Efficacy and trial effectiveness of weekly and daily iron supplementation among pregnant women in rural Bangladesh: disentangling the issues[1–3]

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ABSTRACT

Background: According to our current understanding, iron absorption with weekly iron supplements is not higher than that with daily supplements (ie, there is no mucosal block). However, community-based trials have repeatedly shown that a weekly regimen is as effective as a daily one. Furthermore, when differences in absorption are found, they are commonly smaller than would be expected on the basis of differences in the amount of iron provided. The possibility of differential compliance between the regimens needs to be evaluated to explain these findings.

Objective: Taking compliance into account, we compared the efficacy and trial effectiveness of weekly and daily iron supplementation during pregnancy.

Design: In Bangladesh, 50 antenatal centers were randomly assigned to prescribe either 2 doses of 60 mg Fe once weekly or 1 dose of 60 mg Fe/d. Compliance was monitored by using a pill bottle equipped with an electronic counting device. Hemoglobin concentrations were measured at baseline and after 4, 8, and 12 wk of supplementation.

Results: There was no differential effect per iron tablet between weekly and daily regimens. A 12-wk daily regimen (68% compliance) produced a small but significantly greater hemoglobin response than did the weekly regimen (104% compliance). The first 20 tablets consumed produced most of the effect; after 40 tablets, there was no further response.

Conclusions: There was no evidence of a mucosal block in the daily regimen. Over 12 wk, 50% of the amount of iron in a daily regimen was sufficient for maximum hemoglobin effect. The weekly regimen provided a large part of this amount, explaining the limited difference in effect. It appears that the current international recommendation for iron supplementation in pregnancy is higher than necessary. *Am J Clin Nutr* 2002;76:1392–1400.

KEY WORDS Iron supplementation, pregnancy, efficacy, trial effectiveness, weekly dose frequency, Bangladesh

INTRODUCTION

The controversy over the so-called "mucosal block" theory in iron supplementation is not yet resolved. It was hypothesized that a first dose of iron would load the mucosa with iron and block subsequent doses from absorption (1, 2). By reducing the dose frequency to once per week, matching the mucosal turnover in man, iron from each tablet would be better absorbed, and consequently a lower amount of iron would be required. Studies in small animals support the hypothesis (2, 3), but studies using radiolabeled iron in humans showed only a small reduction in absorption due to previous administration of iron (4, 5). This suggests that the mucosal block effect is not pronounced in humans. A daily dose frequency should therefore produce a larger hematologic response than that of a weekly dose frequency because of the larger amount of iron the former provides. However, although some of the community-based trials testing the mucosal block theory in practice showed that daily supplementation had a greater effect than did weekly supplementation (6), the difference was less than that expected from the differences in amounts of iron between the regimens.

There are several possible explanations for the limited difference in effect between the supplementation regimens in these community-based trials. First, the population may have responded to a limited extent because of a low prevalence of iron deficiency, low intake of the supplements, or the presence of other limiting factors such as vitamin A deficiency (7) or chronic infection (8). Second, differential compliance between weekly and daily supplementation may have resulted in ingestion of similar amounts of iron. Finally, a third, less discussed explanation is that the amount of iron provided by weekly supplementation was sufficient to produce a maximal effect.

The aim of this study was to compare the response in hemoglobin concentration between daily and weekly iron supplementation and in the process differentiate between biologically and

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behaviorally induced differences such as compliance. Precise information on the intake of supplements was obtained by using a microchip-equipped pill bottle, Medication Event Monitor System (MEMS; Aardex, Zug, Switzerland), that enabled a comparison of the effect per tablet consumed and of compliance with the prescribed regimens. It was also used for an estimation of the amount of iron required for maximal hemoglobin effect. This is the first study designed to evaluate the mucosal block theory in a community trial and to compare both the efficacy and trial effectiveness of weekly and daily iron supplementation regimens.

SUBJECTS AND METHODS

Study population and supplementation groups

The study was implemented in rural areas of Mymensingh thana (subdistrict), Bangladesh. The area is ≈110 km north of the capital, Dhaka, in the rural plain areas typical of Bangladesh. Rice is the dominant food crop and is cultivated by those who have access to land. Approximately two-thirds of the population is functionally landless and have to rely on work as manual day laborers to earn their living (9). Bangladesh has an estimated prevalence of low birth weight of 45% (10), a maternal mortality of 420/100 000 births (11), and a prevalence of anemia of 45% and 50% in nonpregnant and pregnant women, respectively (12), suggesting that there are serious limitations in maternal health and nutrition in the country.

Women attending antenatal care centers run by a national nongovernmental organization, BRAC, participated in the study. In 2 areas (Samvugonj and Dapunia), 50 of 54 BRAC community-based antenatal care centers were selected. Each antenatal care center covers a population of ≈1000 and is serviced monthly. Current services include antenatal controls, health and nutrition education, and provision of iron supplements.

Each of the antenatal care centers was randomly assigned to prescribe one of the interventions: one supplement daily or 2 supplements each Friday. Each supplement contained the equivalent of 60 mg Fe and 250 μg folic acid. The weekly regimen thus provided 28% of the amount of iron in the daily regimen. The tablets were produced in Norway and provided by the United Nations Children's Fund (UNICEF).

All pregnant women in the catchment area of the selected 50 antenatal care centers were identified through household visits. The pregnant women were encouraged to enroll in the services of the antenatal care center. On the day of the scheduled antenatal service, the first 4 women who booked for service at each center and who fulfilled the primary inclusion criteria were invited to participate. The inclusion criteria were having a fundal height between 14 and 22 cm, not having used iron supplements during the pregnancy before the start of the study, and being apparently healthy. Informed consent to participate in the study was obtained, and hemoglobin concentrations were measured in venous blood collected from the women who agreed to participate. If a woman's hemoglobin concentration was < 80 g/L, she was excluded from the study and referred for appropriate investigation and therapy, including daily supplementation and monthly hemoglobin assessments.

During the course of the trial, the women were assessed monthly for low hemoglobin concentrations. If a single measurement was < 75 g/L or 2 consecutive monthly measurements were between 75 and 79 g/L, the woman was excluded from the study, transferred to daily supplementation, and referred to the BRAC health center. Supplementation continued until 6 wk after giving birth.

The study was reviewed and approved by the Research Ethics Committee of the Medical Faculty at Umeå University, Sweden, and the Ethical Board at Bangladesh Medical Research Council, Dhaka, Bangladesh. Informed consent was obtained verbally because of the high rate of illiteracy.

Compliance

Compliance with the recommended dose frequency was assessed by MEMS (13). It consists of an ordinary pill bottle equipped with a cap in which a counting device and a small microprocessor are embedded. Each time the bottle was opened or closed, the time and date were recorded. The information was later retrieved by a special reader connected to a computer. Use of this equipment permitted information on compliance to be continuously collected over an extended period of time. Bottle-opening events that occurred on the first and last day of using MEMS were discarded because they did not provide information on a full day. For the other days, each event was considered as one tablet taken. Compliance (%) was defined as (mean number of tablets taken/prescribed number of tablets) × 100.

The women assigned to the daily regimen received a MEMS pill bottle with 100 tablets and were advised to take 1 tablet every day of the week. The women assigned to the weekly regimen received a MEMS bottle with 30 tablets and were advised to take 2 tablets (one in the morning and one in the evening) every Friday. The supplements were distributed to the women at the antenatal clinic. After 12 wk of supplementation, the women returned the MEMS pill bottle with any remaining tablets. For the rest of the study (until 6 wk after giving birth), iron supplements sufficient for either daily or weekly supplementation were provided in the same pill bottles except that the MEMS caps were replaced with ordinary caps.

The total number of tablets taken at weeks 3, 7, and 11 were collected from MEMS and used to predict hemoglobin concentrations at weeks 4, 8, and 12. The numbers of tablets taken at these earlier time points (ie, weeks 3, 7, and 11) were more strongly associated with hemoglobin response than were the numbers of tablets taken at the time of the hemoglobin assessments. This was probably due to a time lag in the response of hemoglobin concentrations to iron supplementation.

Hemoglobin concentrations

Venous blood samples were collected in evacuated, nontreated tubes at baseline and after 12 wk of supplementation. Hemoglobin concentrations were assessed in the field with the HemoCue system (HemoCue, Ängelholm, Sweden), a portable hemoglobin meter that uses disposable microcuvettes and has good reliability and accuracy (14). The HemoCue photometer was checked daily against its control cuvette. In addition to venous blood samples, capillary blood samples were collected for monthly monitoring of hemoglobin concentration. In pregnancy the hemoglobin concentration in capillary blood is on average 5 g/L lower than that in venous blood (15). To allow for comparison with values from venous blood samples, the capillary hemoglobin concentrations at 4 and 8 wk of supplementation were adjusted by adding 5 g/L. Because all the hemoglobin outcomes were compared across supplementation groups, this correction did not introduce any bias in the comparison between weekly and daily supplementation.

Fundal height

Fundal height was used as an indicator of gestational age. The field assistants received 3 d of training on how to measure symphysis-fundal height at the Obstetric Unit of Mymensingh Medical College. Fundal height was measured at the antenatal care centers on the day of recruitment. Measurements in cm were taken with a standard plastic tape. Each woman was asked to empty her bladder and lie on her back with her legs extended. Measurements were taken along the longitudinal uterine axis to reflect fetal crown-rump length. The women with a fundal height of 14–22 cm, which corresponded to a gestational age of 18–24 wk (16), were included in the study. Information on the last menstrual period as a measure of gestational age was also collected, but because the validity of that information proved to be poor, it was not used in the analyses.

Baseline characteristics

Baseline information on midupper arm circumference, age, parity, and socioeconomic situation (SES) was collected from all the identified pregnant women at the first household visit. Midupper arm circumference was measured at the center to the nearest 1 mm by using a Teaching Aids at Low Cost (TALC) insertion tape (St Albans, Herts, United Kingdom) and following the standard procedure. The questionnaire included 3 binomial indicators of SES: formal education of the woman, household landholding, and perceived household economic status. "With education" was defined as ever being enrolled in a formal school, "with landholdings" was defined as household landholding of ≥0.5 acre, and "economically surplus" was defined as a self-reported perception that the household had not experienced any periods of economic deficit in the preceding year. A SES score was constructed by using a combination of the 3 indicators. The score ranged from 0 to 3 on the basis of the accumulated number of positive attributes.

Analytic approach and statistical methods

Monitoring compliance by using MEMS in this study enabled comparisons of efficacy and effectiveness between weekly and daily supplementation. *Efficacy* is "the extent to which a specific intervention, procedure, regimen, or service produces a beneficial result under ideal conditions" (17). In the context of this study, efficacy was interpreted as the extent to which iron tablets ingested either weekly or daily produce a differential effect on hemoglobin. Precise information on compliance was used to relate responses to amounts of iron consumed. *Effectiveness* is defined as "the extent to which a specific intervention, procedure, regimen, or service, when deployed in the field, does what it is intended to do for a defined population" (17). Effectiveness is affected both by efficacy and by compliance. Thus, in contrast to efficacy, which is affected only by biology, effectiveness is influenced by both behavioral and biological factors (18). It is useful to address both efficacy and effectiveness because biologically and behaviorally induced limitations call for different actions. The concept of effectiveness in public health is often associated with program effectiveness (19), which includes additional programmatic factors that were not dealt with in this research trial. To make the distinction clear between program effectiveness and the measure of effectiveness used in this trial, we propose that the latter should be thought of as trial effectiveness.

Furthermore, when estimating the differences in efficacy on hemoglobin concentration between weekly and daily supplementation, it was necessary to differentiate between the effect per tablet taken and the effect over the entire supplementation period. To differentiate between these 2 effects, we used the concepts dose frequency and supplementation regimen. The relation between these concepts may be expressed as follows:

$$\begin{aligned}\text{Supplementation regimen (mg iron)} &= \text{dose (mg iron/tablet)} \\ &\times \text{dose frequency (number of tablets/wk)} \\ &\times \text{duration of supplementation (wk)} \qquad (1)\end{aligned}$$

Using these concepts, we compared *1*) the estimated efficacy of weekly and daily dose frequencies, *2*) the predicted efficacy of weekly and daily supplementation regimens, and *3*) the estimated trial effectiveness of weekly and daily supplementation regimens.

The comparison of efficacy of dose frequencies tested whether there was a difference in hematologic response per tablet ingested between a weekly dose frequency and a daily one. In the comparison of efficacy of supplementation regimens, the difference in hematologic response over a 12-wk period was predicted as if compliance had been 100%. The comparison of trial effectiveness of supplementation regimens showed the actual difference in hematologic effect over a 12-wk period, including the effect of differential compliance.

The questionnaires were coded and data was entered by using the SPSSWIN statistical package (version 7.5.1; SPSS Inc, Chicago). Data were verified by checking for consistency and range. For data analysis, the SPSSWIN (version 9.0) and STATA 7 (Stata Corporation, College Station, TX) statistical packages were used. The statistical methods included Student's *t* test, analysis of variance (ANOVA), Bonferroni test for multiple comparisons, correlation analysis, three-factor repeated-measures ANOVA, multivariate regression analysis, and hierarchical ANOVA. Statistical significance was set at $P < 0.05$. Variables were tested for normal distribution. For nonnormal distributions, medians and percentiles were presented, and comparisons were made by using Mann-Whitney *U* test statistics. Lowess (20) smoothed plots were used to visualize dose-response relations.

The risk of confounding was fundamentally different between the analyses of efficacy and trial effectiveness. The efficacy analysis depended on differential compliance to produce a range of tablet intakes. The number of tablets taken during the 2 regimens was therefore not randomized but depended on individual behavior and was potentially associated with confounding factors. Careful analyses of potentially confounding factors were therefore essential in the efficacy analyses. In the trial effectiveness analyses, however, the interventions (weekly and daily regimens) were randomized, as were any potentially confounding factors. Control for confounding in this type of analysis is theoretically not necessary but was done nevertheless to increase plausibility (19). All analyses were tested for the confounding effects of maternal age, fundal height, initial hemoglobin concentration, parity, and SES. For a given analysis, potential confounders were identified as those variables with a P value < 0.20 for any linear or nonlinear association between hemoglobin outcomes and tablet ingestion (for efficacy analyses) or for any linear or nonlinear association of hemoglobin outcomes across the daily or weekly regimens (for effectiveness analyses). Potential confounding was accounted for by introducing potentially confounding factors into the crucial analyses as main effects and as pertinent interactions. Confounding by the measured variables was regarded as an unimportant contribution to the main effects if the effect of their linear or nonlinear introductions changed the

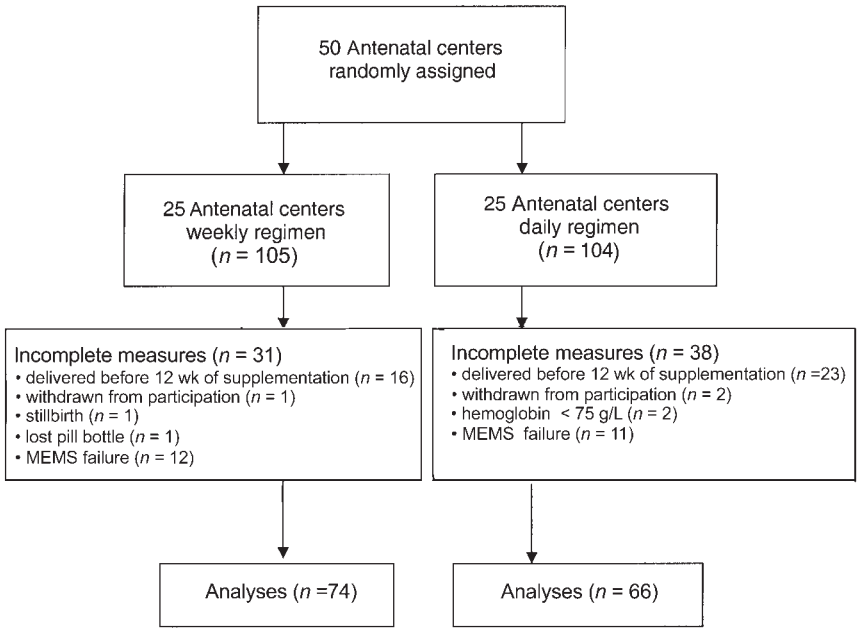

FIGURE 1. Numbers of women assigned to a weekly or daily supplementation regimen, lost to follow-up, and with complete measures for analyses. MEMS, Medication Event Monitor System (Aardex, Zug, Switzerland).

variables describing the dose response or the interaction between daily and weekly regimens by < 10%.

RESULTS

Participation

Among the 209 women enrolled in the study, 140 (67%) had complete information, including hemoglobin concentration after 12 wk of supplementation and information on the number of tablets taken (**Figure 1**). The major reasons for loss to follow-up were giving birth before the scheduled blood sample collection at 12 wk of supplementation and technical problems with the MEMS that resulted in loss of information on compliance. The only differences between those with and without complete data were in initial hemoglobin concentration and fundal height at baseline (**Table 1**). Among the women with incomplete data, a fundal height higher than that of the women with complete data occurred to a similar extent in the daily and weekly supplementation groups. A lower hemoglobin concentration among the women with incomplete data occurred to a greater extent among those in the weekly group than among those in the daily group.

Trial effectiveness of daily and weekly regimens after 12 wk of supplementation

Full sample

The comparison of trial effectiveness between the supplementation regimens included the effect of a potential differential level of compliance with the prescribed supplementation regimens. The comparisons are those between the allocation of daily and weekly regimens, disregarding the level of compliance. This type of analysis is often referred to as intention-to-treat analysis.

There was no significant difference in initial hemoglobin concentration between the weekly and daily supplementation groups at baseline (**Table 2**). After 4, 8, and 12 wk of supplementation,

TABLE 1
Baseline characteristics of women with complete and incomplete measures during the study by weekly or daily supplementation regimen[1]

	Complete			Incomplete		
	All (*n* = 140)	Weekly (*n* = 74)	Daily (*n* = 66)	All (*n* = 69)	Weekly (*n* = 31)	Daily (*n* = 38)
Age (y)	24.0 ± 5.9[2]	23.3 ± 5.6	24.8 ± 6.2	24.3 ± 6.0	25.1 ± 8.1	23.6 ± 5.9
Parity (no.)	1 (0, 3)[3]	1 (0, 3)	1 (0, 3)	2 (0, 3)	2 (0, 3)	1 (0, 3)
Fundal height (cm)	16.5 ± 1.8	16.6 ± 1.8	16.3 ± 1.8	18.3 ± 2.3[4]	18.8 ± 2.3[5]	17.9 ± 2.2[5]
MUAC (mm)	223 ± 18	223 ± 19	223 ± 16	227 ± 22	229 ± 26	224 ± 17
Ascaris (*n* [%])	52 [37]	29 [39]	23 [35]	30 [44]	13 [42]	17 [45]
SES	1 (1, 2)	1 (1, 2)	1 (1, 2)	1 (1, 2)	1 (0, 2)	1 (1, 2)
Hemoglobin (g/L)	110.6 ± 13.3	112.6 ± 13.9	110.4 ± 12.7	107.6 ± 14.9[6]	103.9 ± 10.9[5]	110.6 ± 17.3

[1] MUAC, midupper arm circumference; SES, socioeconomic situation.
[2] $\bar{x}$ ± SD.
[3] Median; 25th and 75th percentiles in parentheses. Mann-Whitney *U* test statistics.
[4,6] Significantly different from complete (Student's *t* test): [4] $P < 0.05$, [6] $P = 0.053$.
[5] Significantly different from complete, $P < 0.05$ (Bonferroni test for multiple comparisons).

TABLE 2
Hemoglobin concentrations at baseline and increments in concentration during 12 wk of supplementation by supplementation group in the full sample and in a subset of women with an initial hemoglobin concentration <115 g/L

	Full sample			Hemoglobin <115 g/L		
	Weekly (n = 74)	Daily (n = 66)	P	Weekly (n = 44)	Daily (n = 46)	P
Hemoglobin (g/L)[1]						
Concentration at baseline	112.6 ± 13.9[2]	110.4 ± 12.7	0.334	103.3 ± 8.4	103.9 ± 8.4	0.728
Increment at 4 wk	1.0 ± 11.3	4.5 ± 11.6	0.068	5.4 ± 8.9	8.2 ± 10.0	0.156
Increment at 8 wk	4.4 ± 11.6	7.7 ± 13.8	0.127	8.9 ± 9.0	13.3 ± 12.0	0.051
Increment at 12 wk	10.0 ± 12.3	14.4 ± 14.1	0.052	11.6 ± 11.0	17.0 ± 13.3	0.043
Concentration at 12 wk	122.6 ± 16.1	124.8 ± 16.1	0.422	115.0 ± 12.4	120.9 ± 15.4	0.047
Tablets at wk 11 (no.)[3]	23 (17, 27)	52 (29, 75)	<0.01	22 (18, 26)	58 (30, 78)	<0.01
Compliance at wk 11 (%)[1]	104	68	<0.01	100	69	<0.01

[1] Student's t tests.
[2] $\bar{x}$ ± SD.
[3] Median; 25th and 75th percentiles in parentheses. Mann-Whitney U test statistics.

hemoglobin concentrations had increased in both groups, but the increments were larger with the daily regimen. However, there was no significant difference between the groups in hemoglobin concentration after 12 wk of supplementation. Mean tablet intakes were 52 and 23 in the daily and weekly supplementation groups, respectively ($P < 0.05$). This corresponded to a compliance of 68% with the daily regimen and 104% with the weekly regimen ($P < 0.05$). The analysis was repeated by using a three-factor repeated-measures ANOVA (initial hemoglobin concentration, time of hemoglobin measurement, and supplementation regimen) including all interactions. As expected, initial hemoglobin concentration had a significant effect on the other hemoglobin measures ($P < 0.05$). Accounting for this and for a clustering effect within subjects, the mean hemoglobin concentration at 12 wk in the daily supplementation group (120.1 g/L) was significantly higher than that in the weekly supplementation group (117.1 g/L; $P < 0.05$).

Hemoglobin concentrations at baseline corresponded to an initial prevalence of anemia of 50% in the daily supplementation group and 42% in the weekly supplementation group. After 4, 8, and 12 wk of supplementation, the prevalence of anemia was 30%, 23%, and 14%, respectively, in the daily supplementation group and 39%, 30%, and 20% in the weekly supplementation group. There was no significant difference between the supplementation regimens in the prevalence of anemia at any time point. Some anemia persisted with both regimens.

At baseline there were no significant differences between the 2 supplementation groups in initial hemoglobin concentration, fundal height, age, parity, or SES. Thus, the trial effectiveness analysis could not have been confounded by these factors.

Subset with a low initial hemoglobin concentration

Women with a low initial hemoglobin concentration (<115 g/L) were selected to compare the trial effectiveness of the daily and weekly regimens because these women were expected to have a larger need for iron supplements. Such exclusion of potential nonresponders may reduce the risk of not identifying a difference in effect.

Hemoglobin concentrations increased faster in the daily supplementation group than in the weekly supplementation group and were significantly different in the 2 groups at week 8 of supplementation. At 12 wk there were significant differences both in the increment in hemoglobin concentration and in the actual hemoglobin concentration, showing a greater trial effectiveness in the women on the daily regimen than in those on the weekly regimen (Table 2).

A similar pattern was also found for the prevalence of anemia. Both groups had an initial prevalence of ≈70%. After 4 wk the prevalence in the daily supplementation group had decreased to 37%, whereas the prevalence in the weekly supplementation group had decreased to 55% ($P = 0.09$). The prevalence continued to decrease. After 8 wk of supplementation, it was 33% in the daily supplementation group and 39% in the weekly supplementation group. After 12 wk of supplementation, 20% of the women in the daily supplementation group were anemic, whereas 34% of the women in the weekly supplementation group were anemic ($P = 0.12$).

In this subset of women, initial hemoglobin concentration, age, and parity (but not SES or fundal height) were associated with hemoglobin concentration at 12 wk. Among these variables, age was also associated with supplementation regimen and could thus potentially confound the analysis. In multivariate regression analysis, potential confounding by age was controlled for by including it as main effect and as its bivariate interaction with supplementation group. The introduction of age did not change the estimated difference between the supplementation groups (data not shown).

Antenatal care center analysis: subset with a low initial hemoglobin concentration

The unit of randomization was the antenatal care center. The trial effectiveness analysis in the subset of women with a low initial hemoglobin concentration was therefore also performed with antenatal care center as the unit of analysis. If a large variation existed between the antenatal care centers, a bias may have been introduced. The mean hemoglobin concentration for each antenatal care center was calculated, and the concentrations obtained at centers prescribing daily supplementation were compared with those obtained at centers prescribing weekly supplementation. The mean (± SD) increment in hemoglobin concentration over 12 wk of supplementation in the centers that prescribed daily supplementation was 17.0 ± 14.7 g/L (n = 19), whereas that in the centers that prescribed weekly supplementation was 10.8 ± 7.9 g/L (n = 21). The difference was not significant ($P = 0.10$), but the power of this comparison was limited. The difference in increment (6.2 g/L) was somewhat higher than that observed in the analyses with individual women as the unit of analysis. The mean hemoglobin concentration at 12 wk was higher in the daily supplementation centers than in the weekly supplementation centers (121.8 ± 15.8 g/L compared with 113.5 ± 10.6 g/L; $P = 0.056$). The difference in effect between daily and weekly supplementation

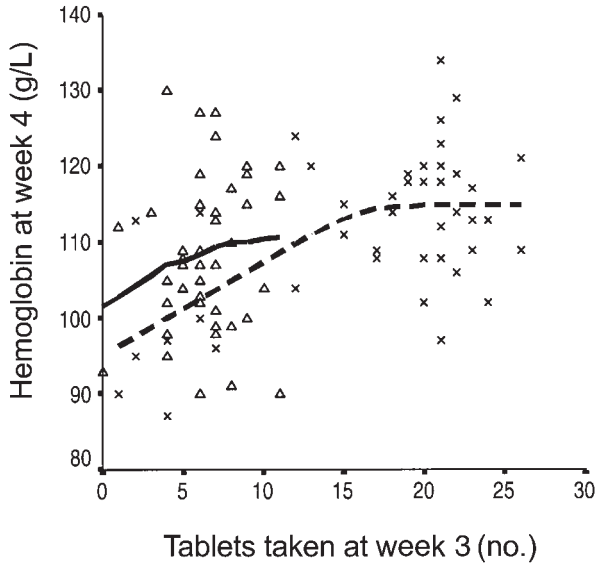

FIGURE 2. Hemoglobin concentrations at week 4 plotted against the number of iron tablets taken at week 3 in a subset of women with an initial hemoglobin concentration < 115 g/L who were assigned either to daily (– –; x) or weekly (——; △)supplementation. The lines through the data points are lowess moving-average fitted lines.

was also confirmed in a hierarchical ANOVA that adjusted for the variation between antenatal care centers ($P = 0.054$). The analyses with antenatal care center as the unit of analysis confirmed the finding at the individual level that the trial effectiveness of 12 wk of daily supplementation was higher than that of 12 wk of weekly supplementation.

Efficacy per tablet with weekly and daily supplementation

The analysis of the difference in the efficacy of dose frequencies was used to evaluate the mucosal block theory. In this analysis, we tested the hypothesis that a dose of iron from tablets administered daily is less efficiently absorbed and incorporated into hemoglobin than is the same dose of iron from tablets taken weekly. The aim of the efficacy analyses was to compare biological differences in response to iron supplementation. The subset of women with a low initial hemoglobin concentration was used for the efficacy analyses.

Information on compliance was used to compare the efficacy per tablet taken between weekly and daily dose frequencies. Two criteria had to be fulfilled to enable an evaluation of the difference in dose effect. First, the comparison had to be made within a range of tablet intakes in which the number of tablets taken and hemoglobin concentrations showed a dose-effect relation. Second, the supplementation groups had to have an overlapping distribution of number of tablets taken.

Hemoglobin concentrations at 4 wk were plotted as a function of the number of tablets taken at week 3, and lowess moving-average lines were fitted to the data (**Figure 2**). These lines showed a steep initial response that leveled off and reached its asymptote at ≈15 tablets. A subset of women who took ≤ 15 tablets, representing the steepest segment of the curve, was selected for the comparison of efficacy per tablet between weekly and daily dose frequencies. The mean (± SD) tablet intakes in the weekly and daily supplementation groups were 6.6 ± 2.0 (range: 0–11; $n = 44$) and 7.5 ± 4.9 (range: 1–15; $n = 14$), respectively, and thus overlapped.

TABLE 3
Regression model of hemoglobin concentration at 4 wk as a function of initial hemoglobin concentration, number of iron tablets taken during the first 3 wk, supplementation group, and the interaction between iron tablet intake and supplementation group in the subset of women with an initial hemoglobin concentration < 115 g/L who took ≤ 15 tablets[1]

Covariate	β ± SEE	*P*
Initial hemoglobin	0.62 ± 0.13	0.01
Number of tablets	1.19 ± 0.48	0.02
Group[2]	6.80 ± 5.88	0.25
Number of tablets × group	−0.47 ± 0.75	0.54
Constant	97.25 ± 4.26	0.01

[1] $n = 58$. $R^2 = 0.4$.
[2] Daily supplementation group coded as 0, and weekly supplementation group coded as 1.

Using ordinary least-squares regression analysis, we tested for a differential dose effect by modeling hemoglobin concentration at 4 wk as a function of initial hemoglobin concentration, number of iron tablets taken during the first 3 wk, supplementation group, and the interaction between supplementation group and number of iron tablets. The regression model indicated that there was no significant difference in response ($P = 0.54$) between weekly and daily dose frequencies and that each of the first 15 tablets taken increased the hemoglobin concentration by 1.2 g/L ($P < 0.05$) (**Table 3**). Iron supplements taken weekly did not have a greater efficacy in increasing hemoglobin concentrations than did those taken daily, and the effect per tablet was the same regardless of dose frequency. Thus, there was no evidence for a mucosal block due to daily dose frequency.

The analysis was tested for possible confounding effects of maternal age, parity, fundal height, SES, and initial hemoglobin concentration as described in Subjects and Methods. In the subset used for this analysis, initial hemoglobin concentration and fundal height were associated with hemoglobin concentration at 4 wk, but because they were not associated with supplement intake, they were not confounding factors in the analysis.

Predicted efficacy of daily and weekly regimens after 12 wk of supplementation

This analysis was used to predict the efficacy of supplementation regimens, ie, the maximal effect on hemoglobin concentration that could have been achieved over 12 wk of supplementation if compliance had been 100%. The subset of women with a low initial hemoglobin concentration was also selected for this analysis.

Hemoglobin concentrations at weeks 4, 8, and 12 were plotted as functions of the number of tablets taken at weeks 3, 7, and 11, respectively (**Figure 3**). A dose effect was shown not only at week 4 but also at weeks 8 and 12. The 3 lines indicating responses after 4, 8, and 12 wk of supplementation were superimposed, implying that the major determinant of dose effect was the number of tablets taken and not the duration of supplementation. The graph suggested that at 12 wk a plateau was reached at ≈40 tablets. To verify the tablet intake required for the maximal effect that could be achieved with iron supplements, the shape of the curve, a steep initial response that ceased at ≈40 tablets, was tested. A categorical variable that divided the women into those who had taken < 40 tablets ($n = 56$) at week 11 and those who had taken ≥ 40 tablets ($n = 34$) was constructed. With the use of an

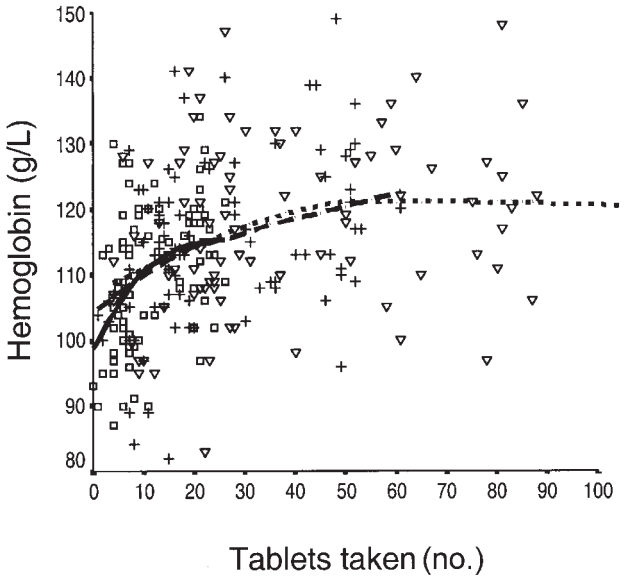

FIGURE 3. Hemoglobin concentrations at weeks 4 (——; □), 8 (– –; +), and 12 (- - - -; ▽) plotted against the number of iron tablets taken at weeks 3, 7, and 11, respectively, in a subset of women with an initial hemoglobin concentration < 115 g/L. The data for the weekly and daily supplementation groups were combined. The lines through the data points are lowess moving-average fitted lines.

ordinary least-squares regression model, the dose effect of the first 39 tablets taken up to week 11was compared with the dose effect of ≥ 40 tablets. The hemoglobin concentration at 12 wk was modeled as a function of initial hemoglobin concentration, total number of tablets taken during the first 11 wk, the 40-tablet categorical variable, and the interaction between number of tablets and tablet category (**Table 4**). An interaction was found ($P < 0.05$), showing a differential response between the first 39 tablets and higher tablet intakes. A dose effect was shown for the first 39 tablets (0.34 g/L per tablet; $P = 0.083$) although such a relation was not present for additional tablets taken, implying that there was no further hemoglobin response when > 39 tablets were taken.

The analysis was tested for potential confounding. Fundal height was not associated with hemoglobin concentration at 12 wk. Apart from the initial hemoglobin concentration already in the model, age, parity, and SES were identified as potentially confounding factors because of their association with hemoglobin concentration at 12 wk. Of these variables, age was associated with tablets ingested and could act as a confounding factor. Introducing age into the model changed the estimated effect and differential response by < 10%, and age was not retained in the model.

With an assumption of 100% compliance, the predicted hemoglobin responses of weekly and daily supplementation over 12 wk were estimated from the dose-effect curve (Figure 3). At 11 wk, if women in the daily supplementation group had taken the 77 tablets prescribed during that period, then their hemoglobin concentration at week 12 would have been 121 g/L. Women in the weekly regimen should have taken 22 tablets during the first 11 wk, which would correspond to a hemoglobin concentration at week 12 of 115 g/L. The estimated increment in hemoglobin concentration would be 11.7 g/L with the weekly regimen and 17.1 g/L with the daily regimen. The predicted efficacy with the weekly regimen was thus 68% of that with the daily regimen although the weekly regimen provided only 28% of the amount of iron provided by the daily regimen. This indicated that for regimens that were 12 wk long and had 100% compliance, a daily supplementation regimen would be more efficacious.

TABLE 4
Regression model of hemoglobin concentration at 12 wk as a function of initial hemoglobin concentration, number of iron tablets taken during the first 11 wk, tablet category (<40 or ≥40 tablets), and the interaction between number of iron tablets and tablet catagory in the subset of women with an initial hemoglobin concentration < 115 g/L[1]

Covariate	β ± SEE	*P*
Initial hemoglobin[2]	0.80 ± 0.16	0.001
Number of tablets	0.345 ± 0.20	0.083
Tablet category[3]	21.37 ± 9.70	0.03
Number of tablets × tablet category	−0.470 ± 0.23	0.047
Constant	108.5 ± 4.44	0.001

[1] $n = 90$. $R^2 = 0.31$.
[2] Value centered at the mean.
[3] Category codes: <40 tablets = 0; ≥40 tablets = 1.

DISCUSSION

Validity

The greater loss to follow-up of women with a low initial hemoglobin concentration in the weekly supplementation group than in the daily supplementation group may have led to both an underestimation and an overestimation of the trial effectiveness of the weekly regimen. An underestimation may have occurred because the increment in hemoglobin concentration may have been lower because of the loss of more responsive women. On the other hand, an overestimation may have occurred because hemoglobin concentrations may have been higher because of the loss of women with lower concentrations. However, because there was no significant difference in initial hemoglobin concentration between the regimens, it is unlikely that this difference biased the results to a significant degree.

All analyses were tested for potentially confounding effects of maternal age, parity, initial hemoglobin concentration, fundal height, and SES. Of particular concern were the use of tablet intake as an independent measure and the potential confounding of gestational age. However, there was no association between fundal height and supplementation group or tablet intake. As described in Subjects and Methods, the procedures to control for confounding were rigorous. The results from the analyses with tablet intake as an independent variable were robust, and the estimated effects were not affected by confounding factors. It is thus unlikely that the results were influenced by confounding of any practical importance.

Efficacy and trial effectiveness of supplementation regimens

During 12 wk of supplementation, the women in the weekly supplementation group were prescribed 28% of the amount of iron prescribed to the women in the daily supplementation group. However, in the case of full compliance, the predicted effect on the increment in hemoglobin concentration with the weekly regimen was 68% of that with the daily regimen. This was explained by the lack of an additional hemoglobin response after the intake

of ≈40 tablets, and consequently almost one-half of the tablets in the daily regimen would not produce any effect. On the basis of the regression model showing a maximal effect at 40 tablets (Table 4) and on the complete superposition of the dose-effect curves over time (Figure 3), it is reasonable to believe that the efficacy of the weekly regimen would have approached that of the daily regimen in regimens longer than 12 wk. The advantage of the daily regimen appears to be the shorter time required for a maximal effect. It is difficult to judge the public health importance of the observed difference in effectiveness. A more easily interpreted and probably more biologically relevant comparison is that involving the prevalence of anemia. Assuming that anemia of the observed degree (mild to moderate) is associated with a negative pregnancy outcome, there appears to be an advantage in using a daily regimen because it resulted in fewer anemic women at the end of the trial (low initial hemoglobin subset) and treated anemia faster and thus earlier in pregnancy. Epidemiologic studies provided evidence that anemia in early pregnancy, rather than in late pregnancy, is associated with a poor pregnancy outcome (21–24), suggesting that anemia should be controlled early in pregnancy.

Several other trials compared weekly and daily iron supplementation of pregnant women. Although these trials may appear to have been similar in design, they were conceptually different and answered different research questions, making comparisons difficult. Studies done in Pakistan (25), Indonesia (26), and Malawi (27) evaluated the trial effectiveness of supplementation regimens because there were no valid measures of compliance. Intention-to-treat analyses were performed without clarifying whether the lack of difference in trial effectiveness (26, 27) was because of limitations in efficacy or compliance or both. In a trial in China (28), tablet intake was supervised, and thus the efficacy of supplementation regimens was evaluated.

Efficacy of dose frequencies

A comparison of the efficacy per tablet administered during weekly and daily regimens was performed in a subset of women with similar tablet intakes. In this subset, women in the daily supplementation group had a higher average tablet intake than did those in the weekly supplementation group. The estimation of the effect per tablet in the 2 groups was thus based on different mean numbers of tablets taken. It is expected that the efficacy per iron tablet decreases with the number of tablets taken because absorption decreases with improved hematologic status. This bias may have decreased the estimated efficacy of daily supplementation. Despite this bias in favor of a weekly dose frequency, efficacy was not higher in the weekly supplementation group than in the daily supplementation group. The conclusion that there is no improved efficacy with a weekly dose frequency is in agreement with clinical trials using radiolabeled iron (4, 5). Although these trials showed a difference, it was judged to be too small to be of biological importance (4).

Amount of iron required

Over the 12 wk of supplementation, 2400 mg Fe (40 tablets × 60 mg) was sufficient to produce a maximal hemoglobin response. This amount is ≈50% of the amount recommended by the World Health Organization for the same period. Results of an iron supplementation study in pregnant women in Tanzania indicated a maximal response with a similar amount of iron (29). Compliance with daily doses of 120 mg Fe was monitored for 1 mo with MEMS, and hemoglobin concentrations were assessed after 12 wk. Approximately 50% compliance was apparently sufficient to reach the maximal effect. With our recent understanding that most of the effect occurs during the first month of supplementation, the estimated 50% compliance for a maximal response can be reinterpreted as 50% of the first month's amount of iron. This corresponds to 1800 mg Fe (50% × 30 d × 120 mg Fe) and is similar to the 2400 mg estimated to be required in the Bangladeshi population.

The current recommendation by the World Health Organization is 60 mg Fe daily during 6 mo of pregnancy (30), which equals ≈10 800 mg Fe. On the basis of the complete superposition of the dose-effect curves of tablets taken during the course of gestation, it is reasonable to assume that only a small additional amount of iron is required to maintain the maximal potential hemoglobin concentration once it has been reached. The estimated requirements of 2400 mg in Bangladesh and 1800 mg in Tanzania may thus be sufficient for the full length of pregnancy. Thus, the amount of iron apparently required is 22% and 17%, respectively, of the current World Health Organization recommendation.

Program implications and future research needs

Our results indicate that the current international recommendation on iron supplementation is inappropriately high. Because of the potential negative effects of iron amounts that are larger than necessary, there is an urgent need for further research establishing what is required, particularly in populations where parasite infestation aggravates the severity of iron deficiency.

We suggest that the focus should be on estimating the total amount of iron required during pregnancy, and a supplementation strategy should be developed to deliver this amount. However, because the expected positive effect may occur early in pregnancy, a strategy with an initially larger (daily?) dose of iron to correct an existing deficit seems biologically justifiable, although a lower (weekly?) dose may be needed to maintain the achieved hemoglobin concentration. The initial dose should be set as high as possible without producing side effects that may compromise compliance. The duration of the supplementation regimen needs to be adjusted accordingly to provide the required amount of iron. Regardless of the amount of iron that will be recommended finally, limited compliance is likely to be an issue and needs to be addressed in research.

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