**Online Supporting Material**

Supplemental Methods

Development of Folate Model to Predict Serum 25-Hydroxyvitamin D Change
Between Pre- and Post-Fortification
Alicia L. Carriquiry

- Estimates of the serum folate distribution can be obtained from the NHANES database for the pre- fortification period (1988-1994) and early post-fortification period (1999-2004). *See Subject and Methods section of accompanying publication for data specifics.*

  - The change in the distribution from one time period to the next was quantified by mapping percentiles between the two time periods and then fitting a model to describe the map.

  - Changes in the serum folate distribution due to fortification were described by calculating how much each percentile in the pre-fortification period increased after fortification. To avoid issues of scale, we determined how much we would need to multiply each pre-fortification percentile to obtain the corresponding post-fortification percentile. See **Supplemental Figure 1.** A larger multiplicative factor for serum folate concentrations was required at the lower end of the distribution than at concentrations above approximately 20nmol/L.

- Observed—baseline for this study—serum 25-hydroxyvitamin D (25(OH)D) data are available from the 2001-2006 NHANES (*See Subject and Methods section of accompanying publication for data specifics).*To predict the change for serum 25(OH)D under similar circumstances of fortification as for folate, the following modeling approach was carried out:

  - Step 1: Fit a curve to the serum folate data illustrated in Supplemental Figure 1. The best fitting curve resulted in the fitted equation of yhat = c + a /(x-b); estimates of parameters a, b, and c are ahat = 28.28 ± 0.37), bhat = -2.28 ± 0.06), and chat = -1.14 ± 0.02). This equation, which depended on only three parameters, was termed the <u>Folate Model</u>.

  - Step 2: Determine the extent to which the relationship between pre-fortification between serum folate concentrations (1988-1994) and serum 25(OH)D concentrations (2001-2006) is linear

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- See **Supplemental Figure 2.** The relationship is not linear, but is readily approximated by a piecewise linear function with a knot at about 14 units of serum folate. The fitted equation is:
  folate percentile = -6.342 + 0.414 x serum 25(OH)D percentile + 6.56 x (serum 25(OH)D percentile – 54.17).
  This equation was termed the <u>Vitamin D Model</u>.

- Step 3: Apply the Vitamin D Model to rescale the serum 25(OH)D concentrations.

- Step 4: Apply the Folate Model to the rescaled serum 25(OH)D concentrations to predict the change in the serum 25(OH)D distribution post-fortification. Because percentiles are non-linear functions of observations, the multiplicative factors were applied to rescaled serum 25(OH)D concentrations in the corresponding intervals defined by the values of 25(OH)D at the percentiles. See **Supplemental Figure 3.**

- Step 5: Compare the Folate Model-predicted serum 25(OH)D shift against baseline serum 25(OH)D. See **Supplemental Figure 4**.
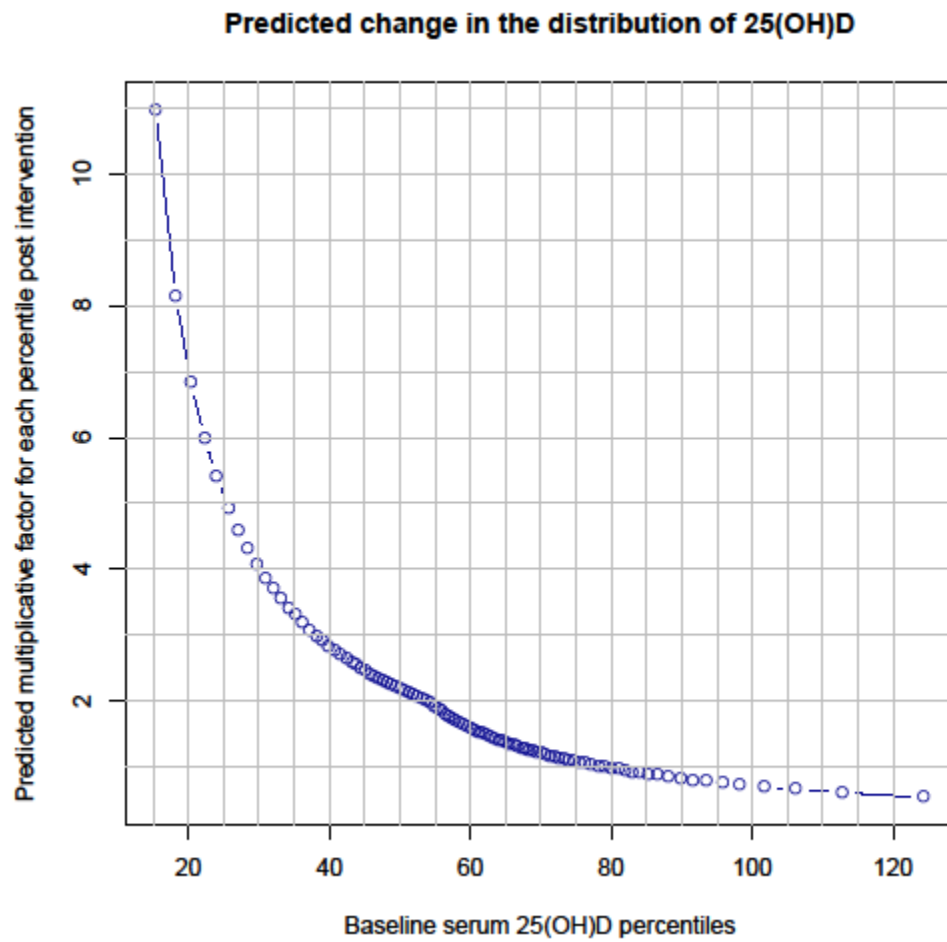
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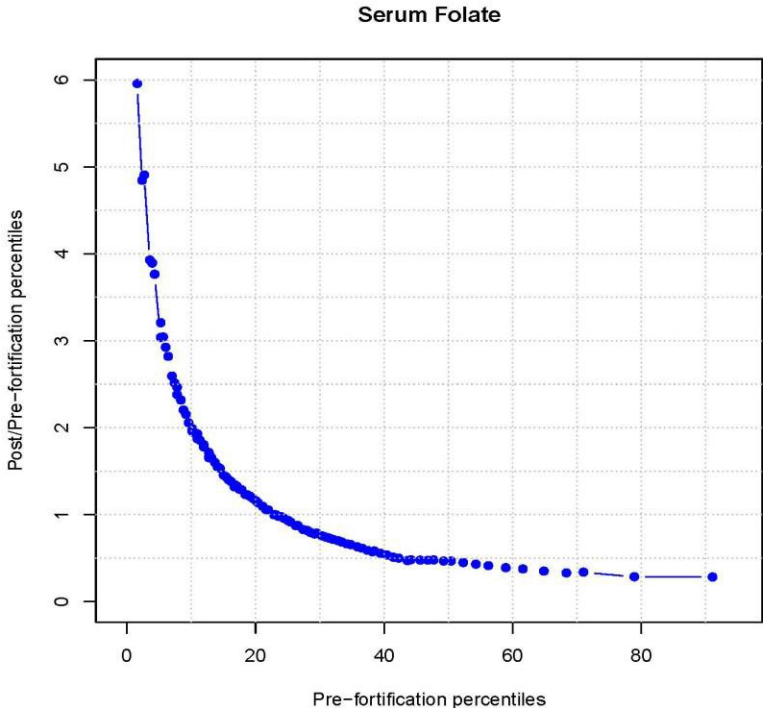
Serum Folate

Supplemental Figure 1. Serum folate percentiles for NHANES 1988-1994 (persons >4 y; n=23,703) are shown on the x-axis, plotted against the ratio on the y-axis of the serum folate percentiles for NHANES 1999-2004 (persons >4 y; n=23,200) over the serum folate percentiles prior to fortification. NHANES survey weights were incorporated for the serum folate measures.
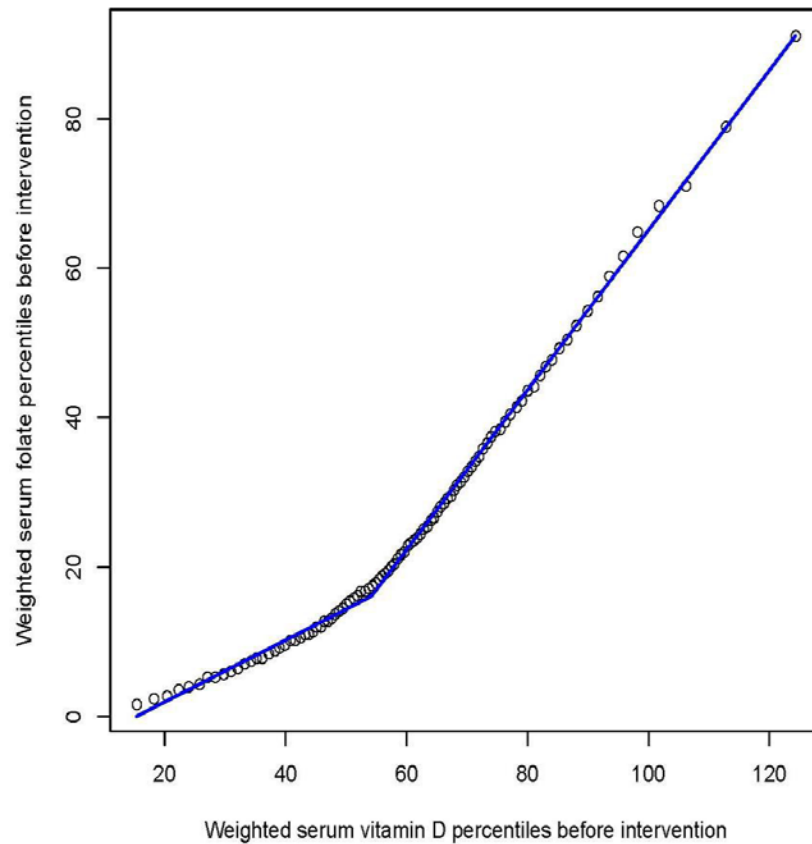
Supplemental Figure 2. Graphic illustrating the nature of the relationship between pre- fortification serum folate concentrations NHANES 1988-1994; persons ≥4 y; n=23,703) and serum 25(OH)D concentrations (NHANES 2001-2006; persons ≥1 y; n=24,411). Serum 25(OH)D concentrations are shown on the x-axis and serum folate concentrations on the y-axis. The weighting refers to the use of NHANES survey weights.

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Supplemental Figure 3. Baseline serum 25(OH)D percentiles appear on the x-axis and the y- axis reflects the factor by which each would be multiplied if an intervention of the magnitude of folate fortification were to occur.

Fortification Prediction for Serum 25(OH)D Based on Serum Folate Change
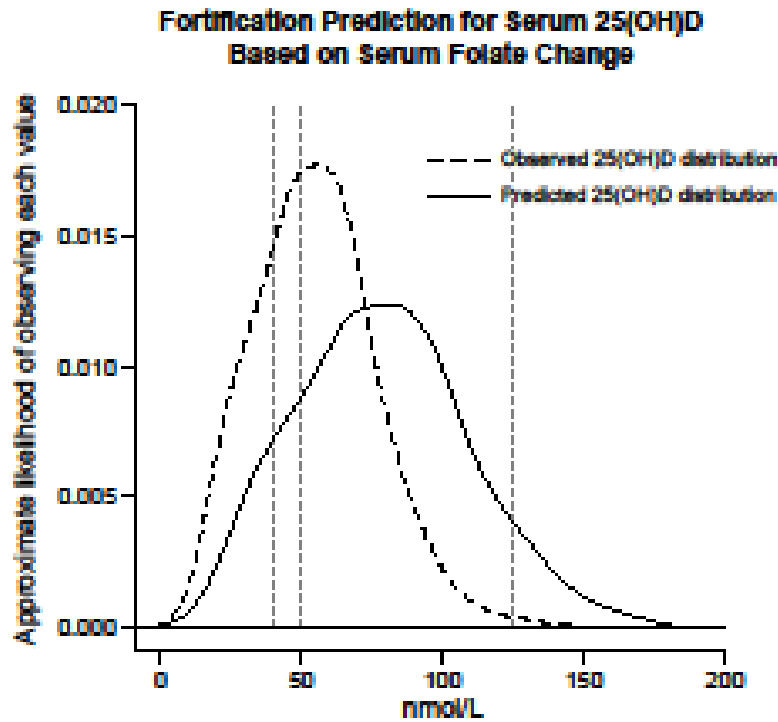
Supplemental Figure 4. Observed (baseline) versus the Folate Model-predicted serum 25(OH)D distribution; NHANES 2001-2006, persons ≥1 y, n=24,411. Serum concentrations go to 0 due to statistical smoothing of data. Vertical lines from left to right reflect, respectively, Institute of Medicine average reference value, 97.5th reference value, and upper limit reference value for serum 25-hydroxyvitmin D from reference 7 in accompanying publication.