USDA Dietary Supplement Ingredient Database Release 3.0 (DSID-3)

Non-prescription Prenatal Multivitamin/mineral (MVM) Dietary Supplement Study

Research Summary

Prepared by

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1. Introduction

Dietary supplementation is commonly recommended during pregnancy and lactation (1). In the United States, more than 70% of pregnant women reported use of a MVM dietary supplement (labeled as a prenatal or adult dietary supplement) (2, 3). The collection of MVM usage data is critical to help implement future recommendations concerning supplement use to ensure adequate micronutrient intake during pregnancy and lactation.

Some prenatal MVM products available in the United States are manufactured for sale as non-prescription dietary supplements (DS) (meaning that purchasers do not need a prescription from a healthcare provider). Others are manufactured for sale only with a prescription (4). The analytical content of these two categories of prenatal MVMs has not been studied systematically.

A single serving of a DS may contain amounts of nutrients or other bioactive compounds that exceed their concentration in foods. During the manufacturing of DS, ingredients may be added in amounts exceeding the label claims in order to compensate for losses during shelf life. However, these amounts are not standardized for specific ingredients or among the different manufacturers. DSID pilot studies have also identified a number of ingredients in a variety of product categories with mean content below label claims. Thus, actual ingredient amounts are unknown to consumers and researchers. Epidemiological studies of nutrient intake and health currently use the manufacturer's label as the source of information on ingredient content in dietary supplements.

In order to provide a tool to more accurately estimate intakes from dietary supplements, an analytically validated database for high priority ingredients in dietary supplement products has been developed. The Dietary Supplement Ingredient Database (DSID; https://dsid.usda.nih.gov) is a collaboration of the Agricultural Research Service (ARS)/ Nutrient Data Laboratory (NDL), and the National Institutes of Health (NIH)/Office of Dietary Supplements (ODS) with other federal partners (National Center for Health Statistics of the Centers for Disease Control and Prevention, Food and Drug Administration, National Cancer Institute of the National Institutes of Health and National Institute of Standards and Technology [NIST] of the Department of Commerce). ODS is the primary funder of the DSID, which builds on the well-recognized strengths of the NDL in developing databases that support assessments of intakes of nutrients from foods. For national DSID studies, representative supplement products are purchased and tested by experienced laboratories for their ingredient content.

Analytically derived estimates of nutrient content in DS could support studies of total nutrient intake of women who use these supplements during pregnancy and lactation (4). This study focused on the analytical content of non-prescription prenatal MVMs. A future study is planned to address the analytical content of prescription prenatal MVMs.

2. Overview of the Non-prescription Prenatal MVM Study

A study of non-prescription prenatal MVMs was conducted to estimate the relationship between label and analytical values for 20 vitamins and minerals in a nationally representative sample. Non-prescription prenatal MVMs were defined for this study as products containing at least three vitamins, with or without minerals or other bioactive components, sold for prenatal use and available for purchase without a health care provider's prescription.

Protocols established in previous DSID MVM studies were applied where appropriate. Products identified as representative of the US market were purchased from retail outlets and through direct-to-consumer sales channels. Samples of multiple lots of these products were sent to qualified laboratories for analysis of ingredients using validated methods and appropriate quality assurance measures. The final analytical dataset was statistically analyzed using regression techniques to estimate relationships between label claims and analytically measured ingredient content and to analyze sources for predicted content variability.

These study results and their National Health and Nutrition Examination Survey (NHANES) application tables were released for the first time in DSID-3 (http://dsid.usda.nih.gov) in 2015.

3. Sampling Plan

NDL scientists consulted with statisticians to set up a sampling frame for purchasing non-prescription prenatal dietary supplement samples to ensure they were representative of the US market. The purpose of the sampling plan was to select sample units from multiple geographic areas of the United States that, when analyzed, could provide reliable and representative estimates of means and variability for ingredient content. Using a multistage probability-proportional-to-size approach with US Census data (5), locations for product sampling were selected.

To identify commonly reported and representative products for purchase, NDL researched non-prescription prenatal MVM products reported by NHANES 2005-06 respondents. The number of reported products was small, some had been discontinued or recalled and others were prescription MVM products. Moreover, several discrepancies were identified between products reported in NHANES 2005-06 and those found in stores in 2009. The market for non-prescription prenatal MVMs was apparently changing (and possibly growing) rapidly. Thus, the NHANES product and market share information was not sufficient as a basis for a national sampling plan for this study.

Store surveys showed that a larger variety and amount of products were available at natural food and other specialty retailers (e.g., Whole Foods and GNC) than at mass-market retailers (e.g., CVS and Target). In collaboration with statisticians, a retail sampling plan was developed based on estimates of market channel distribution from

the Nutrition Business Journal (6). It was concluded that purchasing a large variety of brands and products would best represent non-prescription prenatal MVMs available on the US market.

Six US counties were identified as purchase locations in Alabama, California, Colorado, Michigan, Missouri and New York. Contracted shoppers in each state purchased samples of all prenatal products on the store shelves (up to 40 products total from up to 15 different stores). Shoppers purchased a minimum of 180 tablets (or 32 ounces for liquids) of the same lot of each product that met this study's definition of a prenatal MVM product.

An evaluation of the direct sales market identified many products available online and through multi-level (network) marketers (e.g., Amway, Melaleuca and Herbalife). Non-prescription prenatal MVMs sold through direct channel sources were scored based on a number of factors, including how many websites sold the product and whether the product was sold by one of the 32 top direct channel companies (6). The 12 most commonly sold (high scoring) and 11 randomly chosen non-prescription prenatal MVMs were purchased.
In total, multiple lots of 71 different non-prescription prenatal MVM products were purchased in 2009-2010 and analyzed in 2009-2011.

4. Laboratory Analysis and Quality Control
The purchased products were sent to NDL for processing. Relevant information on each product (e.g., ingredient names and levels, lot number, purchase location and date, and expiration date) was recorded in NDL's in-house database. Samples were repackaged and sent for laboratory analysis in defined batches.

Qualified contracted laboratories analyzed the sample sets using validated sample-handling protocols and appropriate methods to obtain analytical information about ingredient levels (Table 1).

Table 1: Analytical Methods

Nutrients	Analytical Method(s) Used
Calcium Copper Iron Magnesium Manganese Phosphorus Potassium Zinc	Multi-element inductively coupled plasma spectrometry (ICP) after wet ashing
Chromium	Atomic absorption spectroscopy (AAS), with a matrix-matched standard
Iodine	Two methods: Thiosulfate titration and ICP-mass spectrometry (MS)
Selenium	Hydride generation with AAS
Beta-carotene Riboflavin Thiamin	High-performance liquid chromatography (HPLC) with ultraviolet detection
Niacin Vitamin B-6	Two methods: HPLC with UV detection and microbiological
Vitamin D	Two methods: HPLC with UV detection and HPLC- MS/MS
Retinol	HPLC with UV detection for quantification and fluorescence detection for confirmation
Folic acid Vitamin B-12	Microbiological
Vitamin E	HPLC with fluorescence detection

Results for 10 vitamins and 10 minerals are reported in this study. The major components of vitamin A (retinol and beta-carotene) were measured separately, converted to international units (IU) and combined to calculate total vitamin A for comparison to label levels. In some cases, more than one method of analysis was used to accurately measure unusual ingredient forms or low ingredient levels or to replace older methods with newer technologies. Methods for the analysis of chromium and iodine improved during this study. The single-element standard initially used to measure chromium by atomic absorption spectroscopy was replaced by a multiple-element material that matched the product matrix. The iodine analysis method changed from titration to a more consistent ICP spectrometry/mass spectroscopy method.

Quality control (QC) materials were added to each batch of prenatal MVM products to evaluate laboratory precision and accuracy on an ongoing basis. NIST Standard Reference Material (SRM) 3280, an MVM matrix with certified values for vitamins and minerals, was sent in each batch. In addition, each batch included a set of product

duplicates and at least two in-house control materials that were analyzed for all ingredients in the study. Each product sample contained at least 20 units (tablets, capsules or liquid serving amounts) of the MVM product. Labs were instructed to homogenize at least 20 sample units before sub-sampling for analysis (per the United States Pharmacopeia recommendations for the analysis of dietary supplements). For each in-house control material, a case of a single lot of an MVM product with a similar matrix to the study samples was purchased.

Analytical retests were conducted to check unusually high or low results, high variability among product lots, or questionable data in batches where QC results showed a bias. For each sample analyzed, laboratory results reported in mg/g or µg/g were compared to label levels and a percent difference from the label levels was calculated.

5. Statistical Analysis

Ingredient data from the laboratory analysis were prepared for statistical analysis. Observations were equally weighted. To identify overly influential supplement observations, a jackknife technique was used to calculate Cook's distances and restricted likelihood distances.

Relationships between the label and percent difference from label across the range of label levels analyzed were estimated by regression with a SAS® mixed model procedure. For each supplement ingredient, the label value was the independent variable and the percent difference from the label level (based on the laboratory analysis) was the dependent variable. Percent differences from label were calculated: ((analytical value – label value)/label value) × 100%. Three models (mean, linear and quadratic) were used to fit the data for all ingredients, and the most complex and statistically significant model was selected. Lab, supplement within label level and lot within supplement were modeled as random sources of variation. The accuracy of the models' predictions was assessed with validation techniques.

The selected regression equations were used to predict mean analytical levels for each ingredient in non-prescription prenatal MVMs: label value × (1 + predicted percent difference/100). In the DSID-3 files, these mean predictions are shown in application tables as predicted percent differences from the label level or as predicted values in international units (IU), mg, or µg per serving. The mean predictions were linked to label levels for each ingredient and were not specific to any brand or supplement of this prenatal MVM category.

In addition, the standard error of the mean (SEM), 95% confidence intervals (CI) for the mean, and the standard error (SE) of an individual observation were calculated at each label level. Because the regression equation could be used to predict ingredient values of independent supplement samples, SE were adjusted to reflect this expected greater prediction variability.

6. Results and Discussion

In this study, regression results are reported for the following 20 vitamins and minerals: folic acid, niacin, riboflavin, thiamin, vitamin A, vitamin B-12, vitamin B-6, vitamin C, vitamin D, vitamin E, calcium, chromium, copper, iodine, iron, magnesium, manganese, potassium, selenium and zinc.

Regression results for mean predicted percent differences from label levels and the associated SE and 95% CI varied by ingredient and are reported by ingredient level. Detailed results for this study, including all regression equations and applications to NHANES dietary supplement data files, are listed in the "Data Files" page on the DSID website.

The regression results for the most common label level in the prenatal MVM study are summarized in Tables 2 and 3 below. Table 2 lists the predicted mean percent differences from label values for vitamins and Table 3 does the same for minerals.

Table 2: Predicted Means for Vitamins in Non-prescription Prenatal MVMs

Ingredient	Range of Predicted Mean Percent Differences from Label Levels	Most Common Label Level per Serving	Predicted Mean Percent Differences at Most Common Label Level	Predicted SEM at Most Common Label Level
Folic acid	0.5%*	800 mcg	0.5%	2.1%
Niacin	-16.9% to 1.5%	20 mg	-0.4%	1.3%
Riboflavin	0.9%*	1.7 mg	0.9%*	2.8%
Thiamin	-9.2%	1.8 mg	-9.2%	1.9%
Vitamin A	2.4%*	4,000 IU	2.4%	3.5%
Vitamin B-6	-3.0%*	2.6 mg	-3.0%*	2.1%
Vitamin B-12	-18.9% to 7.1%	8 mcg	2.9%	2.8%
Vitamin C	3.6%*	120 mg	3.6%*	2. 5%
Vitamin D	13.1%	400 IU	13.1%	4. 9%
Vitamin E	5.7%*	30 IU	5.7%	2.8%

IU: international units; *Not statistically significantly different from label

Table 3: Predicted Means for Minerals in Non-prescription Prenatal MVMs

Ingredient	Range of Predicted Mean Percent Differences from Label Levels	Most Common Label Level per Serving	Predicted Mean Percent Differences at Most Common Label Level	Predicted SEM at Most Common Label Level
Calcium	-4.3 to 14.3%	200 mg	11.8%	1.8%
Copper	8.6%	2 mg	8.6%	2.9%
Iodine	25.9%	150 mcg	25.9%	4.7%
Iron	1.1%*	30 mg	1.1%*	0.9%
Magnesium	3.4%	100 mg	3.4%	1.6%
Manganese	6.8%	2 mg	6.8%	2.4%
Potassium	20.2%	10 mg	20.2%	4.0%
Selenium	13.1%	100 mcg	13.1%	2.2%
Zinc	-4.1 to 7.6%	15 mg	3.9%	1.2%
Chromium	10.0 to 85.0%	120 mcg	48.5%	6.4%

* Not statistically significantly different from label.

When analyzed across all label levels, the predicted mean percent differences from label levels for folic acid, riboflavin, vitamin A, vitamin B-6, vitamin C, vitamin E and iron were not significantly different from the label value. Predicted mean percent differences within a 3 to 15% above label range were found for vitamin D and for the majority of minerals (calcium, copper, magnesium, manganese, selenium and zinc). Phosphorus was analyzed in these MVMs. However, since only 8 products claimed phosphorus content, the analytical results were not analyzed by regression.

Predicted mean percent differences from label levels were substantially higher than label values for three ingredients. For iodine and potassium, analytical levels were predicted to be 20 to 26% above label amounts and predicted mean overages for chromium reached 85% at the lower labeled levels.

Three vitamins have predicted mean values substantially below label levels. The mean analytical prediction for thiamin was 9.2% below label. At their higher label levels, analytical levels of niacin and vitamin B-12 were predicted to be significantly below label levels (-16.9% and -18.9%, respectively).

These data can provide researchers with more accurate estimates of nutrient intake for non-prescription prenatal MVM than the label levels.

7. Use of DSID data

The regression equations for non-prescription prenatal MVMs released in the DSID predict the mean percent differences from label levels for 20 ingredients in non-

prescription prenatal MVM supplements sold in the United States. The predicted amounts are linked to labeled levels for each ingredient and are not specific to any brand or supplement. These estimates are applicable to MVMs in *large* population surveys of dietary supplement use. Measures of variability are reported with predicted means, as discussed previously.

Results predicted by regression for the mean percent difference from label level and SE have been assigned linking codes that may be applied to NHANES DS data files or used for other studies of DS intake. The predicted results from the DSID can be used to replace information from labels to more accurately assess ingredient intakes from dietary supplements in large population surveys.

Documentation about the DSID data files and instructions for appropriate use of the files are described in the report, *DSID-4 Data File Documentation,* available on the "Data Files" page of the website. Please refer to that report for additional information on how best to interpret and use each data file.

An online, interactive, *Non-Prescription Prenatal MVM Calculator* was released with DSID-3. This calculator should only be used to generate estimates for non-prescription prenatal MVMs because the results from this study may not reflect the analytical content of prescription prenatal MVMs. This calculator allows the user to enter ingredient information from MVM labels and generate the appropriate predicted mean values, SE and 95% CI for those label levels.

8. Future Research

Additional DSID studies are underway to evaluate ingredient quantities in prescription prenatal MVMs and green tea dietary supplements.

9. References

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