Modeling the Proximate Determinants of Hemoglobin Concentration of Bangladeshi Women: Is Iron Fortification of Rice a Viable Strategy for Reducing Iron Deficiencies?

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ABSTRACT Iron deficiency anemia (IDA) affects a large number of women in developing countries especially during child bearing years. Because hemoglobin concentration is a useful for identifying IDA, this paper developed a comprehensive longitudinal model for the proximate determinants of hemoglobin concentration using 3 repeated observations on 514 free living women in Bangladesh. Initially, 900 households were surveyed 4 times at 4-month intervals at 3 sites in Bangladesh. Hemoglobin was measured in the last 3 survey rounds. Dietary intakes at 4 meals per day were measured by the 24-hour recall method in all 4 survey rounds. Socioeconomic, anthropometric, and morbidity variables were measured in all 4 survey rounds. The explanatory variables in the model for hemoglobin concentration were the bioavailable iron intake calculated using a corrected version of the algorithm by Tseng et al. (1997), anthropometric measurement, morbidity involving blood loss, and intake of iron tablets. Socioeconomic factors affecting iron intake from meat, fish, and poultry and from animal source were also modeled. The main results were that bioavailable iron, women's height and mid upper arm circumference, and intake of iron tablets were significant predictors of hemoglobin concentration. Inhibitory effects of phytates on iron absorption were evident in this population. Increases in household incomes were associated with higher intake of iron from meat, fish, and poultry and from animal sources. From a policy standpoint, increased iron intake achieved via breeding iron dense rice and subsidizing fish cultivation would enhance the subjects' iron status; iron tablets would be important for pregnant women. A cost benefit analysis and equity considerations favored a plant breeding strategy.

KEY WORDS Bioavailable iron, hemoglobin, iron deficiency anemia, socioeconomic factors, plant breeding, longitudinal data, dynamic random effects models, cost-benefit analysis, Bangladesh

Running title Hemoglobin concentration of Bangladeshi women

Iron deficiencies are widely prevalent especially in low and middle income countries. It is estimated that 3.5 billion people suffer from iron deficiency anemia (IDA; UNICEF/WHO, 1999). IDA hinders human functions in all age groups. For example, IDA lowered labor productivity of Indonesian rubber trappers (Basta et al., 1979) and Sri Lankan tea pickers (Gardner et al., 1977), adversely affects birth outcomes (Bhargava, 2000), and impairs cognitive development of children (Pollitt, 1993, Scrimshaw, 1996). Productivity loss and human costs associated with IDA are enormous (UNICEF/UNU/WHO/MI, 1999).

Poor diet quality and low bioavailability of dietary iron are important factors contributing to IDA (Tatala et al. 1998); iron loss due to parasitic infection and menstrual bleeding and pregnancy can exacerbate IDA in women. For the design of effective policies, it is essential to adopt a multifactorial approach for analyzing the proximate determinants of IDA under actual living conditions. Thus, for example, the success of food fortification programs is likely to vary with the intakes of meat, fish, poultry, and vitamins A and C that enhance iron absorption (Hallberg et al, 1989, 1997, Monsen et al., 1978).

The algorithms developed for calculating iron bioavailability in the presence of enhancers such as meat and vitamin C were recently extended to incorporate the inhibitory effects of phytates that chelate iron thereby reducing its absorption (Tseng et al., 1997). While iron intake by the poor in Bangladesh comes from staple foods such as rice, contamination iron from pots, and small quantities from green and yellow vegetables and animal products, the phytate content of the meal is typically very high. Thus, algorithms for calculating iron bioavailability taking into account the enhancers and inhibitors of iron absorption at each meal are specially useful for subjects in developing countries. However, the inhibitory effects of phytate intakes were calculated using data on healthy subjects with adequate iron stores (Hallberg et al., 1989), which raises the issue of whether alternative equations need to be developed for poor populations. Moreover, there was a technical error in the algorithm by

Tseng et al (1997) that was repeated in the recent work by Du et al. (2000).

Hemoglobin concentration is a widely used measure for assessing iron deficiencies (UNICEF/WHO, 1999, Khusun et al., 1999). While there are many other causes of anemia, where prevalence rates are high, most anemia is due to iron deficiency. Although measures such as serum ferritin and erythrocyte protoporphyrin provide additional insights into iron deficiencies among under-nourished subjects, there are logistics and other difficulties in transporting venous blood samples from a large number of subjects living in remote areas of developing countries. Thus, modeling the proximate determinants of hemoglobin concentration measured in the field through a finger prick can be valuable for deciding the allocation of resources between instruments such as iron fortification of rice, governmental policies encouraging meat, fish, and poultry production, provision of elemental iron tablets, etc.

Thus far, only a few studies in the nutrition literature have attempted to quantify the effects of nutrient intakes on serum iron measures (Doyle et al., 1999, Du et al., 2000). However, previous analyses did not incorporate other confounding factors and did not address in detail issue surrounding the bioavailability of iron. Dietary intakes and supplements, morbidity from infectious disease, and genetic factors are likely to affect hemoglobin concentration; there is also considerable within subject variation in dietary measures. Therefore, we estimated comprehensive longitudinal models for the proximate determinants of hemoglobin concentration of Bangladeshi women. Because the success of food supplementation programs will be influenced by the way in which dietary intakes change with incomes, we estimated a model for assessing the impact of increases in household incomes on iron intake from meat, fish and poultry and for the total iron intake from animal sources.

METHODS

Subjects

The study was conducted at Jessore, Mymensingh, and Saturia in Bangladesh. One of the major purpose of the research was to investigate the nutritional impact of adoption of improved seeds distributed by the Asian Vegetable Research Development Center (Manila, Philippines) in Saturia, and fish pond management strategies in Jessore and Mymensingh. At each site, the new technologies were introduced through Non Governmental Organization (NGO) programs that provided credit and training to women. Selection of households was done in a complex manner to ensure that the selected households were representative of the 3 sites.

In selecting households at the 3 sites, villages were categorized as those where the new technologies had been introduced by the NGO's (type A) and where they had not been introduced due to resource constraints (type B). Similarly, households were categorized into those that had adopted the new technologies (type A) and those had not (type B). In addition, a random sample of type C households were selected from the pool of adopting and non-adopting villages. Approximately, 110 households were included in each of the categories A, B, and C at the 3 sites; there were 990 households of which 10% were expected to be lost due to attrition. In fact, a total of only 50 households did not participate in the 4 survey rounds and were dropped from the sample.

Overall, there were 16 villages surveyed in Jessore, 10 in Saturia, and 18 in Mymensingh. In preparation for the selection of A, B, and C type households, a census of all households in these villages was undertaken; the census included questions regarding household composition, access to land, and education, among other variables (Bouis et al., 1998). A comparison of the households in the census sample with nationally representative surveys for Bangladesh (Rahman, Hossain, and Sen, 1996) indicated that households in our sample villages had smaller land holdings on average. Broad socioeconomic and demographic comparisons were made for the households in the census (site averages) and those selected for the surveys.

The sample means of variables such as household size, age of the head of the household, land owned, size of the homestead plot, and education of the head of the household and the spouse were very similar to the overall means for the type A, B, and C households selected for the survey (Bouis et al., 1998). Although average landholding and education level in some of the households in Mymensingh owning fish ponds were slightly higher than site averages, per capita expenditures that are a reliable measure of household incomes were quite similar across type A, B, and C households in all 3 sites due to highly diversified sources of incomes. It therefore seemed reasonable to treat our sample as representative of the households in the 3 sites and not atypical of rural areas of Bangladesh.

The study design was approved in 1996 by a Human Subjects Committee of the Bangladesh Medical Research Council in Dhaka. The surveys began in June 1996 and the fourth survey round was completed by September, 1997. Because hemoglobin concentration was measured in survey rounds 2, 3, and 4, we analyzed the data from these rounds. Overall, there were 927 women in the data set for whom longitudinal data, from 3 observations separated by 4-month intervals, were available.

Economic, Demographic and Morbidity variables

Background information was compiled on household members' age, relationship with the head of the household, occupation, education, etc. Detailed information was gathered in the 4 survey rounds on economic variables such as households' assets, incomes, food and non-food expenditures, wages, etc; a variable was constructed for the average *per capita* monthly expenditures for each round. Agricultural income and profits from fish cultivation were recorded; typically, in developing countries expenditures data are more reliable measures of economic well-being than household incomes.

The reproductive history of each woman was investigated and the current pregnancy and lactation status were recorded. Ages of all children in the household were in the data set. Sanitation and hygiene practices of the household were assessed in the surveys by investigating the type of toilet used; source of drinking water was recorded. In each survey round, the women were questioned about symptoms such as fever, cough, diarrhea, and any diseases in the past 2 weeks; presence of mucous and blood in the diarrhea was recorded. Although no stool examinations were done for intestinal parasites (Stoltzfus et al., 1997), the ongoing surveys in Saturia record detailed information on intestinal parasites for all household members. Work loss due to chronic illness during the year was also assessed in the morbidity questionnaire.

Anthropometry and Hematology

Weight and arm circumferences of the women were measured in each survey round; height was measured at the start of the study. Spring scales were used to measure the subjects' weight in light clothing, accurate to 0.25 kg in the 4 survey rounds. An adjustable wooden measuring board was used to measure height to the nearest 0.1 cm with the woman standing in upright position. A paper tape was used to measure the mid upper arm circumference (MUAC). Hemoglobin concentration was measured in the 3 survey rounds in a finger-tip sample of capillary blood obtained by a physician using PHM system microlances. The blood was collected in a microcuvette and analyzed immediately using a portable *photmeter* (HemoCue; HemoCue Inc, Mission Viejo, CA). For a subset of 71 women, hemoglobin concentration was measured on 4 consecutive days in the fourth survey round. An analysis of the 4-day measurements was also included in this study as it provided insights in interpreting the variances in the comprehensive model for hemoglobin concentration.

Nutritional intakes and the bioavailable iron

In each of the 3 survey rounds, food intakes were measured using the 24-h recall method for the 4 meals consumed i.e. breakfast, lunch, dinner, and snacks. The person primarily responsible for preparing the meals was questioned about the recipes, ingredients, source of the ingredients (e.g. own-farm, market, etc), and amounts of the dishes consumed by household members and guests. The women's intakes of 40 nutrients at each meal were estimated using food composition table of Murphy et al. (1991) based on data from 6 countries. The tables for India were mainly utilized. In the statistical modeling of the data, nutrient intakes were also averaged over the 3 survey rounds to reduce within subject variation (Liu et al., 1976).

For modeling women's hemoglobin concentration, taking into account nutrient interactions at each meal, we focused on the intakes of total dietary iron, iron from animal source, ascorbic acid, vitamin A, phytates, and tannins from tea. Because most women did not consume any snacks (typically between lunch and dinner), the data on snacks were aggregated with nutrient intakes at lunch. Moreover, since tea consumption was negligible in this population, the inhibitory effects of phytates were the prime concern. For calculating the bioavailability of iron, algorithms due to Monsen et al. (1978), Monsen and Balintfy (1982), and Tseng et al. (1997) were suitably extended. The enhancing effects of meat, fish, poultry, and ascorbic acid, and inhibitory effects of phytates in the meal were programmed to calculate bioavailable iron at each meal under the assumptions that the women's iron body stores were 0, 250, and 500 mg; absorption rates for heme and non-heme iron were 50% or more higher for subjects with iron stores of 0 mg than for normal subjects with body stores 500 mg (Monsen et al., 1978; Table 1). Mathematical formulae for body stores 0 and 250 mg have not been used in previous studies and are believed to be more realistic for under-nourished populations.

Further, the algorithm due to Tseng et al. (1997), that assumed 500 mg body stores, contained a mathematical error. The correct formulae for the 0, 250, and 500 mg iron stores are presented as equations (3)-(5) below. A Fortran program for meal-by-meal calculations is available from the first author; the program also sums the bioavailable iron (and other nutrients) at 3 or more meals to produce figures for the 24-h period.

In the equations that follow, iron intake from meat, fish and poultry is denoted by FeMFP, the total iron intake by FeTOT and the bioavailable iron by FeBIO. Heme iron was assumed to constitute 40% of FeMFP. Using the notation of Monsen and Balintfy (1982), let EF (enhancing factor) for a meal be given by

$$EF = (M + F + P) + AA \qquad (1)$$

where M, F and P are, respectively, the edible quantities of meat, fish and poultry in g, and AA is the intake of ascorbic acid in mg. If $EF > 75$, then EF was assumed to be 75.

To take account of the inhibitory effects of phytates, using the data of Hallberg et al. (1989), Tseng et al. (1987) calculated the "correction term" CT ($0 \leq CT \leq 1$) that gives the proportion of bioavailable iron. However, Tseng et al (1997) defined CT incorrectly when phytate intake was ≤ 2.88 mg because in the data of Hallberg et al. (1989), phytate intakes were either zero or were > 2.88 mg. Let PHY be the total phytate intake in mg during the meal. Then, for $PHY \leq 2.88$mg, we define $CT = 1$. By contrast, the algorithm of Tseng et al. (1997) would lead to an incorrect value of $CT > 1$. For $PHY > 2.88$, CT was defined, as in Tseng et al. (1997), by

$$CT = 10^{[-0.2869 \log_{10}(PHY) + 0.1295]} \qquad (2),$$

where $\log_{10}$ is logarithm to the base 10.

Assuming that body iron stores were 0, 250, and 500 mg, the bioavailable iron (FeBIO) can be calculated, respectively, from the following 3 equations:

$$FeBIO\ (0) = 0.140\ FeMFP + [5 + 26.804 \log_n \{(EF+100)/100\}]\ CT\ [FeTOT-0.4FeMFP]/100 \quad (3)$$

$$FeBIO\ (250) = 0.112\ FeMFP + [4 + 14.296 \log_n \{(EF+100)/100\}]\ CT\ [FeTOT-0.4FeMFP]/100 \quad (4)$$

$$FeBIO\ (500) = 0.092\ FeMFP + [3 + 8.93 \log_n \{(EF+100)/100\}]\ CT\ [FeTOT\text{-}0.4FeMFP]/100 \quad (5),$$

where $\log_n$ is natural logarithm (to the base e).

Lastly, note that because absorption of heme iron was assumed to be unaffected by the presence of other nutrients in the meal, the formulae for calculating bioavailable iron were unique in the sense that the effects of enhancers and inhibitors of iron absorption were simultaneously taken into account.

Overall, the data contained information on over 600 variables for 780 women (one selected from each household) in the age group 15-49 years at 3 points in time separated by 4 month intervals. The wife of the head of the household was generally selected; in some households where the wife was absent, a daughter or a daughter-in-law was chosen. Thus, data were available on 757 women in 3 time periods. Because observations on nutritional, anthropometric, and other variables were missing for some women in the survey rounds, models for hemoglobin concentration were estimated using the complete data on 514 women. Missing observations on hemoglobin concentration and the indicator variable for whether the women were taking iron supplements were prime reasons for reduction in sample size. The models for women's iron intake from meat, fish, and poultry and for iron intake from all animal sources were estimated using data on 733 women. For the simple models estimating for autocorrelations and between and within subject variations in hemoglobin concentration, data were available on 664 women in 3 survey rounds; hemoglobin concentration data were available on a subset of 71 women on 4 consecutive days in the fourth survey round.

An empirical model for the proximate determinants of hemoglobin concentration

The hemoglobin concentration of a woman is influenced by the history of nutritional intakes, sicknesses, pregnancy and lactation status, dietary supplements, and genetic factors. The intake of heme iron, and interactions between non-heme iron and ascorbic acid, vitamin A, β-carotene, and phytates in the meal affect iron absorption and, ultimately, the hemoglobin concentration (Hunt, 1996). While these effects take place gradually over time, one is more likely to detect the effects of nutrient interactions on hemoglobin concentration in under-nourished subjects. In developing countries, economic constraints on households' food consumption adversely affect the quality of diet thereby hindering iron absorption. Determination of within subject variability in 24 h recall data at a given time requires 2 or 3 random repetitions within a limited time period. However, in our study we obtained useful information on within subject variability over the entire study by using the women's nutrient intakes in each of the 3 survey rounds.

Longitudinal studies measuring subjects' nutrient intakes over extended periods of time would be prohibitively expensive. It is therefore important to introduce anthropometric variables such as the mid upper arm circumference (MUAC), height, and weight in models explaining hemoglobin concentration; anthropometric variables reflect the history of nutritional intakes and infections. Moreover, recent sicknesses especially those involving blood loss can reduce hemoglobin concentration (Scrimshaw et al., 1959). By contrast, iron supplements can raise hemoglobin concentration within a short time frame (Viteri, 1999); the model should account for all these factors.

The effects of nutritional intakes and other variables on hemoglobin concentration can be analyzed using longitudinal data by estimating dynamic regression models that include previous measurement on hemoglobin concentration as an explanatory variable. Denoting the i th subject's hemoglobin concentration in time period t by Hb_{it} ($i=1,2,\ldots,n$; $t=2,3$), we postulated the model

$$Hb_{it} = \sum_{j=1}^{m} z_{ij}\,\tau_j + \sum_{j=1}^{k} x_{ijt}\,\beta_j + \alpha\, Hb_{it-1} + \lambda_1\, HT_i + \lambda_2\, W_{it} + \lambda_3\, AM_{it} + \lambda_4\, M_{it} + u_{it} \qquad (6).$$

Here, HT, W, AM and M represent, respectively, the height, weight, arm circumference, and morbidity of subject i in survey round t (height was assumed to be fixed during the observation period). The z's were time invariant variables such as the subject's age in the first survey round; the x's contained variables such as the bioavailable iron that changed with the survey rounds. Greek letters in equation (6) denote coefficients of the explanatory variables.

There were several attractive features of the model in equation (6) for modeling Bangladeshi women's hemoglobin concentration using 3 repeated observations in an 8 month period. First, the short run or the immediate impact of a change in a variable such as the bioavailable iron on hemoglobin concentration was given by (say) β_1; the long run impact was $[\beta_1/(1-\alpha)]$. Generally, one would expect that $0<\alpha<1$, so that the long run effect would be greater than the short run impact. Moreover, for small estimated values of α, the 8-month observation period was sufficiently long for much of the long-term effect to materialize.

Second, the error term u_{it} affecting equation (6) can be decomposed in a simple random effects fashion as:

$$u_{it} = \delta_i + v_{it} \qquad (7)$$

where δ's are women specific random effects and v's are independently distributed random variables. Because hemoglobin concentration is influenced by genetic factors (Garner et al., 2000) and other unobserved characteristics, one would expect the between subject variance (of δ_i) to be an important parameter in the model explaining differences in hemoglobin concentration.

Third, hemoglobin concentration using capillary blood often exhibits large within subject variation that can obscure the effects of explanatory variables (Morris et al., 1999, Liu et al., 1976). In contrast with a single observation on the subjects, however, maximum likelihood estimates computed using repeated observations in a random effects framework can alleviate some of these problems. Fourth, interesting hypotheses can be tested using the model given in equation (6). For example, one might test if economic variables such as household incomes or expenditures directly affect the hemoglobin concentration. Economic factors critically affect diet quality of the Bangladeshi women. Diet quality in turn would determine the quantity of bioavailable iron that would be expected to predict hemoglobin concentration.

A dynamic model for iron intake from meat, fish and poultry and iron intake from animal sources

From the standpoint of investigating the indirect effects of economic variables on hemoglobin concentration, we postulated a dynamic model for the intake of iron from meat, fish and poultry (FeMFP) (a similar model was estimated for iron intake from animal sources):

$$\mathrm{FeMFP}_{it} = \sum_{j=1}^{m^2} z_{ij}\,\phi_j + \sum_{j=1}^{k^2} x_{ijt}\,\psi_j + \alpha_2\,\mathrm{FeMFP}_{it-1} + \mu_1\,\mathrm{BMI}_{it} + u_{2it} \qquad (8).$$

Here, the z's contained background variables such as household size; women in poor households with a large number of children were likely to consume inadequate quantities of absorbable iron. Economic variables such as households' per capita total expenditures that changed with the survey rounds were included in the x's. The short and long run impact of households' expenditures on iron intakes would provide important information to policy makers about the likely improvements in nutrient intakes with rises in incomes (Bhargava, 1991). Women's height and weight were introduced in the model since they reflected the energy requirements (James and Schofield, 1990); micronutrient deficiencies can affect the calculation of energy needs (Bhargava and Reeds, 1995). In equation (8), height and weight were combined as the BMI due to the results of a statistical test (Bhargava, 1994). BMI is a measure of chronic under-nutrition and is associated with a variety of adverse outcomes (e.g. James and Ralph, 1994).

Econometric Procedure

Because there were only 3 time observations available on the women, statistical estimation was based on the large number of women (n) and the fixed number of survey rounds. Thus, initial observations on the dependent variables were treated as endogenous variables (correlated with the errors, Bhargava and Sargan, 1983). The errors on equations (6) and (8) were assumed independent across women, but correlated over time with a positive definite variance-covariance matrix. The random effects decomposition for the u's given in equation (7) was a special case of this model.

The joint determination of the 3 observations on hemoglobin concentration (or iron intakes) and the possibility that some of the explanatory variables were correlated with the random effects implied that econometric techniques used for simultaneous equations were likely to be useful in this application. Details of the maximum likelihood estimation method are presented elsewhere (Bhargava and Sargan, 1983). Here, we note that the profile log-likelihood functions of the models in equations (6) and (8) were optimized using a numerical scheme E04 JBF in (NAG, 1989); asymptotic standard errors of the parameters were obtained by approximating second derivatives of the function at the maximum. The maximized values of the logarithm of the likelihood function were used to test hypotheses regarding the coefficients of the variables included in the models for hemoglobin concentration and iron intakes. Moreover, the unobserved random effects (δ_i) can sometimes be correlated with explanatory variables. The null hypothesis of zero correlation ("exogeneity") between the random effects (δ_i) and certain explanatory variables was tested using likelihood ratio statistics.

RESULTS

Descriptive Statistics

The sample means of selected variables in 3 survey rounds for 514 Bangladeshi women are reported in Table 1. Approximately 5% of the women were pregnant during the observation period. A striking features of the nutrient intakes data was the low intake of iron from meat, fish, and poultry (MFP) and the high phytate intake. For example, in the first survey round, the average daily total iron intake was 6.93 mg (SD=3.00) of which only 0.33 mg (SD 0.47) was from meat, fish and poultry. Further, assuming 0 mg iron body stores and taking into account enhancers of iron absorption, the average daily bioavailable iron was estimated to be 0.85 mg (SD=0.54). However, this figure was reduced to 0.20 mg (SD=0.13) when the effect of phytate intake was incorporated. Similarly, assuming 250 mg iron stores, the average daily bioavailable iron fell from 0.56 mg (SD=0.33) to 0.14 mg (SD=0.09) when phytate intakes were taken into account. These tabulations suggested that phytate intakes play an important role in hindering iron absorption.

Autocorrelations and between and within subject variations in hemoglobin concentration

Table 2 presents the results for a simple dynamic model for the natural logarithm of the hemoglobin concentration; the constant term was the only explanatory variable in equation (6). There were 3 observations available in the survey rounds on 664 women; the measurements on 4 consecutive days in the fourth survey round were available for a subset of 71 women. The point estimate of the coefficient of the lagged dependent variable using the data for the 3 survey rounds was 0.18 (SE=0.058) was smaller than the corresponding estimate 0.248 (SE=0.112) for the 4 day observations; both estimates were statistically significant. The difference in estimates seemed reasonable because nutritional and other factors such as women's pregnancy and lactation status and anthropometric indicators changed over the 3 survey rounds.

The ratio of between to within variance was 0.536 (SE=0.133) for the 3 survey rounds and was 0.417 (0.207) for 4 consecutive day observations on the subset of women. The between subject variation in hemoglobin concentration were relatively large; individual characteristics such as the subjects' height, MUAC, and weight may account for some these differences. Underlying genetic factors (Garner et al., 2000) may also have contributed to the between-women differences. A comparison of the results in Table 2 with those for the model where anthropometric variables were included as explanatory variables (Tables 3-5) can yield insights into this issue. Lastly, the within subject variance was large especially in comparison to studies where simple random effects models were estimated for venous blood samples (Morris et al., 1999). However, the autocorrelations were not estimated in previous studies.

Results from the dynamic model explaining Bangladeshi women's hemoglobin concentration by demographic and nutritional variables

The results from estimating the dynamic model given in equation (6) for women's hemoglobin concentration are presented in Tables 3-5; body iron stores were assumed to be 0, 250, and 500 mg in Tables 3, 4, and 5, respectively. Also, the 3 tables present results for the cases where only the enhancers of iron absorption were taken into account and where the inhibitory effects of phytates in the meal were also incorporated in calculating the bioavailable iron. The subjects' age, height, MUAC, weight, bioavailable iron, and the previous measurement on hemoglobin concentration were transformed into natural logarithms. The logarithmic transformation reduced the internal variation in the data (Nelson et al., 1989). Also, the estimated coefficients of the variables in logarithms were short-run "elasticities" (proportionate change in the dependent variable resulting from a 1% change in an independent variable). The long run elasticity with respect to an explanatory variable can be obtained by dividing the short run elasticity by ($1-\alpha$), where α is the coefficient of the lagged dependent variable.

First considering the results in Table 3 where body iron stores were assumed to be 0 mg, there was a decline in hemoglobin concentration with age, though the coefficient was not statistically significant. By contrast, hemoglobin concentration of pregnant women was significantly lower ($p<0.05$). An additional indicator (0-1) variable was included for women who were lactating during the survey round. However, it was not statistically significant. Pregnancy was observed to impose additional demands on the iron status of these women.

The women's height and MUAC were positively associated with hemoglobin concentration; the coefficients were statistically significant. Height is a good indicator of early nutrition whereas MUAC can approximate lean body mass in some applications (Bhargava, 1999). It was not surprising that these two variables were positively associated with hemoglobin concentration. By contrast, weight was negatively associated which may have been due to the presence of pregnant women in the sample.

In the first column, where only the enhancers of iron absorption were used for calculating the bioavailable iron, the coefficient of bioavailable iron was positive and significant at the 5% level. Moreover, this coefficient showed a 50% increase in the second column where the inhibitory effects of phytate intakes were also taken into account. The maximized value of the logarithm of the likelihood function was higher when the inhibitory effects of phytate intake were included. While the overall effect of bioavailable iron detected on hemoglobin concentration was small, the results suggested that excessive phytate intakes were hindering iron absorption in this population.

The indicator variable for woman with bloody diarrhea was negatively associated with hemoglobin concentration but was not statistically significant. However, the indicator variable for women taking iron tablets during the survey round was positive and significant. In Bangladesh, iron tablets were often sold together with birth control pills. The results reported in Table 3 (and Tables 4 and 5) set the indicator variable for iron tablets to one only when the women answered affirmatively the specific question regarding iron tablets intake.

The coefficient of the lagged dependent variables were comparable to that obtained for the simple model without explanatory variables in Table 2 for the data from the 3 survey rounds; the coefficients were smaller than the estimates using measurements on 4 consecutive days (Table 2). However, the between to within variance ratios were of a similar order of magnitude in Tables 2 and 3 suggesting that there were unknown factors such as genetic differences among the subjects that accounted for some of the differences in hemoglobin concentration.

The results in Tables 4 and 5 were broadly similar to those reported in Table 3. The importance of phytate intakes was evident when the results in the two respective columns of Table 4 and 5 were compared; the coefficient of bioavailable iron was invariably smaller when phytate intake was not taken into account. This also confirmed the robustness of the results from our modeling approach that related bioavailable iron to hemoglobin concentration. Moreover, maximized values of the logarithms of the likelihood functions were slightly higher when it was assumed that the women had 0 mg body iron stores (Table 3) than in the cases where the iron stores were assumed to be 250 or 500 mg (Tables 4 and 5). While this was not direct evidence for the actual iron stores, the results suggested that the average woman probably had iron stores lower than 250 mg.

Results from the dynamic model explaining Bangladeshi women's intake of iron from meat, fish and poultry and iron from animal source by economic, demographic and nutritional variables

The results from estimating the model in equation (8) for iron intake from meat, fish and poultry (MFP) and for iron from animal source are presented in Table 6. Because some women did not consume any meat, fish, or poultry in one or more survey rounds but consumed milk or other animal products, the two sets of results give a better description of the food consumption patterns. The zero intakes of iron by some women were set to 0.01 mg prior to the logarithmic transformation; a sensitivity analysis was performed to investigate if this assumption had induced distortions in the results but it did not. The interesting aspects of the results in Table 6 were:

Firstly, that age had a large negative coefficient that was statistically significant in both models; older women consumed lower quantities of animal foods. This finding was also consistent with the decline in hemoglobin concentration with age in Tables 3, 4 and 5, though the coefficients in the model for hemoglobin concentration were not statistically significant. Secondly, there was a non-linear effect of household size on iron intakes from MFP and from animal sources. This was an expected finding because larger households typically comprise of a greater number of children growing up in poverty; the diet quality in such households is generally poor (Mata, 1978).

Thirdly, the coefficients of monthly average per capita expenditure were large and significant; a 1% increase in households' monthly expenditure (or income) was associated with 0.56% and 0.64% increases in iron intake from MFP and iron from animal source, respectively. Thus, the short run income elasticities of iron intake from MFP and animal source were 0.56 and 0.64, respectively; the corresponding long run elasticities were 0.62 and 0.76. These results suggested that women's iron intakes improve significantly with increases in household incomes. However, the extent to which these gains materialize would depend on economic growth in these regions.

Fourthly, the subjects' BMI was a significant predictor of iron intakes from MFP and from animal source. Possible reverse causality from higher iron intakes from MFP to higher BMI was investigated by testing the null hypothesis that the correlation between the random effects (δ_i) in equation (8) and BMI was zero; the hypothesis was accepted for iron intakes from MFP (likelihood ratio statistic= 0.30, degrees of freedom=3; p=0.999). Similar results were obtained for iron intake from animal source. These findings were not surprising because BMI (i.e. body weight) was more likely to be influenced by current energy intakes (Bhargava, 1991).

Lastly, the coefficients of lagged dependent variables were 0.095 and 0.158, respectively, in the models for iron intake from MFP and animal source; both coefficients were statistically significant. One would expect the coefficient in the model for iron from animal source to be larger because these data were in a more aggregated form. The between to within variance ratios were not statistically significant in either model. This was in part due to the large within subject variation in iron intake from animal foods; such foods are relatively expensive in less developed countries and market purchases are often beyond the budgets of the poorest households.

DISCUSSION

Iron deficiencies are widely prevalent in poor and middle income countries (UNICEF/WHO, 1999). Using a recent longitudinal survey from Bangladesh, proximate determinants of hemoglobin concentration of Bangladeshi women were analyzed. For example, the mean intake of bioavailable iron in survey Round 2 was 0.20 mg/d, taking into account the meal-by-meal nutrient interactions and assuming negligible body iron stores; bioavailable iron was reduced to 0.14 mg/d, if 250 mg body stores were assumed. More importantly, high phytate intake reduced iron bioavailability from 0.85 mg/d to 0.20 mg/d assuming zero iron stores. These quantities of bioavailable iron seem very low; it would have been useful to use alternative models for calculating iron bioavailability. Anand and Seshadri (1995) developed a model for predicting iron bioavailability in Indian diets. However, the phytate content of the meal was not incorporated in the analysis.

For illustrating the magnitude of iron deficiencies in the Bangladeshi sample, we used average iron intakes in Table 1 for survey Round 2 and assumed zero iron body stores. Average iron intake was 6.93 mg/d, iron from MFP was 0.33 mg/d, bioavailable iron was 0.85 mg/d if only the effects of enhancers were taken into account; bioavailable iron was 0.20 mg/d when phytate intake was incorporated. Thus, from equation (3), 14% of the iron from MFP was bioavailable; bioavailability of non-heme, however, ranged from 1% to 11%, depending on the *actual* inhibitory effects of phytate intake on iron absorption i.e. on the appropriate value of CT defined by equation (2). A knowledge of the actual absorption rates in under-nourished populations is of critical importance for assessing the efficacy of alternative food policies. For example, if the body is capable of absorbing greater quantities of non-heme iron because of low body stores, then iron fortification of staple foods should be effective in alleviating iron deficiencies. By contrast, higher intake of MFP would be necessary for improving subjects' iron (and protein) status if the absorption rates were closer to those calculated for healthy populations (Hallberg et al., 1989).

Iron deficiencies among the poor partly result from high prices of animal foods that reduce the intake of heme iron. Also, prices of fresh vegetables and fruits show large seasonal fluctuations making them less affordable outside the harvest season. Thus, the enhancing effects of vitamins A and C for iron absorption may be applicable primarily to the well-off groups in developing countries and not to those most likely to be iron deficient. Cereal staples such as rice in Bangladesh have low iron and a high phytate content. Taken together, these factors suggest that improving women's iron status will not be a simple task. For the formulation of effective policies, one would need to simultaneously address instruments such as iron fortification of rice, increased consumption of MFP, and for women of reproductive age, easier access to iron tablets.

Iron fortification of rice

An international effort to breed for micronutrient-dense staple food crops as a means to reduce micronutrient deficiencies has been ongoing since 1995 (Welch and Graham, 1999; Ruel and Bouis, 1998). Much progress has been made for rice. Current modern varieties of rice developed by the International Rice Research Institute (Manila, Philippines) and widely grown throughout Asia contain on average approximately 8 mg/kg of iron in milled rice. Research under the CGIAR micronutrient projects suggests that the iron content could be increased to 16 mg/kg using conventional plant breeding techniques; such varieties are also profitable and hence attractive to adopt by farmers.

The cost of plant breeding research is typically a one-time fixed investment, costing a few million dollars for one country for a specific crop and nutrient (Bouis, 1999). For example, an upper bound for fixed developmental costs for iron fortified rice for Bangladesh may be set at $13 million. The average intake of rice by women in our sample was approximately 500 g/d. Thus, improved rice variety would have increased non-heme iron intake from 6.6 mg/d to approximately 10.6 mg/d. This would constitute an increase between 0.04 mg to 0.44 mg of bioavailable iron, since the absorption rates were between 1% and 11%. Assuming that the improved rice variety has similar yield and input requirements as current varieties and the development costs are amortized over a period of 10 years, the per capita cost of iron fortification for Bangladesh's 130 million people would range from approximately $0.01 per year if the variety was universally adopted; the costs would be $0.02 per capita if half the population was covered.

The benefits of iron fortification of rice would depend on absorption rates that are applicable to the Bangladeshi population. For example, if the deleterious effects of phytates decline after a threshold, then improved varieties of rice can alleviate iron deficiencies. By contrast, if the negative effects show little signs of tapering off, greater resources would be necessary for other interventions such as encouraging fish production in artificial ponds and the cultivation of dark green and yellow vegetables. The estimated parameters of our model for hemoglobin concentration in Table 3 showed that a doubling of bioavailable iron was associated with a 1.5% increase in hemoglobin concentration in the short run; the long run increase would be 1.8%. These increases are likely to be under-estimated because of the within subject variation in iron intakes results in measurement errors that bias the estimated coefficients downwards (Liu et al., 1976). However, there is evidence that even minor improvements in hemoglobin concentration have beneficial effects on child bearing, time allocation, morbidity from infections, work productivity and cognition (UNICEF/UNU/WHO/MI, 1999). Thus, the breeding of iron dense rice and iron fortification of rice would have high benefits compared to the costs because they are such low-cost interventions.

Household incomes, nutrition and fish cultivation

The long-run expenditure (or income) elasticities of iron intake from meat, fish, and poultry (MFP) and iron intake from animal source for the Bangladeshi women were 0.62 and 0.76, respectively. The fact that an overwhelming majority of the households were not intentionally vegetarians implied that intake of heme iron would increase with incomes. However, the average daily iron intake from MFP in the 3 survey rounds at percentiles 1, 5, 10, 15, 25, 50, 75, and 90 were, respectively, 0.009, 0.030, 0.058, 0.080, 0.111, 0.209, 0.338, and 0.513 (in mg). Comparing these results with average heme iron intake by Russian women in the age group 14-54 y (Tseng et al., 1987, Table 4) revealed that Bangladeshi women at the 90th percentile were consuming lower quantities of heme iron. Moreover, as reported in Table 6, there was a decline in iron intake from MFP with age. Presumably, this was a behavioral response by older women to allow younger members in the household to consume more nutritious foods. Thus, while increases in household incomes will alleviate iron deficiencies among Bangladeshi women, the time frame in which this can be achieved is likely to be long, especially for pregnant and aged women in poor households.

In Jessore and Mymensingh the cultivation of "small" and "big" fish in small sized ponds was encouraged. This resulted in an increase in fish consumption in households that had not previously owned such ponds (Bouis et al., 1998). Although fish consumption did not change significantly in households that already owned ponds, there was substitution of small fish by larger ones. Logistic and other difficulties were observed in pond-sharing by groups of households. The effects of increased fish consumption needs further investigation. While a large scale effort promoting fish cultivation in Bangladesh is likely improve iron and protein status of the population, such an intervention would be quite expensive compared with the breeding effort to produce rice of a higher iron content. From cost-benefit and equity standpoints, therefore, it would be effective to fortify rice with iron so that the benefits reach the poorest sections of the Bangladeshi society.

Methodological and other aspects

The models estimated for Bangladeshi women's hemoglobin concentration showed significant effects of the bioavailable iron when the enhancers were used in the calculations; coefficients of the bioavailable iron increased by 50% (to 0.015) and was more precisely estimated ($p=0.03$) when phytate intake was taken into account. Venous blood samples might have improved the results by reducing the within subject variation. The within subject variation in hemoglobin concentration and in iron intake was investigated by re-estimating the models under two alternative sets of assumptions. First, the data for each of the 3 survey rounds were averaged and a cross-sectional regression explaining the average hemoglobin concentration by the average levels of the explanatory variables was estimated. In the second case, the models in Table 3 were estimated with the bioavailable iron intake averaged over the 3 survey rounds. The results from these two specifications, however, were similar. The coefficient of the bioavailable iron in the model assuming zero iron stores was estimated to be 0.016 in the cross-sectional model, and 0.015 in the model where only the bioavailable iron intake in the 3 survey rounds was averaged.

Recently, Du et al. (2000) have related bioavailable iron to hemoglobin concentration of 42,606 Chinese subjects in a cross-sectional framework controlling for the subjects' gender and residence in urban or rural areas. Bioavailable iron, estimated using the algorithm by Tseng et al. (1997), implied a regression coefficient (elasticity) of 0.07; a residual based method for estimating bioavailable iron increased this coefficient to 0.11 (Du et al., 2000, Table 6). While the regression based method was specific to the Chinese diets, the results showed a stronger relationship between hemoglobin concentration and bioavailable iron when the nutrient composition of Chinese diets was taken into account. Further, in our dynamic multivariate framework, we averaged the 3 measurements on 514 women's hemoglobin concentration, total iron intakes, bioavailable iron intake estimated without incorporating the inhibitory effects of phytates, and bioavailable iron intake that took into account the enhancers and inhibitors. Simple models of the type used by Du et al. (2000) were estimated to explain hemoglobin concentration by iron intake. While the regression coefficient of the total iron intake (or the bioavailable iron estimated without the inhibitors) was not significant, the coefficient of bioavailable iron using the corrected Tseng et al. (1997) algorithm was significant (coefficient =0.0249; SE=0.012). These results showed the importance of estimating bioavailable iron in poor societies where the diets have a high phytate content. It would therefore seem reasonable to conclude that the title of the paper by Du et al. (2000) i.e. "Current methods for estimating dietary iron bioavailability do not work in China" did not accurately reflect the contents of their study.

Finally, the hemoglobin concentration was approximately 5% lower for pregnant women. Moreover, women taking elemental iron tablets had approximately 2.5% higher hemoglobin concentration. The costs of daily iron supplementation of pregnant women were estimated to be $2.50 per pregnancy per woman by the World Bank (Levin et al., 1993). Because even small improvements in hemoglobin concentration were found to significantly affect birth outcomes (UNICEF/WHO, 1999, Bhargava, 2000), daily or weekly iron supplementation to pregnant women and to women of child bearing age would be a cost effective strategy to reduce some of the harmful effects of iron deficiencies (Beard, 1998).

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TABLE 1

Sample means ± standard deviation of selected variables in 3 survey rounds of Bangladeshi women from Saturia, Mymensingh and Jessore sites

Variable	Round 2	Round 3	Round 4
Age, y	33.29 ±7.80[1]		
Pregnant (Yes=1,No=0)	0.05 ±0.22		
Height, m	1.50 ±0.05		
Hemoglobin concentr., g/dL	11.73 ±1.43	11.94 ±1.46	11.54 ±1.36
Mid upper arm circ., cm	22.88 ±2.17	22.91 ±2.19	23.25 ±2.22
Weight, kg	42.03 ±6.11	42.26 ±6.07	42.83 ±6.32
Fe tablets (Yes=1,No=0)	0.22 ±0.41	0.22 ±0.42	0.27 ±0.44
Energy intake, kcal/d	2325 ±773	2136 ±613	2412 ±653
Protein intake, g/d	52.58 ±16.99	49.15 ±16.27	54.19 ±16.0
Phytate intake, mg/d	2298± 817	2229± 838	2328± 705
Ascorbic acid, mg/d	41.85 ±39.31	61.33 ±47.04	79.31 ±103.1
Fe, mg/d	6.93 ±3.00	7.99 ±4.36	7.91± 3.60
Fe (Meat,Fish,Poultry),mg/d	0.33 ±0.47	0.23 ±0.31	0.26 ±0.36
Fe (bioavailable:enhancers; zero body Fe stores)[2], mg/d	0.85 ±0.54	0.98 ±0.68	0.99 ±0.63
Fe (bioavailable:enhancers +inhibitors; zero body Fe stores), mg/d	0.20 ±0.13	0.22 ±0.13	0.22 ±0.13
Fe (bioavailable:enhancers; 250mg body Fe stores), mg/d	0.56 ±0.33	0.64 ±0.41	0.64 ±0.38
Fe (bioavailable:enhancers +inhibitors; 250mg body Fe stores), mg/d	0.14 ±0.09	0.14 ±0.09	0.15 ±0.08
Fe (bioavailable:enhancers; 500mg body Fe stores), mg/d	0.39 ±0.22	0.44 ±0.28	0.45 ±0.26
Fe (bioavailable:enhancers +inhibitors; 500 mg Fe stores), mg/d	0.10 ±0.07	0.10 ±0.06	0.11 ±0.06
n	514	514	514

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[1] $\bar{x}$ ± SD.

[2] See equations (3)-(5) in the text for modifications to the algorithms by Monsen et al. (1978) and Tseng et al. (1997).

TABLE 2

Maximum likelihood estimates of a simple first order autoregressive model with random effects for the natural logarithm of Bangladeshi women's hemoglobin concentration measured in 3 survey rounds and hemoglobin concentration measured for a subset of the women on 4 consecutive days in the third survey round [1]

Dependent variable	Hemoglobin concentration, g/dL			
Model	3 survey rounds[2]		4 consecutive days[3]	
Independent variable	Coefficient	SE	Coefficient	SE
Constant	2.009*	0.143	1.844*	0.274
Lagged dependent variable, g/dL	0.180*	0.058	0.248*	0.112
Between/Within variance	0.536*	0.133	0.417*	0.207
Within variance	0.0093		0.0056	
Proportion of variance due to between subject differences	0.435		0.409	
2(log-likelihood function)	8591.62		1393.33	
n	664		71	

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[1] Values are slope coefficients ± standard errors. * $p<0.05$.
[2] Hemoglobin concentration measured at 3-month intervals.
[3] Hemoglobin concentration measured on 4 consecutive days in the third survey round.

TABLE 3

Maximum likelihood estimates of a first order autoregressive model with random effects for Bangladeshi women's hemoglobin concentration in 3 survey rounds explained by anthropometric and morbidity variables and by the intake of bioavailable Fe assuming 0 mg body Fe stores [1]

Dependent variable	Hemoglobin concentration, g/dL			
Model	Fe Enhancers only		Fe Enhancers+inhibitors	
Independent variable	Coefficient	SE	Coefficient	SE
Constant	1.720*	0.199	1.744*	0.193
Age^2, y	-0.021	0.018	-0.020	0.018
Pregnant (Yes=1;No=0)	-0.048*	0.015	-0.047*	0.014
Height^2, m	0.338*	0.134	0.337*	0.130
MUAC^2, cm	0.323*	0.022	0.318*	0.018
Weight^2, kg	-0.201*	0.023	-0.201*	0.020
Fe bioavailable^{2,3},mg/d	0.011*	0.006	0.015*	0.006
Diarrhea with blood (Yes=1; No=0)	-0.037	0.045	-0.038	0.044
Indicator for Fe tablets (Yes=1; No=0)	0.026*	0.009	0.026*	0.009
Lagged dependent variable^2, g/dL	0.168*	0.060	0.172*	0.051
Between/Within variance	0.472*	0.140	0.465*	0.121
Within variance	0.0089		0.0089	
2(log-likelihood function)	6769.95		6772.08	
n	514		514	

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[1] Values are slope coefficients ± standard errors. * $p<0.05$; $^+$$<0.10$.

[2] These variables and dependent variable were in natural logarithms.

[3] See equation (3) in the text for the algorithm.

TABLE 4

Maximum likelihood estimates of a first order autoregressive model with random effects for Bangladeshi women's hemoglobin concentration in 3 survey rounds explained by anthropometric and morbidity variables and by the intake of bioavailable Fe assuming 250 mg body Fe stores [1]

Dependent variable	Hemoglobin concentration, g/dL			
Model	Fe Enhancers only		Fe Enhancers+inhibitors	
Independent variable	Coefficient	SE	Coefficient	SE
Constant	1.722*	0.199	1.750*	0.196
Age[2], y	-0.021	0.018	-0.020	0.018
Pregnant (Yes=1;No=0)	-0.049*	0.015	-0.047*	0.015
Height[2], m	0.338*	0.125	0.338*	0.133
MUAC[2], cm	0.323*	0.026	0.318*	0.020
Weight[2], kg	-0.201*	0.023	-0.201*	0.023
Fe bioavailable[2,3], mg/d	0.011*	0.006	0.015*	0.006
Diarrhea with blood (Yes=1; No=0)	-0.036	0.045	-0.037	0.045
Indicator for Fe tablets (Yes=1; No=0)	0.026*	0.009	0.026*	0.009
Lagged dependent variable[2], g/dL	0.168*	0.060	0.171*	0.061
Between/Within variance	0.473*	0.139	0.466*	0.139
Within variance	0.0089		0.0089	
2(log-likelihood function)	6769.22		6771.57	
n	514		514	

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[1] Values are slope coefficients ± standard errors. * $p<0.05$; +<0.10.

[2] These variables and dependent variable were in natural logarithms.

[3] See equation (4) in the text for the algorithm.

TABLE 5

Maximum likelihood estimates of a first order autoregressive model with random effects for Bangladeshi women's hemoglobin concentration in 3 survey rounds explained by anthropometric and morbidity variables and by the intake of bioavailable Fe assuming 500 mg body Fe stores [1]

Dependent variable	Hemoglobin concentration, g/dL			
Model	Fe Enhancers only		Fe Enhancers+inhibitors	
Independent variable	Coefficient	SE	Coefficient	SE
Constant	1.709*	0.111	1.749*	0.089
Age[2], y	-0.020	0.017	-0.019	0.013
Pregnant (Yes=1;No=0)	-0.048*	0.014	-0.047*	0.013
Height[2], m	0.339*	0.133	0.338*	0.131
MUAC[2], cm	0.324*	0.020	0.317*	0.017
Weight[2], kg	-0.203*	0.013	-0.201*	0.013
Fe bioavailable[2,3], mg/d	0.011*	0.006	0.015*	0.006
Diarrhea with blood (Yes=1; No=0)	-0.036	0.044	-0.037	0.044
Indicator for Fe tablets (Yes=1; No=0)	0.026*	0.009	0.026*	0.009
Lagged dependent variable[2], g/dL	0.175*	0.006	0.174*	0.016
Between/Within variance	0.458*	0.060	0.460*	0.067
Within variance	0.0090		0.0089	
2(log-likelihood function)	6768.94		6771.57	
n	514		514	

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[1] Values are slope coefficients ± standard errors. * $p<0.05$; $^+<0.10$.
[2] These variables and dependent variable were in natural logarithms.
[3] See equation (5) in the text for the algorithm.

TABLE 6

Maximum likelihood estimates of a first order autoregressive model with random effects for Bangladeshi women's iron intake from meat, fish and poultry (MFP) and iron intake from animal source in 3 survey rounds explained by anthropometric, demographic and economic variables[1]

Dependent variable	Fe intake, mg			
Model	Fe from MFP, mg		Fe from animal source,mg	
Independent variable	Coefficient	SE	Coefficient	SE
Constant	-5.207*	0.155	-6.028*	0.343
Age[2], y	-0.516*	0.024	-0.406*	0.039
Household size[2]	0.446*	0.030	0.602*	0.044
Household size squared[2]	-0.033*	0.026	-0.105*	0.023
Monthly average percapita[2] expenditure, Taka[3]	0.561*	0.039	0.639*	0.066
BMI[2], kg/m^2	0.210*	0.015	0.325*	0.016
Lagged dependent variable[2], mg	0.095*	0.034	0.158*	0.024
Between/Within variance	0.043	0.039	0.001	0.100
Within variance	2.0282		1.7668	
2(log-likelihood function)	-1655.42		-1273.21	
n	733		733	

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[1] Values are slope coefficients ± standard errors. * $p<0.05$; $^+<0.10$.
[2] These variables and dependent variable were in natural logarithms;
[3] $ 1.00 = 42 Taka.