Regular Consumption of a High-Phytate Diet Reduces the Inhibitory Effect of Phytate on Nonheme-Iron Absorption in Women with Suboptimal Iron Stores¹,²

Seth M Armah,³ Erick Boy,⁴ Dan Chen,³ Priscila Candal,³ and Manju B Reddy³*

³Department of Food Science and Human Nutrition, Iowa State University, Ames, IA; and ⁴HarvestPlus/International Food Policy Research Institute, Washington, DC

Abstract

Background: High phytate (HP) consumption is a concern in developing countries because of the high prevalence of iron deficiency in these countries.

Objective: We investigated whether habitual consumption of an HP diet reduces the inhibitory effect of phytate on nonheme-iron absorption.

Methods: Thirty-two nonanemic females, 18–35 y of age, with normal body mass index but with suboptimal iron stores (serum ferritin, ≤30 mg/L), were matched for serum ferritin concentration and randomly assigned to HP and low-phytate (LP) groups, in a parallel design study. Each subject consumed HP or LP foods with at least 2 of their daily meals for 8 wk, resulting in a change in phytate intake (from 718 to 1190 mg/d in the HP group and 623 to 385 mg/d in the LP group). The serum iron response over 4 h after a test meal containing 350 mg of phytate was measured at baseline and postintervention. Ferritin, transferrin receptor, and hepcidin concentrations were measured at baseline and 8 wk.

Results: Twenty-eight subjects completed the study (n = 14 per group). The serum iron response to the test meal increased in the HP group at postintervention, resulting in a 41% increase in the area under the curve (AUC; P < 0.0001). However, no effect was observed in the LP group (21% decrease in AUC; P = 0.76). The postintervention serum iron response was lower (P < 0.0001) in the LP group than in the HP group after controlling for the baseline serum iron response and hepcidin concentration, reflecting in a 64% lower AUC.

Conclusions: We found that habitual consumption of an HP diet can reduce the negative effect of phytate on nonheme-iron absorption among young women with suboptimal iron stores. Future studies are needed to explore possible mechanisms. This trial was registered at clinicaltrials.gov as NCT02370940.

Keywords: iron bioavailability, phytate, serum iron curve, hepcidin, iron status

Introduction

Numerous studies have been conducted to investigate the effects of dietary factors on iron absorption (1–7). These dietary factors include meat, calcium, ascorbic acid, tea/polyphenols, phytic acid, and nonheme iron. These, along with an individual’s iron status, are well-recognized determinants of nonheme-iron absorption based on single-meal absorption studies (8, 9). However, studies have suggested that the effects of dietary factors may be dampened when nonheme-iron absorption is measured from the whole/complete diet. In one study, Cook et al. (10) demonstrated that the effects of these dietary factors on iron absorption are exaggerated in single-meal studies. They reported that modifying the usual diet of subjects to include high amounts of meat and other enhancers resulted in only a small increase in nonheme-iron absorption from this diet, whereas an exaggerated increase was observed in the corresponding single meal. Similarly, an inhibitory diet, limited in meat and ascorbic acid and generous in phytic acid, calcium, and polyphenols, consumed over a 2-wk period resulted in a relatively lower reduction in iron absorption compared with what was observed in the corresponding single meal. In other studies, the effects of meat and ascorbic acid on nonheme-iron absorption from a complete diet were shown to be marginal, compared with the reported enhancing effects in single meals (1, 4). This is corroborated by other studies that have shown that regular inclusion of enhancers or inhibitors of iron absorption in the diet does not affect iron status (11–13).
The dampened effects of dietary factors in long-term studies may be caused by the interaction of other meal components or effects of residual components from the previous meals. Various studies, including both human and animal studies, have investigated the latter and resulted in conflicting outcomes (14–16). In this study (NCT02370940), we focused on the inhibitory effect of nonheme-iron absorption from an HP test meal as assessed by the serum iron curve. We investigated whether regular consumption of phytate dampens its negative effect on nonheme-iron absorption. The serum iron curve method was validated by Conway et al. (17) by showing a strong positive correlation with erythrocyte nonheme-iron absorption, as assessed by the serum iron curve. The serum iron curve method was validated by Conway et al. (17) by showing a strong positive correlation with erythrocyte incorporation of isotopic iron. We hypothesized that fractional nonheme-iron absorption from an HP test meal will increase in subjects consuming an HP diet and decrease or not change among those consuming a low-phytate (LP) diet for 8 wk.

Methods

Subjects. Female subjects, 18–35 y old, were recruited for this study by sending mass e-mails to all female students at Iowa State University in the spring of 2013. A total of 113 students responded; however, only 97 participated in the initial screening (Figure 1). Before screening, participants were required to read and sign an informed consent document, and all procedures and potential risks and benefits were explained to them. Potential subjects were required to go through 3 different stages of screening. At the first screening, they completed health and medical history questionnaires and their height and weight were measured for BMI assessment. Subjects who were eligible came back for an iron eligibility assessment (≥30 μg/L) and then for a pregnancy test. We determined that 14 subjects were needed in each group to determine a change in the AUC for serum iron by one-third as statistically significant with 80% power and an α-level of 0.05 (17). To be included in the study, subjects must be female, 18–35 y of age, with serum ferritin concentrations of 30 μg/L, and BMI within the normal range (18.5–24.9 kg/m²). Additionally, subjects must be nonsmoking, nonlactating, nonpregnant, and not taking any drug that interferes with iron absorption and should not have any gastrointestinal disease/condition that can affect absorption. Of the 97 individuals who participated in the initial screening, 65 did not meet these criteria (Figure 1). Thus, a total of 32 subjects were enrolled in the study. Subjects who were anemic (hemoglobin, <120 g/L) were referred to their personal physician and were not included in the study. Subjects were instructed not to use dietary supplements during the study period. The 32 subjects were first matched for serum ferritin concentration and then randomly assigned to either the HP or the LP group (n = 16 per group). The assignment of subjects was performed by a person who was not involved in the study, using a computer-based random-number generator (Microsoft Excel 2010, RAND command; Microsoft Corp.). From each pair, the subject with the higher random number was assigned to the HP group and the one with the lower number was assigned to the LP group.

Study protocol. After recruitments, subjects were asked to keep a 3-d weighed dietary record (2 weekdays and a weekend) before reporting to the Nutrition and Wellness Research Center (NWRC) at Iowa State University for baseline absorption measurements. Iron absorption from an HP test meal, to which 10 mg of iron as iron (II) sulfate has been added, was measured using the serum iron curve (17). The test meal contained 350 mg of phytate and was composed of 1 small corn tortilla (27.2 g), 100 g of black beans (drained from can), 50 g of cooked white rice, 30 g of salsa, and 120 g of orange juice. Subjects were asked to fast overnight before coming to the NWRC for the first absorption measurement. An indwelling catheter was used by a nurse practitioner to collect multiple venous blood samples. An initial baseline blood sample was collected before the test meal administration. After consuming the test meal, blood samples were collected every 30 min for 4 h. After the first absorption measurement, subjects participated in an 8-wk dietary intervention in which each subject consumed either HP or LP diets. Postintervention iron absorption from the same test meal that was administered at baseline was measured for each subject after the 8-wk dietary modification.

All foods needed for the dietary modification were supplied to subjects. Their first 2-wk food supply was given on the day they came for the baseline multiple blood draw. Subjects reported to the NWRC every 2 wk to pick up food. The HP group received whole grain ready-to-eat cereals, whole wheat pasta/spaghetti, tortillas, bagels, bread and dinner rolls, corn tortillas, brown rice, canned black beans, edamame, and tofu and was encouraged to consume generous amounts of nuts and other legume products high in phytate. The LP group received similar foods made from refined wheat and white rice, eggs, and cheese and was instructed to avoid HP foods. The phytate concentration of the food provided for the HP group ranged from 210 mg/100 g to 1500 mg/100 g with a mean of 390 mg/100 g, and those for the LP group ranged from 0 mg/100 g to 420 mg/100 g with a mean of 90 mg/100 g. At weeks 4 (data not shown) and 7 of the study, the subjects were asked again to keep 3-d dietary records. All dietary records were for 3 nonconsecutive days (2 weekdays and a weekend). Subjects were provided with weighing scales and were required to weigh and record all foods consumed during these days. Dietary data were analyzed using the Nutrition Data System for Research (University of Minnesota).

Four subjects dropped out during the study (one got an internship and could not continue with the study, one got sick but not related to the treatment, one did not want to follow the dietary modification and opted to quit, and the last one had difficulty with the multiple blood draws and decided to quit), leaving 28 who completed the study (14 in each group). To assess compliance, subjects were required to write down daily all of the foods they consumed from what they were provided. They were required to incorporate the foods they were provided in at least 2 of their daily meals. Also, at the end of the study, they were asked to provide information on how frequently they consumed specified HP foods listed in an FFQ. The FFQ was designed to contain a list of selected HP foods, belonging to the following categories: nuts and their products, whole

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5 Abbreviations used: CRP, C-reactive protein; HP, high phytate; LP, low phytate; NWRC, Nutrition and Wellness Research Center.
grain cereals and their products, and legumes and legume products. Compliance was estimated as the percentage of days in which they included the foods they were provided in at least 2 of their daily meals. There were no restrictions on the amount to be included in the meals. On the FFQ, subjects were required to select the frequency of consumption of each listed food. These frequencies were tallied to estimate the number of times they consumed each listed HP food over the 8-wk period as a measure of compliance to the study protocol. The study protocol was approved by the Institutional Review Board at Iowa State University (no. 12-470).

**Blood sample analysis.** Before administering the test meal, 2 separate fasting blood samples were collected, one for the measurement of plasma hepcidin and the other for measurement of initial (T0) serum iron, serum C-reactive protein (CRP), serum transferrin receptor, and serum ferritin concentrations. Multiple blood samples collected after the test meal was consumed were analyzed for serum iron by a certified laboratory (Quest Diagnostics, Inc.), and results were used to construct a serum iron curve for each subject. Blood samples were centrifuged and serum or plasma was divided into microcentrifuge tubes and frozen until they were analyzed. Aprotinin (Fisher Scientific) was added to the hepcidin samples and they were frozen at −80°C. Samples for measurement of CRP, transferrin receptor, and ferritin were stored at −20°C until the end of the study. Serum CRP, plasma hepcidin (hepcidin-25), serum ferritin, and serum transferrin receptor were measured using ELISA. Kits for the measurements were obtained from American Laboratory Products Company (ALPCO Diagnostics) for CRP, Peninsula Laboratories for hepcidin-25, and Ramco Laboratories, Inc., for ferritin and transferrin receptor. The intra- and interassay CVs for the different assays were 6.6% and 8.9% for serum transferrin receptor, 6.9% and 11.4% for serum CRP, 9.3% and 8.1% for plasma hepcidin, and 10.6% and 6.9% for serum ferritin.

**Statistical analysis.** Data were analyzed using the GraphPad Prism 6 (GraphPad Software, Inc.) and R Statistical Software, version 3.1.2 (R Foundation for Statistical Computing) (18). For all variables, analyses included all 28 subjects who completed the study (14 per group). The serum iron response to the test meal was our primary outcome. We estimated total body iron using the equation by Cook et al. (19). Means (95% CIs) were reported for body iron, weight, height, and BMI and the geometric means (95% CIs) for serum ferritin, serum transferrin receptor, nutrients intake, serum CRP, and AUC. Comparisons between groups (baseline or postintervention measurements) were performed using independent t tests and paired t tests were used to compare baseline and postintervention values within each group. F tests for nonlinear regression models with second-order polynomials, controlling for subject effects, were used to compare the serum iron curves within groups (baseline and final) and also to compare the postintervention serum iron curves between the 2 groups controlling for the baseline serum iron response and plasma hepcidin concentration. ANCOVA was used to determine the effect of dietary intervention on iron status biomarkers using respective baseline values as covariates after testing for homogeneity of regression slopes. The significance level was set at 0.05.

**Results**

Of the 28 subjects who completed the study, 22 were white, 3 were Asian, 2 were black, and 1 was Latino. Their ages ranged from 18 to 33 y. The means (95% CIs) for height, weight, and BMI of all 28 subjects at baseline were 167 cm (164, 170), 62 kg (60, 64), and 22.3 kg/m² (21.7, 22.9), respectively. Table 1 compares the baseline and postintervention dietary intakes of subjects by group. Fat intake tended to decrease in the LP group (P = 0.06). Phytate intake increased by 66% in the HP group and decreased by 38% in the LP group (P < 0.01). This was corroborated by the compliance results from the FFQ, which indicated that the HP group consumed HP foods much more frequently (a mean of 271 times over the 8-wk period) than the LP group who rarely consumed any HP foods (a mean of 27 times over the intervention period).

### Table 1: Nutrients intake of women who consumed HP or LP diets for 8 wk

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>HP (n = 14)</th>
<th>LP (n = 14)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
</tr>
<tr>
<td>Fat, g·1000 kcal⁻¹·d⁻¹</td>
<td>36 (33, 39)</td>
<td>36 (33, 40)</td>
<td>35 (32, 40)</td>
</tr>
<tr>
<td>Protein, g·1000 kcal⁻¹·d⁻¹</td>
<td>36 (31, 42)</td>
<td>37 (33,41)</td>
<td>38 (34, 43)</td>
</tr>
<tr>
<td>Vitamin C, mg/d</td>
<td>89 (61, 128)</td>
<td>76 (49, 117)</td>
<td>61 (35, 106)</td>
</tr>
<tr>
<td>Calcium, mg/d</td>
<td>783 (638, 960)</td>
<td>806 (684, 949)</td>
<td>729 (531, 999)</td>
</tr>
<tr>
<td>Iron, mg/d</td>
<td>13.2 (10.1, 17.2)</td>
<td>14.1 (11.7, 17.0)</td>
<td>13.4 (11.2, 16.0)</td>
</tr>
<tr>
<td>Phytate, mg/d</td>
<td>718 (548, 941)</td>
<td>1190 (1030, 1380)</td>
<td>623 (482, 804)</td>
</tr>
</tbody>
</table>

1 Values are geometric means (95% CIs). *Different from baseline, P ≤ 0.01. HP, high phytate; LP, low phytate.

### Table 2: Iron status, inflammatory markers, and AUCs for serum iron (unadjusted) in women who consumed HP or LP diets for 8 wk

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>HP (n = 14)</th>
<th>LP (n = 14)</th>
<th>P²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
<td>Baseline</td>
</tr>
<tr>
<td>Serum ferritin, μg/L</td>
<td>17.4 (16.4, 18.5)</td>
<td>18.7 (17.5, 20.0)</td>
<td>20.7 (18.2, 23.4)</td>
</tr>
<tr>
<td>Serum transferrin receptor, μg/mL</td>
<td>5.0 (4.6, 5.4)</td>
<td>4.7 (4.4, 5.1)</td>
<td>5.5 (4.9, 6.1)</td>
</tr>
<tr>
<td>Body iron, mg/kg body weight</td>
<td>3.0 (2.0, 4.1)</td>
<td>3.5 (2.3, 4.7)</td>
<td>3.3 (1.3, 5.3)</td>
</tr>
<tr>
<td>Plasma hepcidin, μg/L</td>
<td>1.5 (1.2, 1.9)</td>
<td>1.6 (1.3, 2.0)</td>
<td>3.6 (3.0, 4.3)</td>
</tr>
<tr>
<td>Serum CRP, mg/L</td>
<td>0.53 (0.43, 0.64)</td>
<td>0.67 (0.55, 0.82)</td>
<td>1.13 (0.88, 1.44)</td>
</tr>
<tr>
<td>AUC for serum iron, μmol·L⁻¹·h⁻¹</td>
<td>640 (556, 724)</td>
<td>905 (805, 1020)</td>
<td>337 (288, 393)</td>
</tr>
</tbody>
</table>

1 Values are geometric means (95% CIs); means (95% CIs) for body iron. *Different from baseline, P ≤ 0.05. CRP, C-reactive protein; HP, high phytate; LP, low phytate.

2 Values refer to comparison between HP and LP groups using t test.

3 P values are based on F test for nonlinear regression models of the serum iron curves.
Compliance, as estimated by the percentage of days in which subjects incorporated foods provided to them in their daily meals, was 87% in the LP group and 96% in the HP group. Based on the results, we estimate that the HP diet provided approximately an extra 470 mg phytate/d. None of the nutrient intakes differed between the 2 groups, at both baseline and postintervention, except for postintervention phytate intake, which was 68% lower in the LP group ($P < 0.0001$).

Serum ferritin, transferrin receptor, CRP, and body iron did not differ between the HP and LP groups at both baseline and postintervention ($P > 0.05$). Plasma hepcidin concentration was higher in the LP group than in the HP group by 2.1 μg/L at baseline and 2.2 μg/L at postintervention ($P < 0.05$; Table 2). Serum ferritin, serum transferrin receptor, plasma hepcidin, and serum CRP concentrations as well as body iron did not change within groups ($P > 0.05$). Figure 2 shows the serum iron curves for the HP and LP groups at baseline and postintervention. In the HP group, the AUC increased by 41% compared to baseline ($P < 0.0001$), and in the LP group, there was no significant change ($P = 0.76$). Baseline iron absorption, as assessed by the serum iron curve, was significantly lower ($P < 0.0001$) in the LP group than in the HP group even though subjects were randomly assigned to the groups. At postintervention, absorption was higher in the HP group than in the LP group after controlling for the baseline serum iron response and hepcidin concentration ($P < 0.0001$), resulting in a 64% lower AUC in the LP group (Table 3). As shown in Table 3, the dietary intervention did not show any significant effect on any of the iron status biomarkers ($P > 0.05$).

**Discussion**

Phytate is known as one of the major inhibitors of nonheme-iron absorption (20). Higher inositol phosphates such as inositol hexaphosphate and inositol pentaphosphate, in particular, are known to bind to iron and make it unavailable for absorption (21). Hallberg et al. (22) have reported a dose-dependent inhibition of sodium phytate on iron absorption from meals consumed together with radiolabeled wheat rolls. A key reason for the concerns about phytate is that it is a major component of staple foods in many developing countries where iron deficiency anemia prevalence is high (23). Interestingly, studies that have measured nonheme-iron absorption in complete diets suggested that the effect of dietary factors may be dampened when the whole diet is considered (10). Particularly for phytate, data are scarce on its effect on nonheme-iron absorption measured from the whole diet. Brune et al. (14) compared the effect of bran on iron absorption between a vegetarian group and a nonvegetarian control group and found no significant difference between the 2 groups. However, the mean daily phytate intake among the vegetarian group was 323 mg/d, which is well below what has been reported for many countries. For example, median phytate intake in the United Kingdom was estimated to be 809 mg/d (24). In most developing countries, daily phytate intake values are even higher (25). Moreover, their study included both males and females with a wider serum ferritin concentration range. In this study, we investigated whether habitual HP diet consumption reduces the inhibitory effect of phytate on nonheme-iron absorption among female subjects with serum ferritin concentrations of ≤30 μg/L.

Generally, daily nutrient intakes were lower than recommendations (26). However, this observation is not unexpected among college female students. For example, mean daily iron intakes at baseline for subjects in our study (13.2 and 13.4 mg) were similar to the intakes among college female students 18–28 y of age reported in our previous study (13). Baseline phytate intakes were 718 and 623 mg/d for the HP and LP groups, respectively, which are comparable with the reported mean intake of 750 mg/d for US adults (27).

The results of this study indicate no effect of intervention on the biomarkers of iron status (serum ferritin, serum transferrin receptor, and body iron) because none of them changed significantly after the 8-wk dietary modification. These results are similar to the observation by Hunt and Roughead (15), who found that 10-wk dietary intervention with a high or low bioavailability diet did not affect blood indexes of iron status. Based on our previous studies, we found that long-term consumption of soy protein rich in phytate reduced ferritin concentration in postmenopausal women with high iron status (28) but consuming soy foods showed no significant effect in premenopausal women with low iron status (13). These studies suggest that the effect of phytate depends on baseline iron status of subjects. Hence, our results may apply to only populations with suboptimal iron stores and cannot be generalized to all populations. Also, because mean hepcidin concentration was higher in the LP group than in the HP group, and based on its inverse relation with iron absorption, it was not surprising to see that baseline absorption was lower in the LP group than in the HP group.

**FIGURE 2** Iron bioavailability from an HP test meal in young women who had consumed an LP or HP diet for 8 wk. Values are means ± SEMs, $n = 14$. The serum iron response for curves with different letters are significantly different ($P < 0.0001$) based on $F$ test comparisons of nonlinear regression models. HP, high phytate; LP, low phytate.

**TABLE 3** Adjusted postintervention means of iron status, inflammatory markers, and AUCs for women who consumed HP or LP diets for 8 wk

<table>
<thead>
<tr>
<th></th>
<th>HP ($n = 14$)</th>
<th>LP ($n = 14$)</th>
<th>$P^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum ferritin, μg/L</td>
<td>19.6 (15.8, 24.2)</td>
<td>17.8 (14.4, 22.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>Serum transferrin receptor, μg/mL</td>
<td>4.8 (3.9, 5.8)</td>
<td>5.4 (4.5, 6.6)</td>
<td>0.33</td>
</tr>
<tr>
<td>Body iron, mg/kg body weight</td>
<td>3.6 (2.4, 4.8)</td>
<td>2.9 (1.7, 4.1)</td>
<td>0.40</td>
</tr>
<tr>
<td>Plasma hepcidin, μg/L</td>
<td>2.1 (1.4, 3.0)</td>
<td>3.0 (2.0, 4.4)</td>
<td>0.20</td>
</tr>
<tr>
<td>Serum CRP, mg/L</td>
<td>0.87 (0.57, 1.33)</td>
<td>1.12 (0.74, 1.72)</td>
<td>0.39</td>
</tr>
<tr>
<td>AUC for serum iron, μmol · L⁻¹ · h</td>
<td>815 (527, 1260)</td>
<td>296 (192, 459)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Values are postintervention geometric means (95% CIs) after adjusting for the respective baseline values. For each variable, the $P$ value was based on ANCOVA with baseline values as covariates. CRP, C-reactive protein; HP, high phytate; LP, low phytate.

2 Values are for treatment effect.

3 Values are means (95% CIs).

4 Values were adjusted for both baseline AUC and baseline hepcidin concentration. $P$ value is based on $F$ test for nonlinear regression models of the serum iron curves.
Hepcidin is known as a key regulator of iron absorption. It regulates iron absorption through ferroportin, the main iron exporter from intestinal cells and macrophages. Hepcidin binds to ferroportin and prevents the export of iron, resulting in the internalization of the ferroportin and subsequent reduction in iron absorption. Thus, higher hepcidin concentration leads to lower iron absorption and vice versa (29–32). After controlling for the baseline values for hepcidin concentration and serum iron response, because they differed between the 2 groups at baseline, the serum iron response was significantly greater in the HP group than in the LP group at postintervention, suggesting that the inhibitory effect of phytate on nonheme-iron absorption is dampened with habitual HP diet consumption among females with suboptimal iron stores. The effect of dietary intervention was not explained by any of the measured biomarkers of iron status. Future studies with longer intervention periods and multiple midpoint measurements of iron status biomarkers and markers of oxidative stress, infection, and inflammation are needed to understand the mechanism, taking into account other possible factors such as genetic factors that influence iron absorption and status.

We acknowledge the limitations of this study. First, assessment of compliance to the dietary intervention was based solely on records kept by subjects because it was not a controlled feeding study. Although subjects in both groups were not required to consume specific amounts of HP or LP foods they were provided, phytate intake was significantly higher in the HP group during the intervention, as expected. We also recognize that there could be large variations in the amount of provided food intake, which has a potential of influencing the outcome of the study. This was implemented in part to create a condition similar to a real-life scenario and to accommodate individual differences in food consumption. In conclusion, the results of this study suggest that regular consumption of an HP diet may reduce the inhibitory effect of phytate on iron absorption among women with suboptimal iron stores.

Acknowledgments

We acknowledge the Nutrition Coordinating Center of the University of Minnesota for providing us with the student license version of the Nutrition Data System for Research software for dietary intake analysis because the first author was then a PhD student. SMA, EB, and MBR were responsible for the overall research design of the study and modified the final paper; DC was responsible for the laboratory analysis; PC was involved throughout the study, especially with subject recruitment, dietary intervention, meal preparation, and dietary data analysis. SMA conducted the research, performed statistical analysis, and wrote the first draft of the paper. MBR provided oversight of the study and had primary responsibility for the final content. All authors read and approved the final manuscript.

References