Clin Nutr. 2001; 73:613–21. [PubMed: 11237940]
17. Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, Tverdal A, Tell GS, Nygård O, Vollset SE. The Hordaland Homocysteine study: a community-based study of homocysteine, its determinants, and associations with disease. J Nutr. 2006; 136:1731S–40S. [PubMed: 16702348]
18. Bates CJ, Mansoor MA, van der Pols J, Prentice A, Cole TJ, Finch S. Plasma total homocysteine in a representative sample of 972 British men and women aged 65 and over. Eur J Clin Nutr. 1997; 51:691–7. [PubMed: 9347290]
19. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Elevated serum methylmalonic acid concentrations are common among elderly Americans. J Nutr. 2002; 132:2799–2803. [PubMed: 12221248]
20. Bates CJ, Schneede J, Mishra G, Prentice A, Mansoor MA. Relationship between methylmalonic acid, homocysteine, vitamin B12 intake and status and socio-economic indices, in a subset of participants in the British National Diet and Nutrition Survey of people aged 65 y and over. Eur J Clin Nutr. 2003; 57:349–57. [PubMed: 12571671]
21. Vogiatzoglou A, Oulhaj A, Smith AD, Nurk E, Drevon CA, Ueland PM, Vollset SE, Tell GS, Refsum H. Determinants of plasma methylmalonic acid in a large population: implications for assessment of vitamin B12 status. Clin Chem. 2009; 55:2198–206. [PubMed: 19833840]
22. Hsu C, McCulloch CE, Curhan GC. Epidemiology of anemia associated with chronic renal insufficiency among adults in the United States: results from the Third National Health and Nutrition Examination Survey. J Am Soc Nephrol. 2002; 13:504–10. [PubMed: 11805181]
23. Hsu C, McCulloch CE, Curhan GC. Iron status and hemoglobin level in chronic renal insufficiency. J Am Soc Nephrol. 2002; 13:2783–6. [PubMed: 12397050]

24. Pirtle CJ, Schoolwerth AC, Giles WH, Brown DW, Mokdad AH, Ford ES. State-specific trends in chronic kidney failure – United States, 1990–2001. Morb Mort Wkly Rep. 2004; 53:918–20.
25. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999–2010. J Am Med Assoc. 2012; 307:491–7.
26. US Centers for Disease Control and Prevention. Crude and age-adjusted percentage of civilian, noninstitutionalized population with diagnosed diabetes, United States, 1980–2010. [cited 2013 Feb 24]. Available from: http://www.cdc.gov/diabetes/statistics/prev/national/figage.htm
27. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes: vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. Washington, D.C.: National Academy Press; 2001.
28. U.S. Centers for Disease Control and Prevention. About the National Health and Nutrition Examination Survey. [cited 2013 Feb 24]. Available from: http://www.cdc.gov/nchs/nhanes/about_nhanes.htm
29. U.S. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey 2003–2004 Lab Methods. [cited 2013 Feb 24]. Available from: http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/lab_methods_03_04.htm
30. U.S. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey 2005–2006 Lab Methods. [cited 2013 Feb 24]. Available from: http://www.cdc.gov/nchs/nhanes/nhanes2005-2006/lab_methods_05_06.htm
31. Sternberg MR, Schleicher RL, Pfeiffer CM. Regression modeling plan for twenty-nine biochemical indicators of diet and nutrition measured in NHANES 2003–2006. J Nutr. 2013 in print.
32. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Am J Clin Nutr. 2004; 80:1678S–88S. [PubMed: 15585788]
33. Ascherio A, Willett WC, Rimm EB, Giovannucci EL, Stampfer MJ. Dietary iron intake and risk of coronary disease among men. Circulation. 1994; 89:969–74. [PubMed: 8124837]
34. Törnqvist M, Fred C, Haglund J, Helleberg H, Paulsson B, Rydberg P. Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. J Chromatogr B. 2002; 778:279–308.
35. Shelnutt SR, Cimino CO, Wiggins PA, Badger TM. Urinary pharmacokinetics of the glucuronide and sulfate conjugates of genistein and daidzein. Cancer Epidemiol Biomarkers Prev. 2000; 9:413–9. [PubMed: 10794486]
36. Setchell KDR, Faughnan MS, Avades T, Zimmer-Nechemias L, Brown NM, Wolfe BE, Brashear WT, Desai P, Oldfield MF, et al. Comparing the pharmacokinetics of daidzein and genistein with the use of C-13-labeled tracers in premenopausal women. Am J Clin Nutr. 2003; 77:411–9. [PubMed: 12540402]
37. Kritchevsky SB, Bush AJ, Pahor M, Gross MD. Serum carotenoids and markers of inflammation in nonsmokers. Am J Epidemiol. 2000; 152:1065–71. [PubMed: 11117616]
38. 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. [PubMed: 12947436]
39. Gruber M, Chappell R, Millen A, LaRowe T, Moeller SM, Iannaccone A, Kritchevsky SB, Mares J. Correlates of serum lutein plus zeaxanthin: Findings from the Third National Health and Nutrition Examination Survey. J Nutr. 2004; 134:2387–94. [PubMed: 15333733]
40. Chun OK, Chung SJ, Claycombe KJ, Song WO. Serum C-reactive protein concentrations are inversely associated with dietary flavonoid intake in US adults. J Nutr. 2008; 138:753–60. [PubMed: 18356331]
41. Pellegrini N, Valtuena S, Ardigo D, Brighenti F, Franzini L, Del Rio D, Scazzina F, Piatti PM, Zavaroni I. Intake of plant lignans matairesinol, secoisolariciresinol, pinoresinol, and lariciresinol in relation to vascular inflammation and endothelial dysfunction in middle age-elderly men and post-menopausal women living in Northern Italy. Nutr Metabol Cardiovasc Dis. 2010; 20:64–71.
42. Beard, J. WHO/CDC. Assessing the iron status of populations: report of a joint World Health Organization/Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level. Geneva: World Health Organization; 2007. Indicators of the iron status of the population: free erythrocyte protoporphyrin and zinc protoporphyrin; serum and

plasma iron, total iron binding capacity and transferrin saturation; and serum transferrin receptor. [cited 2013 Feb 24]. Available from: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107.pdf

43. Hodson L, Skeaff CM, Fielding BA. Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. Prog Lipid Res. 2008; 47:348–80. [PubMed: 18435934]
44. Ahmad I, Zhan M, Miller M. High prevalence of C-reactive protein elevation with normal triglycerides (100–149 mg/dL): Are triglyceride levels below 100 mg/dL more optimal in coronary heart disease risk assessment? Am J Med Sci. 2005; 329:173–7. [PubMed: 15832099]
45. Arnadottir M, Hultberg B, Nilsson-Ehle P, Thysell H. The effect of reduced glomerular filtration rate on plasma total homocysteine concentration. Scand J Clin Lab Invest. 1996; 56:41–6. [PubMed: 8850171]
46. Institute of Medicine, Food and Nutrition Board. Dietary reference intakes: Vitamin C, vitamin E, selenium, and carotenoids. Washington, D.C.: National Academy Press; 2000.
47. Milman N. Iron and pregnancy – a delicate balance. Ann Hematol. 2006; 85:559–65. [PubMed: 16691399]
48. Murphy MM, Molloy AM, Ueland PM, Fernandez-Ballart JD, Schneede J, Arija V, Scott JM. Longitudinal study of the effect of pregnancy on maternal and fetal cobalamin status in healthy women and their offspring. J Nutr. 2007; 137:1863–7. [PubMed: 17634256]
49. Milman N, Byg K-E, Bergholt T, Eriksen L, Hvas A-M. Cobalamin status during normal pregnancy and postpartum: a longitudinal study comprising 406 Danish women. Eur J Haematolog. 2006; 76:521–5.
50. Shields RC, Caric V, Hair M, Jones O, Wark L, McColl MD, Ramsay JE. Pregnancy-specific reference ranges for haematological variables in a Scottish population. J Obstet Gynaecol. 2011; 31:286–9. [PubMed: 21534746]
51. Johnston CS, Thompson LL. Vitamin C status of an outpatient population. J Am Coll Nutr. 1998; 17:366–70. [PubMed: 9710847]
52. Kang SS, Wong PW, Zhou JM, Cook HY. Total homocyst(e)ine in plasma and amniotic fluid of pregnant women. Metabolism. 1986; 35:889–91. [PubMed: 3762398]
53. Murphy MM, Scott JM, McPartlin JM, Fernandez-Ballart JD. The pregnancy-related decrease in fasting plasma homocysteine is not explained by folic acid supplementation, hemodilution, or a decrease in albumin in a longitudinal study. Am J Clin Nutr. 2002; 76:614–9. [PubMed: 12198008]
54. Ginde AA, Sullivan AF, Mansbach JM, Camargo CA. Vitamin D insufficiency in pregnant and nonpregnant women of childbearing age in the United States. Am J Obstet Gynecol. 2010; 202:436.e1–8. [PubMed: 20060512]
55. Milman N, Hvas A-M, Bergholt T. Vitamin D status during normal pregnancy and postpartum. A longitudinal study in 141 Danish women. J Perinat Med. 2012; 40:57–61. [PubMed: 22098305]
56. Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr. 2007; 137:447–52. [PubMed: 17237325]
57. Dati F, Schumann G, Thomas L, et al. Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP reference material (CRM 470). Eur J Clin Chem Clin Biochem. 1996; 34:517–20. [PubMed: 8831057]
58. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, Hogg RJ, Perrone RD, Lau J, Eknoyan G. National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med. 2003; 139:137–47. [PubMed: 12859163]

Table 1

List of dependent and independent variables including stratification

Nutrient classes and biomarkers of diet and nutrition (dependent variables)
Water-soluble vitamins (WSV) and related metabolites (RM):
• Serum folate (S-FOL)
• RBC folate (RBC-FOL)
• Serum pyridoxal-5′-phosphate (PLP)
• Serum 4-pyridoxic acid (4PA)
• Serum total cobalamin (B-12)
• Plasma total homocysteine (tHcy)
• Plasma methylmalonic acid (MMA)
• Serum ascorbic acid (VIC)
Fat-soluble vitamins (FSV) and micronutrients (MN):
• Serum retinol (VIA)
• Serum *alpha*-tocopherol (VIE)
• Serum carotenes (CAR, sum of 3 analytes)
• Serum xanthophylls (XAN, sum of 3 analytes)
• Serum 25-hydroxyvitamin D (25OHD)
Fatty acids:
• Plasma saturated (SFA, sum of 6 analytes)
• Plasma monounsaturated (MUFA, sum of 7 analytes)
• Plasma polyunsaturated (PUFA, sum of 11 analytes)
• Plasma total fatty acids (tFA, sum of 24 analytes)
Trace elements:
• Serum ferritin (FER)
• Serum soluble transferrin receptor (sTfR)
• Serum body iron (BI, calculated from the ratio of sTfR to FER)
• Urine iodine (uI)
Phytoestrogens:
• Urine genistein (GEN)
• Urine daidzein (DAZ)
• Urine equol (EQU)
• Urine O-desmethylangolensin (DMA)
• Urine enterodiol (ETD)
• Urine enterolactone (ETL)
Acrylamide hemoglobin adducts:
• Hemolyzed blood acrylamide hemoglobin adduct (HbAA)
• Hemolyzed blood glycidamide hemoglobin adduct (HbGA)
Physiologic variables (independent variables)
Fasting status:

Nutrient classes and biomarkers of diet and nutrition (dependent variables)
• <3 h • 3–<8 h • ≥8 h **Inflammation** (based on C-reactive protein [CRP]): • No, CRP <5 mg/L • Yes, CRP ≥5 mg/L (57) **Renal function** (defined by estimated glomerular filtration rate [eGFR] using the National Kidney Foundation classification system to determine stages of chronic kidney disease (CKD [58]): • Normal, eGFR ≥60 mL/(min·1.73 m^2) and absence of albuminuria • Stage 1 or 2 of CKD, eGFR ≥60 mL/(min·1.73 m^2) and presence of albuminuria • Stage 3–5 CKD, eGFR <60 mL/(min·1.73 m^2) **Pregnancy** (ascertained at the time of the health examination by the value from urine pregnancy test and from self-reported pregnancy status; for women who were interviewed but not examined, pregnancy status could not be determined): • No • Yes

Table 2

Unadjusted mean biomarker concentrations by fasting categories for adults ≥20 y, NHANES 2003–2006[1,2,3,4,5]

Analyte (matrix)[6], *unit*	Overall	Fasting			*P*-value[7]
		<3 h	3–<8 h	≥8 h	
Water-soluble vitamins and related metabolites					
FOL (S), *μg/L*	11.9 (11.6 – 12.2)	12.7[a] (12.1 – 13.4)	12.2[a] (11.8 – 12.6)	11.5[b] (11.2 – 11.9)	<0.0001
FOL (RBC), *μg/L*	271 (266 – 276)	281[a] (274 – 288)	273[a,b] (264 – 281)	267[b] (261 – 273)	0.0021
PLP (S), *nmol/L*	50.1 (47.4 – 52.9)	53.7[a] (50.0 – 57.6)	48.0[b] (43.7 – 52.8)	48.5[b] (45.3 – 51.9)	0.028
4PA (S), *nmol/L*	35.6 (33.3 – 38.0)	41.0[a] (37.2 – 45.2)	37.1[a] (33.9 – 40.5)	31.7[b] (29.3 – 34.2)	<0.0001
B-12 (S), *ng/L*	465 (454 – 476)	475 (457 – 494)	467 (452 – 482)	461 (448 – 475)	0.26
tHcy (P), *μmol/L*	8.21 (8.06 – 8.37)	7.92[a] (7.71 – 8.14)	8.69[b] (8.50 – 8.88)	8.12[a] (7.95 – 8.29)	<0.0001
MMA (P), *nmol/L*	139 (132 – 146)	163[a] (140 – 189)	146[a] (140 – 152)	134[b] (126 – 142)	<0.0001
VIC (S), *μmol/L*	54.0 (52.4 – 55.5)	55.9[a] (53.6 – 58.2)	56.9[a] (54.6 – 59.1)	52.0[b] (50.3 – 53.8)	<0.0001
Fat-soluble vitamins and (micro)nutrients					
VIA (S), *μg/dL*	58.2 (57.3 – 59.2)	58.3 (57.2 – 59.4)	57.9 (56.1 – 59.8)	58.3 (57.2 – 59.5)	0.86
VIE (S), *mg/dL*	1.18 (1.15 – 1.21)	1.21[a] (1.17 – 1.24)	1.18[a,b] (1.16 – 1.20)	1.16[b] (1.13 – 1.19)	0.0483
CAR (S), *μg/dL*	59.7 (58.2 – 61.3)	62.0[a] (59.5 – 64.6)	57.8[b] (55.4 – 60.3)	58.8[b] (56.7 – 61.1)	0.0342
XAN (S), *μg/dL*	22.8 (21.9 – 23.7)	23.1 (21.9 – 24.3)	23.0 (21.8 – 24.2)	22.5 (21.4 – 23.6)	0.63
25OHD (S), *nmol/L*	58.8 (57.0 – 60.7)	59.1 (56.7 – 61.6)	58.4 (56.5 – 60.2)	58.9 (56.8 – 61.0)	0.63
SFA (P), *mmol/L*	3.68 (3.59 – 3.77)	No data	No data	3.68 (3.59 – 3.77)	n/a
MUFA (P), *mmol/L*	2.58 (2.52 – 2.65)	No data	No data	2.58 (2.52 – 2.65)	n/a
PUFA (P), *mmol/L*	4.82 (4.73 – 4.91)	No data	No data	4.82 (4.73 – 4.91)	n/a

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Analyte (matrix)[6], *unit*	Overall	Fasting			*P*-value[7]
		<3 h	3–<8 h	≥8 h	
tFA (P), *mmol/L*	11.1 (10.9 – 11.4)	No data	No data	11.1 (10.9 – 11.4)	n/a
Trace elements					
FER (S), *μg/L*	39.9 (38.2 – 41.7)	40.4 (37.1 – 44.0)	39.2 (35.1 – 43.7)	40.0 (37.7 – 42.4)	0.89
sTfR (S), *mg/L*	3.46 (3.39 – 3.53)	3.36 (3.22 – 3.50)	3.52 (3.39 – 3.65)	3.47 (3.38 – 3.57)	0.22
BI (S), *mg/kg*	5.65 (5.47 – 5.83)	5.78 (5.41 – 6.15)	5.51 (5.03 – 6.00)	5.65 (5.43 – 5.88)	0.65
uI (U), *μg/L*	146 (139 – 153)	154 (137 – 173)	148 (136 – 160)	142 (135 – 150)	0.32
Phytoestrogens					
GEN (U), *μg/L*	28.6 (26.5 – 30.9)	27.5[a,b] (22.5 – 33.6)	23.2[a] (20.6 – 26.2)	31.6[b] (28.4 – 35.2)	0.0022
DAZ (U), *μg/L*	61.1 (56.2 – 66.5)	61.8[a,b] (49.1 – 77.7)	50.1[a] (44.2 – 56.7)	66.3[b] (58.6 – 74.9)	0.0108
EQU (U), *μg/L*	7.43 (6.85 – 8.05)	6.61[a] (5.77 – 7.57)	6.07[a] (5.30 – 6.95)	8.40[b] (7.53 – 9.37)	0.0009
DMA (U), *μg/L*	4.32 (3.92 – 4.75)	3.84[a,b] (2.77 – 5.32)	3.35[a] (2.74 – 4.10)	4.99[b] (4.33 – 5.76)	0.0131
ETD (U), *μg/L*	38.2 (34.9 – 41.7)	32.6[a] (26.8 – 39.6)	28.5[a] (24.1 – 33.8)	45.4[b] (40.3 – 51.2)	<0.0001
ETL (U), *μg/L*	282 (255 – 312)	255[a,b] (208 – 314)	246[a] (207 – 294)	309[b] (273 – 349)	0.0378
Acrylamide hemoglobin adducts					
HbAA (B), *pmol/g Hb*	62.1 (58.6 – 65.9)	66.5 (60.7 – 72.8)	61.3 (56.3 – 66.9)	62.2 (58.3 – 66.4)	0.33
HbGA (B), *pmol/g Hb*	58.2 (55.3 – 61.2)	58.0 (52.0 – 64.7)	57.5 (52.9 – 62.6)	58.5 (55.6 – 61.5)	0.89

[1] Biomarker concentrations represent geometric means (arithmetic means for vitamin C, vitamin D, and body iron) and 95% CI. Within a variable, labeled means in a row without a common letter differ based on pairwise comparison, $P<0.05$.

[2] 25OHD, 25-hydroxyvitamin D; 4PA, 4-pyridoxic acid; B-12, total cobalamin; BI, body iron; CAR, carotenes; DAZ, daidzein; DMA, O-desmethylangolensin; EQU, equol; ETD, enterodiol; ETL, enterolactone; FER, ferritin; FOL, folate; HbAA, acrylamide hemoglobin adduct; HbGA, glycidamide hemoglobin adduct; GEN, genistein; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; sTfR, soluble transferrin receptor; tFA, total fatty acids; tHcy, total homocysteine; uI, urine iodine; VIA, retinol; VIC, ascorbic acid; VIE, *alpha*-tocopherol; XAN, xanthophylls.

[3] SI conversion factors are as follows: B-12, ×0.738 (pmol/L); DAZ, ×3.93 (nmol/L); DMA, ×3.87 (nmol/L); EQU, ×4.13 (nmol/L); ETD, ×3.31 (nmol/L); ETL, ×3.35 (nmol/L); FER, ×2.247 (pmol/L); FOL, ×2.266 (nmol/L); GEN, ×3.70 (nmol/L); uI, ×7.88 (nmol/L); VIA, ×0.03491 (μmol/L); VIE, ×23.218 (μmol/L).

[4] MMA, SFA, MUFA, PUFA, tFA, HbAA, and HbGA data only available for NHANES 2003–2004; PLP, 4PA, VIA, VIE, CAR, and XAN data only available for NHANES 2005–2006.

[5] Sample sizes for each biomarker by covariate categories can be found in Supplemental Table 1.

[6] S, serum; P, plasma; U, urine; B, whole blood.

[7] *P*-value based on Wald F test, which tests whether at least 1 of the means across the categories is significantly different.

Table 3

Unadjusted mean biomarker concentrations by inflammation and renal function categories for adults ≥20 y, NHANES 2003–2006[1,2,3,4,5]

Analyte (matrix)[6], *unit*	Overall	Inflammation[7]		*P*-value[9]	Overall	Renal function[8]			*P*- value[9]
		No	Yes			Normal	CKD stage 1 or 2	CKD stage 3–5	
Water-soluble vitamins and related metabolites									
FOL (S), *μg/L*	11.9 (11.6 – 12.2)	12.0 (11.7 – 12.4)	11.5 (11.1 – 12.0)	0.0084	11.9 (11.6 – 12.2)	11.6[a] (11.3 – 11.9)	11.7[a] (11.3 – 12.1)	15.8[b] (14.6 – 17.1)	<0.0001
FOL (RBC), *μg/L*	271 (266 – 276)	268 (262 – 273)	281 (275 – 288)	<0.0001	271 (266 – 277)	265[a] (259 – 270)	276[b] (267 – 285)	340[c] (326 – 354)	<0.0001
PLP (S), *nmol/L*	50.1 (47.4 – 53.0)	55.2 (52.6 – 57.8)	36.5 (33.6 – 39.6)	<0.0001	50.3 (47.6 – 53.2)	51.5[a] (48.5 – 54.6)	41.8[b] (38.2 – 45.8)	51.6[a] (46.1 – 57.8)	0.0001
4PA (S), *nmol/L*	35.6 (33.3 – 38.1)	36.1 (33.6 – 38.7)	34.2 (31.5 – 37.2)	0.17	35.7 (33.4 – 38.1)	33.0[a] (30.9 – 35.2)	33.4[a] (30.0 – 37.2)	83.0[b] (72.7 – 94.8)	<0.0001
B-12 (S), *ng/L*	465 (454 – 476)	466 (455 – 477)	461 (447 – 476)	0.42	465 (454 – 476)	461[a] (449 – 473)	474[a,b] (453 – 496)	497[b] (477 – 519)	0.002
tHcy (P), *μmol/L*	8.22 (8.06 – 8.37)	8.25 (8.10 – 8.40)	8.11 (7.87 – 8.36)	0.20	8.21 (8.06 – 8.36)	7.88[a] (7.75 – 8.02)	8.35[b] (8.10 – 8.61)	12.1[c] (11.7 – 12.6)	<0.0001
MMA (P), *nmol/L*	138 (132 – 145)	139 (132 – 145)	138 (128 – 149)	0.89	138 (132 – 145)	131[a] (126 – 137)	144[b] (133 – 156)	226[c] (212 – 241)	<0.0001
VIC (S), *μmol/L*	54.0 (52.4 – 55.5)	55.7 (54.0 – 57.4)	48.2 (46.6 – 49.7)	<0.0001	54.0 (52.5 – 55.6)	53.9[a] (52.1 – 55.6)	50.7[b] (48.1 – 53.3)	59.9[c] (57.2 – 62.7)	<0.0001
Fat-soluble vitamins and (micro)nutrients									
VIA (S), *μg/dL*	58.2 (57.3 – 59.2)	59.5 (58.5 – 60.6)	54.1 (52.6 – 55.7)	<0.0001	58.3 (57.3 – 59.3)	57.2[a] (56.2 – 58.2)	56.9[a] (54.6 – 59.2)	72.6[b] (70.3 – 75.1)	<0.0001
VIE (S), *mg/dL*	1.18 (1.15 – 1.21)	1.18 (1.15 – 1.21)	1.19 (1.15 – 1.23)	0.61	1.18 (1.16 – 1.21)	1.15[a] (1.13 – 1.17)	1.23[b] (1.18 – 1.29)	1.41[c] (1.33 – 1.50)	<0.0001
CAR (S), *μg/dL*	59.7 (58.2 – 61.3)	62.7 (60.4 – 64.9)	50.8 (48.4 – 53.2)	<0.0001	59.9 (58.3 – 61.6)	60.8[a] (59.0 – 62.7)	55.9[b] (52.2 – 59.7)	56.8[b] (53.5 – 60.4)	0.0052
XAN (S), *μg/dL*	22.8 (21.9 – 23.7)	23.9 (23.0 – 24.9)	19.3 (18.4 – 20.4)	<0.0001	22.8 (21.9 – 23.7)	22.8 (21.8 – 23.8)	22.4 (20.6 – 24.3)	23.5 (22.5 – 24.6)	0.48
25OHD (S), *nmol/L*	58.9 (57.0 – 60.7)	60.2 (58.4 – 62.0)	54.4 (52.0 – 56.9)	<0.0001	58.9 (57.0 – 60.8)	59.5[a] (57.5 – 61.5)	55.1[b] (52.7 – 57.5)	57.9[a] (55.5 – 60.4)	<0.0001
SFA (P), *mmol/L*	3.68 (3.59 – 3.77)	3.60 (3.50 – 3.69)	3.98 (3.87 – 4.09)	<0.0001	3.68 (3.60 – 3.77)	3.65[a] (3.56 – 3.74)	3.88[b] (3.70 – 4.07)	3.82[b] (3.65 – 3.99)	0.0177
MUFA (P), *mmol/L*	2.58 (2.52 – 2.65)	2.52 (2.44 – 2.61)	2.81 (2.69 – 2.93)	0.0001	2.58 (2.52 – 2.65)	2.53[a] (2.46 – 2.60)	2.81[b] (2.60 – 3.04)	2.90[b] (2.73 – 3.07)	<0.0001

Analyte (matrix)[6], *unit*	Overall	Inflammation[7]		*P*-value[9]	Overall	Renal function[8]			*P*- value[9]
		No	Yes			Normal	CKD stage 1 or 2	CKD stage 3–5	
PUFA (P), *mmol/L*	4.82 (4.73 – 4.91)	4.78 (4.69 – 4.87)	4.97 (4.83 – 5.11)	0.0016	4.82 (4.73 – 4.91)	4.84 (4.75 – 4.92)	4.73 (4.38 – 5.11)	4.80 (4.60 – 5.00)	0.83
tFA (P), *mmol/L*	11.1 (10.9 – 11.4)	11.0 (10.7 – 11.2)	11.8 (11.4 – 12.1)	<0.0001	11.1 (10.9 – 11.4)	11.1[a] (10.8 – 11.3)	11.5[a,b] (11.0 – 12.0)	11.5[b] (11.0 – 12.1)	0.0379
Trace elements									
FER (S), *μg/L*	39.9 (38.2 – 41.7)	37.5 (35.8 – 39.3)	46.5 (43.0 – 50.3)	<0.0001	39.9 (38.2 – 41.7)	40.5 (38.4 – 42.8)	34.2 (29.4 – 39.8)	NR[8]	0.11
sTfR (S), *mg/L*	3.46 (3.39 – 3.53)	3.41 (3.35 – 3.48)	3.56 (3.44 – 3.69)	0.0108	3.46 (3.38 – 3.53)	3.42[a] (3.34 – 3.49)	3.82[b] (3.63 – 4.03)	NR	0.0009
BI (S), *mg/kg*	5.65 (5.47 – 5.83)	5.46 (5.26 – 5.66)	6.09 (5.75 – 6.44)	0.0028	5.65 (5.47 – 5.83)	5.74[a] (5.51 – 5.98)	4.73[b] (4.07 – 5.39)	NR	0.0388
uI (U), *μg/L*	146 (139 – 153)	144 (137 – 150)	154 (143 – 167)	0.0289	146 (140 – 153)	144[a] (137 – 152)	127[b] (114 – 140)	211[c] (179 – 249)	<0.0001
Phytoestrogens									
GEN (U), *μg/L*	28.5 (26.4 – 30.8)	28.6 (25.9 – 31.6)	28.2 (23.9 – 33.3)	0.90	28.3 (26.2 – 30.7)	27.7 (25.3 – 30.4)	29.2 (22.9 – 37.4)	34.4 (28.9 – 40.9)	0.13
DAZ (U), *μg/L*	60.9 (56.0 – 66.3)	61.8 (55.1 – 69.3)	58.2 (50.8 – 66.7)	0.54	60.6 (55.6 – 66.2)	60.1 (54.2 – 66.6)	58.5 (47.0 – 72.9)	69.8 (57.0 – 85.5)	0.41
EQU (U), *μg/L*	7.45 (6.90 – 8.05)	7.44 (6.80 – 8.15)	7.48 (6.82 – 8.21)	0.93	7.43 (6.86 – 8.05)	7.57 (6.93 – 8.28)	6.73 (5.16 – 8.78)	6.90 (5.80 – 8.20)	0.42
DMA (U), *μg/L*	4.31 (3.92 – 4.74)	4.72 (4.18 – 5.32)	3.23 (2.78 – 3.76)	0.0005	4.29 (3.89 – 4.73)	4.21 (3.73 – 4.76)	3.78 (2.81 – 5.09)	6.06 (4.26 – 8.62)	0.09
ETD (U), *μg/L*	38.3 (35.0 – 41.9)	40.0 (35.9 – 44.6)	33.3 (28.4 – 39.0)	0.07	38.3 (35.0 – 41.9)	38.1 (34.2 – 42.5)	41.0 (32.5 – 51.6)	37.8 (31.6 – 45.2)	0.84
ETL (U), *μg/L*	285 (258 – 315)	317 (290 – 346)	203 (167 – 248)	<0.0001	284 (258 – 313)	286[a,b] (255 – 321)	225[a] (181 – 279)	367[b] (288 – 469)	0.0137
Acrylamide hemoglobin adducts									
HbAA (B), *pmol/g Hb*	62.2 (58.6 – 66.0)	62.8 (59.4 – 66.4)	60.3 (55.1 – 66.0)	0.18	62.2 (58.6 – 66.1)	63.6[a] (59.7 – 67.7)	63.3[a] (58.9 – 68.2)	48.9[b] (44.3 – 54.0)	<0.0001
HbGA (B), *pmol/g Hb*	58.3 (55.4 – 61.4)	57.5 (54.8 – 60.2)	61.4 (56.6 – 66.6)	0.0244	58.5 (55.6 – 61.5)	59.9[a] (56.7 – 63.3)	58.5[a] (54.0 – 63.4)	45.1[b] (41.7 – 48.7)	<0.0001

[1]Biomarker concentrations represent geometric means (arithmetic means for vitamin C, vitamin D, and body iron) and 95% CI. Within a variable, labeled means in a row without a common letter differ based on pairwise comparison, $P<0.05$.

[2] 25OHD, 25-hydroxyvitamin D; 4PA, 4-pyridoxic acid; B-12, total cobalamin; BI, body iron; CAR, carotenes; DAZ, daidzein; DMA, O-desmethylangolensin; EQU, equol; ETD, enterodiol; ETL, enterolactone; FER, ferritin; FOL, folate; HbAA, acrylamide hemoglobin adduct; HbGA, glycidamide hemoglobin adduct; GEN, genistein; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; sTfR, soluble transferrin receptor; tFA, total fatty acids; tHcy, total homocysteine; uI, urine iodine; VIA, retinol; VIC, ascorbic acid; VIE, *alpha*-tocopherol; XAN, xanthophylls.

[3] SI conversion factors are as follows: B-12, ×0.738 (pmol/L); DAZ, ×3.93 (nmol/L); DMA, ×3.87 (nmol/L); EQU, ×4.13 (nmol/L); ETD, ×3.31 (nmol/L); ETL, ×3.35 (nmol/L); FER, ×2.247 (pmol/L); FOL, ×2.266 (nmol/L); GEN, ×3.70 (nmol/L); uI, ×7.88 (nmol/L); VIA, ×0.03491 (μmol/L); VIE, ×23.218 (μmol/L).

[4] MMA, SFA, MUFA, PUFA, tFA, HbAA and HbGA data only available for NHANES 2003–2004; PLP, 4PA, VIA, VIE, CAR, and XAN data only available for NHANES 2005–2006.

[5] Sample sizes for each biomarker by covariate categories can be found in Supplemental Table 1.

[6] S, serum; P, plasma; U, urine; B, whole blood.

[7] C-reactive protein (CRP, mg/L) was used to assess inflammation status (<5, no inflammation; ≥5, inflammation).

[8] Estimated glomerular filtration rate (eGFR) was used to assess renal function; normal was defined as eGFR ≥60 mL/(min·1.73 m^2) and absence of albuminuria; stage 1 or 2 chronic kidney disease (CKD) was defined as eGFR ≥60 mL/(min·1.73 m^2) and presence of albuminuria; stage 3–5 CKD was defined as eGFR <60 mL/(min·1.73 m^2).

[9] *P*-value based on Wald F test, which tests whether at least 1 of the means across the categories is significantly different.

[10] Not reported due to small sample size (n <42).

Table 4

Unadjusted mean biomarker concentrations by pregnancy categories for women 20–49 y of age, NHANES 2003–2006[1,2,3,4,5]

Analyte (matrix)[6], *unit*	Overall	Pregnant		*P*-value[7]
		No	Yes	
Water-soluble vitamins and related metabolites				
FOL (S), *μg/L*	11.2 (10.8 – 11.5)	11.0 (10.6 – 11.3)	14.4 (13.4 – 15.5)	<0.0001
FOL (RBC), *μg/L*	259 (251 – 266)	254 (247 – 261)	347 (330 – 366)	<0.0001
PLP (S), *nmol/L*	43.0 (39.5 – 46.8)	43.0 (39.5 – 46.8)	42.7 (35.6 – 51.2)	0.93
4PA (S), *nmol/L*	26.9 (24.6 – 29.4)	26.2 (24.1 – 28.4)	38.6 (28.6 – 51.9)	0.0047
B-12 (S), *ng/L*	443 (429 – 458)	449 (433 – 464)	372 (348 – 398)	<0.0001
tHcy (P), *μmol/L*	6.67 (6.53 – 6.82)	6.86 (6.71 – 7.02)	4.35 (4.18 – 4.51)	<0.0001
MMA (P), *nmol/L*	120 (113 – 127)	121 (114 – 128)	106 (92.1 – 123)	0.05
VIC (S), *μmol/L*	53.7 (51.3 – 56.1)	53.1 (50.7 – 55.6)	63.3 (60.5 – 66.0)	<0.0001
Fat-soluble vitamins and (micro)nutrients				
VIA (S), *μg/dL*	51.3 (49.9 – 52.7)	52.3 (50.8 – 53.9)	40.3 (37.9 – 42.8)	<0.0001
VIE (S), *mg/dL*	1.07 (1.04 – 1.10)	1.05 (1.02 – 1.08)	1.23 (1.17 – 1.30)	<0.0001
CAR (S), *μg/dL*	61.1 (59.0 – 63.3)	60.7 (58.4 – 63.1)	66.7 (61.4 – 72.5)	0.06
XAN (S), *μg/dL*	22.1 (20.9 – 23.4)	21.7 (20.4 – 23.0)	29.0 (26.7 – 31.6)	<0.0001
25OHD (S), *nmol/L*	59.8 (57.4 – 62.2)	59.2 (56.8 – 61.6)	68.9 (63.7 – 74.2)	0.0003
SFA (P), *mmol/L*	3.53 (3.40 – 3.66)	3.49 (3.35 – 3.63)	4.28 (4.02 – 4.56)	<0.0001
MUFA (P), *mmol/L*	2.36 (2.28 – 2.44)	2.33 (2.26 – 2.42)	2.87 (2.67 – 3.08)	<0.0001
PUFA (P), *mmol/L*	4.76 (4.64 – 4.88)	4.73 (4.61 – 4.85)	5.42 (5.11 – 5.75)	<0.0001
tFA (P), *mmol/L*	10.7 (10.4 – 10.9)	10.6 (10.3 – 10.9)	12.6 (11.8 – 13.5)	<0.0001
Trace elements				
FER (S), *μg/L*	39.9 (38.2 – 41.7)	40.9 (39.1 – 42.8)	27.1 (23.4 – 31.4)	<0.0001
sTfR (S), *mg/L*	3.46 (3.39 – 3.53)	3.46 (3.39 – 3.54)	3.40 (3.24 – 3.55)	0.46
BI (S), *mg/kg*	5.65 (5.46 – 5.83)	5.73 (5.54 – 5.92)	4.30 (3.66 – 4.94)	0.0001
uI (U), *μg/L*	116 (107 – 125)	115 (107 – 123)	137 (100 – 189)	0.22
Phytoestrogens				
GEN (U), *μg/L*	23.0 (19.1 – 27.7)	23.1 (19.0 – 28.1)	21.5 (16.2 – 28.6)	0.64
DAZ (U), *μg/L*	50.7 (42.5 – 60.4)	51.1 (42.5 – 61.4)	45.6 (34.2 – 60.8)	0.47
EQU (U), *μg/L*	7.38 (6.35 – 8.57)	7.34 (6.30 – 8.55)	7.91 (5.60 – 11.2)	0.66
DMA (U), *μg/L*	3.97 (3.26 – 4.82)	4.05 (3.29 – 5.00)	2.99 (1.84 – 4.87)	0.26
ETD (U), *μg/L*	36.7 (31.6 – 42.6)	35.9 (30.5 – 42.1)	48.9 (30.8 – 77.8)	0.22
ETL (U), *μg/L*	266 (224 – 315)	265 (221 – 318)	275 (190 – 399)	0.85
Acrylamide hemoglobin adducts				
HbAA (B), *pmol/g Hb*	63.9 (59.7 – 68.4)	64.9 (60.3 – 69.9)	46.4 (41.4 – 52.0)	<0.0001
HbGA (B), *pmol/g Hb*	64.6 (60.7 – 68.9)	64.9 (60.7 – 69.5)	59.2 (51.4 – 68.1)	0.25

[1] Biomarker concentrations represent geometric means (arithmetic means for vitamin C, vitamin D, and body iron) and 95% CI.

[2] 25OHD, 25-hydroxyvitamin D; 4PA, 4-pyridoxic acid; B-12, total cobalamin; BI, body iron; CAR, carotenes; DAZ, daidzein; DMA, O-desmethylangolensin; EQU, equol; ETD, enterodiol; ETL, enterolactone; FER, ferritin; FOL, folate; HbAA, acrylamide hemoglobin adduct;

HbGA, glycidamide hemoglobin adduct; GEN, genistein; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; sTfR, soluble transferrin receptor; tFA, total fatty acids; tHcy, total homocysteine; uI, urine iodine; VIA, retinol; VIC, ascorbic acid; VIE, *alpha*-tocopherol; XAN, xanthophylls.

[3] SI conversion factors are as follows: B-12, ×0.738 (pmol/L); DAZ, ×3.93 (nmol/L); DMA, ×3.87 (nmol/L); EQU, ×4.13 (nmol/L); ETD, ×3.31 (nmol/L); ETL, ×3.35 (nmol/L); FER, ×2.247 (pmol/L); FOL, ×2.266 (nmol/L); GEN, ×3.70 (nmol/L); uI, ×7.88 (nmol/L); VIA, ×0.03491 (μmol/L); VIE, ×23.218 (μmol/L).

[4] MMA, SFA, MUFA, PUFA, tFA, HbAA and HbGA data only available for NHANES 2003–2004; PLP, 4PA, VIA, VIE, CAR, and XAN data only available for NHANES 2005–2006.

[5] Sample sizes for each biomarker by covariate categories can be found in Supplemental Table 1.

[6] S, serum; P, plasma; U, urine; B, whole blood.

[7] *P*-value based on Wald F test, which tests whether at least 1 of the means across the categories is significantly different.

J Nutr. Author manuscript; available in PMC 2016 March 29.

Table 5

Estimated change in biomarker concentrations by fasting, inflammation, renal function (adults ≥20 y) and pregnancy (women 20–49 y), NHANES 2003–2006[1,2,3]

Analyte (matrix)[4], *unit*	Fasting (<3 vs. ≥8 h)		Inflammation (yes vs. no)[5]		Renal function (CKD stage 3–5 vs. normal)[6]		Pregnancy (yes vs. no)	
	SLR[7]	MLR[8]	SLR	MLR	SLR	MLR	SLR	MLR[9]
Water-soluble vitamins and related metabolites								
FOL (S), %	10.4*	9.5*	−4.2*	−4.5*	36.5*	6.8*	31.4*	17.9*
FOL (RBC), %	5.3*	4.9*	5.1*	5.5*	28.3*	8.5*	36.8*	26.2*
PLP (S), %	10.7*	9.3*	−33.9*	−28.5*	0.4	2.9	−0.7	−12.6
4PA (S), %	29.6*	28.2*	−5.1	−3.9	151.6*	66.3*	47.2*	33.8*
B-12 (S), %	3.1	3.8	−1.0	−1.7	8.0*	5.3*	−17.1*	−21.5*
tHcy (P), %	−2.4	−2.5*	−1.6	−1.5	53.8*	32.3*	−36.7*	−29.6*
MMA (P), %	21.4*	21.0*	−0.3	−1.1	72.4*	42.3*	−11.8	−4.2
VIC (S), *µmol/L*	3.9*	3.4*	−7.5*	−8.2*	6.1*	−1.3	10.1*	3.8*
Fat-soluble vitamins and (micro)nutrients								
VIA (S), %	−0.1	−0.9	−9.2*	−8.2*	27.0*	20.2*	−23.0*	−21.5*
VIE (S), %	3.6*	3.7*	0.7	0.1	22.5*	−0.7	17.1*	22.2*
CAR (S), %	5.4*	3.8	−19.0*	−18.3*	−6.6*	−5.4	9.9	11.9*
XAN (S), %	2.8	2.9	−19.2*	−20.6*	3.3	−3.9	34.1*	32.3*
25OHD (S), *nmol/L*	0.2	−0.5	−5.8*	−3.9*	−1.6	−0.7	9.7*	6.6*
SFA (P), %	N/A[10]	N/A	10.7*	9.5*	4.6*	−4.8*	22.6*	28.0*
MUFA (P), %	N/A	N/A	11.3*	10.9*	14.4*	0.9	22.9*	29.0*
PUFA (P), %	N/A	N/A	4.0*	2.9*	−0.8	−7.5*	14.7*	18.6*
tFA (P), %	N/A	N/A	7.3*	6.1*	4.0*	−4.2	19.0*	22.9*
Trace elements								
FER (S), %	1.0	1.8	24.0*	24.6*	9.1	5.9	−33.8*	−33.1*
sTfR (S), %	−3.4	−2.7	4.4*	2.5	14.0	10.8	−1.9	−3.0

Analyte (matrix)[4], *unit*	Fasting (<3 vs. ≥8 h)		Inflammation (yes vs. no)[5]		Renal function (CKD stage 3–5 vs. normal)[6]		Pregnancy (yes vs. no)	
	SLR[7]	MLR[8]	SLR	MLR	SLR	MLR	SLR	MLR[9]
BI (S), *mg/kg*	0.1	0.1	0.6*	0.7*	−0.2	−0.2	−1.4*	−1.4*
uI (U), %	8.3	26.4*	7.5*	1.6	46.2*	7.1	19.7	8.1
Phytoestrogens								
GEN (U), %	−13.0	4.3	−1.4	−4.4	24.0	2.5	−7.0	2.5
DAZ (U), %	−6.8	11.4	−5.8	−9.1	16.2	−4.9	−10.7	5.1
EQU (U), %	−21.3*	−5.8	0.5	−2.4	−9.0	−17.2	7.8	18.1
DMA (U), %	−23.1	−15.4	−31.4*	−35.5*	43.8	11.8	−26.2	−9.7
ETD (U), %	−28.2*	−14.1	−16.9	−20.8*	−0.9	−18.3	36.5	44.0
ETL (U), %	−17.3	−5.7	−35.7*	−37.9*	28.5	−1.5	4.0	8.9
Acrylamide hemoglobin adducts								
HbAA (B), %	6.9	4.4	−4.0	−4.8*	−23.1*	−5.7	−28.6*	−10.4*
HbGA (B), %	−0.9	1.9	6.9*	5.8*	−24.8*	−8.3*	−8.9	6.3

[1] Change represents percent change in geometric mean for all biomarkers except for vitamin C, vitamin D, and body iron where change in arithmetic mean represents concentration units.

[2] 25OHD, 25-hydroxyvitamin D; 4PA, 4-pyridoxic acid; B-12, total cobalamin; BI, body iron; CAR, carotenes; DAZ, daidzein; DMA, O-desmethylangolensin; EQU, equol; ETD, enterodiol; ETL, enterolactone; FER, ferritin; FOL, folate; GEN, genistein; HbAA, acrylamide hemoglobin adduct; HbGA, glycidamide hemoglobin adduct; MMA, methylmalonic acid; PLP, pyridoxal-5′-phosphate; sTfR, soluble transferrin receptor; tFA, total fatty acids; tHcy, total homocysteine; uI, urine iodine; VIA, retinol; VIC, ascorbic acid; VIE, *alpha*-tocopherol; XAN, xanthophylls.

[3] MMA, SFA, MUFA, PUFA, tFA, HbAA and HbGA data only available for NHANES 2003–2004; PLP, 4PA, VIA, VIE, CAR, and XAN data only available for NHANES 2005–2006.

[4] S, serum; P, plasma; U, urine; B, whole blood.

[5] C-reactive protein (CRP, mg/L) was used to assess inflammation status (<5, no inflammation; ≥5, inflammation).

[6] Estimated glomerular filtration rate (eGFR) was used to assess renal function; normal was defined as eGFR ≥60 mL/(min·1.73 m^2) and absence of albuminuria; stage 1 or 2 chronic kidney disease (CKD) was defined as eGFR ≥60 mL/(min·1.73 m^2) and presence of albuminuria; stage 3–5 CKD was defined as eGFR <60 mL/(min·1.73 m^2).

[7] Simple linear regression (model 1).

[8] Multiple linear regression model controlled for age, sex, race-ethnicity, smoking, supplement use, fasting, inflammation, and renal function (and urine creatinine for urine biomarkers) (model 2).

[9] Multiple linear regression model for women 20–49 y controlled for pregnancy in addition to the above mentioned variables (model 2).

[10] N/A as all subjects were fasting for ≥8 h.

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* Change is significantly different from 0; $P < 0.05$.