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Effect of daily vs twice weekly iron supplementation in Indonesian preschool children with low iron status[1–3]

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ABSTRACT The effect of daily vs twice weekly iron supplementation on iron status was studied in preschool children with low iron status in a randomized double-masked field trial. Subjects ($n = 87$) were selected on the basis of their hemoglobin concentration being < 111 g/L in finger-prick blood, and were divided into two groups. For 8 wk one group received a daily supplement of 30 mg Fe, while the other group received 30 mg Fe twice per week. A complete data set was obtained from 32 children in the group supplemented daily and from 33 children in the group supplemented twice weekly. Hemoglobin, serum ferritin, and protoporphyrin increased significantly in both groups ($P < 0.001$). Changes in hemoglobin and protoporphyrin were correlated with initial hemoglobin concentration ($P < 0.001$). The difference in treatment effect between groups was not significant after correction for the initial hemoglobin concentration. It is concluded that in preschool children with low iron status, twice weekly iron supplementation has an effect on iron status similar to that of daily supplementation. *Am J Clin Nutr* 1995;61:111–5

KEY WORDS Anemia, iron, weekly supplementation, children

Introduction

Iron-deficiency anemia is still a major nutritional and public health problem in developing countries. As opposed to other micronutrient deficiencies such as vitamin A deficiency and iodine deficiency, limited progress has been made in solving the problem during the past decade (1). The prevalence of iron-deficiency anemia among pregnant women and preschool children is particularly high. In Southeast Asia the prevalence among these population groups is estimated to be between 50% and 70% (1, 2). The consequences of iron deficiency are serious and range from reduced work capacity (3) and poor pregnancy outcome in adults (4, 5) to reduced school performance (6, 7), decreased growth rate, and impaired motor development in children (8, 9).

Many countries, such as Indonesia, distribute therapeutic iron supplements through their primary health care system to reduce the prevalence of anemia among pregnant women (10). Generally, similar programs do not exist for preschool children, who only receive iron supplements on the basis of diagnosed anemia (10). However, the efficacy of such interventions is often weak due to factors such as poor efficiency of health services, lack of compliance of the target group, and poor quality of supplement tablets (10–12). The lack of compliance of the target group may reduce the efficacy, even when the logistical side of a program is well organized. Compliance is influenced by undesirable side effects of the iron supplements, which are related to the amount and form of the supplemented iron (13).

Supplementation programs for other micronutrients, such as vitamin A, are more successful, partially because there are few side effects and, unlike iron, daily supplementation is not necessary. However, recently it has been reported that in anemic rats the administration of an iron supplement every third day was as effective in improving the iron status as was a daily supplement (14). The theoretical explanation would be that daily supplementation of iron leads to a strongly reduced absorption several days after initiation, whereas with intermittent supplementation the level of absorption would remain much higher. If this would hold true for humans it would have important implications for the organization and efficacy of iron-supplementation programs.

The aim of this study was to investigate whether twice weekly iron supplementation would improve iron status of anemic human subjects as effectively as daily supplementation. Preschool children were chosen as subjects to avoid the confounding influence of the state of pregnancy. Furthermore, it was considered ethically questionable to take pregnant women out of an established daily supplementation program as long as the effect of twice weekly supplementation was uncertain.

Subjects and methods

The study was carried out from September to December 1993 in the subdistrict Kelurahan Tenga of East Jakarta, in close cooperation with the local health center. The total population of the study area was ≈24 700, with a population density of 12 200 people/km^2.

[1] From Deutsche Gesellschaft fuer Technische Zusammenarbeit, GTZ GmbH, Eschborn, Germany; the Institute for Research and Development in Nutrition, Bogor, Indonesia; and the Regional South East Asian Ministries of Education Organization Center for Community Nutrition at the University of Indonesia, Jakarta, Indonesia.

[2] Carried out in the framework of the German-Indonesian intergovernmental technical cooperation (PN 88.2534.01–100).

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Received March 29, 1994.
Accepted for publication August 29, 1994.

Subjects

Subjects were children aged 2–5 y. Sample size calculations indicated that ≥72 subjects were required to be able to distinguish a difference in hemoglobin of 10 g/L between groups with a 5% significance level and a power of 0.90. The initial selection criterion was a hemoglobin concentration <110 g/L. Because it is difficult to take blood from preschool children, for screening purposes hemoglobin was determined in finger-prick blood. With the help of the health center staff the preschool children aged >2 y were gathered in the health center: 308 children were measured and 80 had a hemoglobin concentration <110 g/L. Because there were no more children available in the area to be screened, 16 children with hemoglobin concentrations of either 110 or 111 g/L were included in the study to compensate for an expected 25% drop-out rate. These 96 children were invited to receive an effective anthelmintic treatment (15) before starting iron supplementation, so as to remove helminthiasis as a possible confounder of hematological status. All children were given anthelmintic treatment because previous research (9) in a similar environment in Jakarta among preschool children indicated that worm infection was highly prevalent (≈65% ascariasis and/or trichuriasis). However, the parents of only 87 children gave their consent for participation in the study.

The research proposal was approved by the Ethical Review Committee of the SEAMEO-TROPMED Center at the University of Indonesia, Jakarta.

Methods

These 87 subjects were assigned at random to two groups. At the start of the study each child received two small bottles (each 80 mL) and two large bottles (each 170 mL), each containing a syrup of similar appearance. The parents and supervising health staff were instructed that each child should take 5 mL from the small bottle on Mondays and Fridays and 5 mL from the large bottle on the remaining days of the week using a standardized spoon for 8 wk. In one group ($n = 44$) both bottles contained glucose syrup (with citric acid a preservative, and strawberry flavoring) in which ferrous sulfate was dissolved (5 mL syrup containing 30 mg elemental Fe). In the other group ($n = 43$) only the small bottle contained ferrous sulfate whereas the large bottle contained only the glucose syrup as a placebo. The design enabled a double-blind supplementation in which one group received a daily supplement of 30 mg elemental Fe whereas the other group received the iron supplement only twice per week. Daily syrup ingestion by subjects took place at home and was supervised by district health workers and one of the researchers (MG).

At the start and finish of the 2-mo supplementation period a 2-mL blood sample was drawn via venipuncture from each subject. Hemoglobin was determined in duplicate by the cyanomethemoglobin method (16) by using a Compur Minilab (Bayer Diagnostic GmbH, München, Germany). Variability based on duplicate measurements was 1.1 g/L (SD). Serum ferritin was determined by enzyme immunoassay with a commercial kit (Boehringer-Mannheim, Mannheim, Germany) (16). Variability was 2.3 μg/L (SD) based on duplicate measurements carried out in 10% of the samples. Erythrocyte protoporphyrin was assessed by measuring zinc protoporphyrin (ZPP) with a hematofluorometer (16) (Protofluor Z, Helena Laboratories, Beaumont, TX). Variability based on duplicate measurements was 3.2 μmol ZPP/mol heme (SD). Anthropometric measurements were carried out at the start and finish of the study. Body weight was measured to the nearest 0.1 kg with an electronic weighing scale (SECA 770 alpha; SECA, Hamburg, Germany). Body height was measured to the nearest 0.1 cm by using a microtoise. *Z* scores of the indicators weight-for-age, height-for-age, and weight-for-height were calculated by using the National Center for Health Statistics reference data (17).

Statistical analysis

Data analysis was carried out by using the multivariate analysis of variance (MANOVA) repeated-measures design of *SPSS/PC+ 4.0* (SPSS Inc, Chicago) (18), with the two treatment types (daily vs twice weekly supplementation) as a between-subjects factor and treatment effect (before vs after supplementation) as a within-subject factor. Additionally, the initial hemoglobin concentration (low, medium, or high) was added as a second between-subjects factor to correct for its confounding influence. Serum ferritin and protoporphyrin were analyzed by a natural logarithm transformation of the values, because the original values were not normally distributed.

Results

A complete set of data was obtained for 33 subjects in the group supplemented twice weekly (group 1) and for 32 subjects in the group supplemented daily (group 2) (**Table 1**). The children who dropped out had average weights, ages, and initial hemoglobin concentrations similar to those of the children who completed the supplementation period. The reason for dropout was that the children refused to take the supplements during a part of the supplementation period. No information was obtained about why the children refused to take the supplements. In the final data set no significant differences existed between the groups in sex distribution, weight, height, age, and anthropometric indexes (Table 1).

The initial selection of subjects was based on hemoglobin determination in finger-prick blood. Although hemoglobin concentrations determined in finger-prick blood and in venous blood were positively correlated ($r = 0.75$, $P = 0.001$), mean hemoglobin from finger-prick blood at the time of screening was 3 ± 7 g/L ($P = 0.01$) lower than the mean hemoglobin determined in venous blood at the start of the supplementation period. Therefore, 15 subjects who had hemoglobin concentra-

TABLE 1

Selected characteristics of subjects at the beginning of the study[1]

	Group 1 (n = 15 M, 18 F)	Group 2 (n = 14 M, 18 F)
Weight (kg)	11.8 ± 1.6	11.9 ± 1.8
Height (cm)	88.7 ± 7.0	89.4 ± 7.6
Age (m)	41.9 ± 11.7	40.2 ± 10.7
Weight-for-age (SD score)	−2.07 ± 0.75	−1.88 ± 0.82
Height-for-age (SD score)	−2.33 ± 1.06	−1.85 ± 1.10[2]
Weight-for-height (SD score)	−0.92 ± 0.73	−0.93 ± 0.65

[1] $\bar{x}$ ± SD. Group 1 was supplemented twice weekly and group 2 daily with 30 mg elemental Fe.

[2] Difference between two groups: $P = 0.09$ (NS).

tions <110 g/L in finger-prick blood were not classified as anemic based on the hemoglobin concentration in venous blood. These subjects were retained in the study and the possible confounding effects of the initial hemoglobin concentration were adjusted for in the analysis of variance. At the start of the supplementation period, there were no significant differences between groups 1 and 2 in mean venous blood concentrations of hemoglobin ($P = 0.103$), serum ferritin ($P = 0.70$), or ZPP ($P = 0.52$) (**Table 2**). Both the daily supplementation and the twice weekly supplementation led to a significant increase in hemoglobin and serum ferritin and to a decrease in ZPP concentration within the respective groups ($P = 0.001$ for all).

To evaluate the differences in treatment effect between the two groups, the initial hemoglobin concentration had to be taken into consideration because it was correlated with the changes in hemoglobin ($n = 65$; $r = -0.67$, $P = 0.001$) and ZPP ($r = 0.55$, $P = 0.001$). By chance, more subjects with low initial hemoglobin concentrations were allocated to the group supplemented daily than to the group supplemented twice weekly. Because the initial hemoglobin concentration could be a confounding factor in analyzing the difference in treatment effects between groups, the initial hemoglobin concentration was included as a factor in the analysis of variance. For this purpose the initial hemoglobin concentration was divided into three classes: <100 g/L ($n = 22$), 100–110 g/L ($n = 18$), and ≥110 g/L ($n = 25$).

After correction for the initial hemoglobin concentration by including it in the analysis of variance, the differences in treatment effects between groups (tested by the interaction between treatment effect and type) for hemoglobin ($P = 0.21$), serum ferritin ($P = 0.15$), and ZPP ($P = 0.13$) were not statistically significant (**Table 3**). This finding indicates that the effects of the daily vs the twice weekly dose were similar. The iron dose per kg body wt which the children received varied between 4 and 2.2 mg. The possible influence of iron dose per kg body wt was checked by entering the dose as a covariant in the analysis of variance. The dose had no effect ($P = 0.59$) and there was no interaction between dose and treatment type.

Figure 1 shows the changes in hemoglobin according to the three initial hemoglobin concentrations. Children with an initial hemoglobin concentration <110 g/L showed biologically significant increases in hemoglobin of 12 ± 9 g/L ($n = 17$, $P < 0.001$) in the group supplemented twice weekly and of 17 ± 11 g/L ($n = 23$, $P < 0.001$) in the group supplemented daily. The difference of 5 g/L between these subgroups was not statistically significant ($P = 0.11$). In this group of 40 children with an initial hemoglobin concentration <110 g/L, it would have been possible to detect a significant difference of 8 g/L with a P value of 0.05 and a power of 0.8.

The proportion of children who went from being anemic (hemoglobin < 110 g/L) to nonanemic during the supplementation trial was 65% in the group supplemented twice weekly and 83% in the group supplemented daily ($\chi^2 = 1.67$, $P = 0.196$).

TABLE 3
Results of analysis of variance: hematological indexes, by treatment effect, treatment type, and initial hemoglobin concentration

Independent factors	Dependent factors (P values)		
	Hemoglobin	Ferritin	Zinc protoporphyrin
Within subjects			
Treatment effect (time)	0.00	0.00	0.00
Between subjects			
Treatment type (daily vs twice weekly)	0.06	0.23	0.08
Hemoglobin class (three classes)	0.00	0.01	0.00
Interactions			
Treatment type × hemoglobin class	0.60	0.84	0.53
Treatment effect × type	0.21	0.15	0.13
Treatment effect × hemoglobin class	0.00	0.36	0.00
Treatment effect × type × hemoglobin class	0.65	0.39	0.06

Discussion

In the present study the hematological status of both the group supplemented daily and the group supplemented twice weekly improved significantly. Generally, the increases we observed in hemoglobin and serum ferritin were similar to the increases in anemic preschool Indonesian children who received a daily supplement of 50 (19) or 30 mg (7) for 2 mo. The general deworming treatment that all children received before the supplementation was started probably did not influence the hematological values in an important way because in a previous study carried out in a similar environment among Jakarta preschool children, very small nonsignificant changes in hemoglobin and serum ferritin occurred as a result of deworming alone (9).

At first sight, daily supplementation seemed to be more efficient than twice weekly supplementation because the in-

TABLE 2
Hematological values at the beginning and end of the supplementation period based on venous blood samples[1]

	Group 1 ($n = 33$)			Group 2 ($n = 32$)		
	Hemoglobin	Ferritin	Zinc protoporphyrin	Hemoglobin	Ferritin	Zinc protoporphyrin
	g/L	*μg/L*	*μmol/mol heme*	*g/L*	*μg/L*	*μmol/mol heme*
Start	108 ± 11	16.9 ± 13.2[11.5]	98 ± 74 [82.7]	104 ± 11	15.4 ± 12.2[10.8]	110 ± 87(88.9)
Finish	114 ± 10	34.9 ± 28.7[25.3]	79 ± 60 [67.7]	117 ± 8	41.2 ± 30.0[31.2]	67 ± 34(61.5)
Difference	7 ± 10[2]	18.0 ± 20.5[13.8][2]	−19 ± 24.6 [15.0][2]	13 ± 12[2,3]	25.8 ± 23.3[20.4][2,3]	−43.0 ± 58(27.4)[2,3]

[1] Arithmetic $\bar{x}$ ± SD; geometrical means in brackets.
[2] Significant within-group change, $P < 0.001$.
[3] There was no significant treatment effect × type interaction after correction for initial hemoglobin concentration (*see* Table 3).

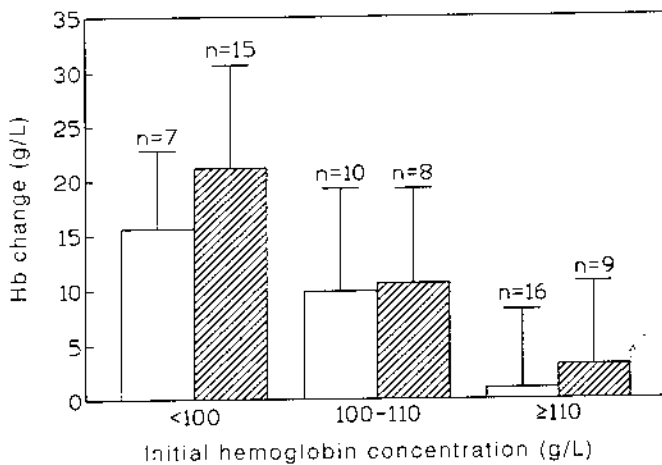

FIG 1. Changes in hemoglobin (Hb) in the two treatment groups according to three initial Hb concentrations. □, Group supplemented twice weekly with 30 mg elemental Fe ($n = 33$); ■, group supplemented daily with 30 mg elemental Fe ($n = 32$).

crease in hemoglobin and the decrease in ZPP appeared to be larger in the group supplemented daily than in the group supplemented twice weekly. However, iron absorption from supplements may depend on physiological needs (20), and the changes in hemoglobin and ZPP caused by the supplementation were related to the initial hemoglobin concentration. After correction for the confounding influence of the initial hemoglobin concentration, no statistically significant difference in treatment effect existed between groups. Furthermore, the final hemoglobin concentrations in both groups were roughly equivalent. Because we enrolled children on the basis of a hemoglobin deficit, we would expect the children to be at the low end of the distribution for that population, even with adequate iron status (21). It is not probable that the hemoglobin concentration after 8 wk of supplementation would have reached values much higher than the ≈115 g/L average observed. Therefore, the result indicates that in iron-deficient children a twice weekly supplementation of 30 mg Fe did not have a significantly different effect on the improvement of the iron status from a daily supplementation of 30 mg during a period of 2 mo.

A study carried out in anemic rats reported that an iron supplement given every third day resulted in an iron status comparable with that after daily supplementation (14). The results of our study support the observation that with twice weekly dosing the level of absorption remains high whereas with daily dosing the level of absorption decreases rapidly after a few days. Assuming that young children have ≈75 mL blood volume/kg body wt and that 1 g hemoglobin has 3.4 mg Fe (22), the following calculation was made. The amount of absorbed iron to account just for the increase in hemoglobin in the blood circulation was 4.4% in the group supplemented twice weekly and 2.3% in the group supplemented daily. For children with an initial hemoglobin concentration <110 g/L, the absorbed rates were 7.9% and 2.9%, respectively.

Iron supplementation in the form of tablets is still the preferred intervention in anemic individuals (23). In many developing countries, where the prevalence of anemia among pregnant women and preschool children is especially high, iron supplements are distributed through the primary health care system (23). However, sustained efficacy is uncommon due to factors such as irregular tablet distribution and poor compliance (10–12). A less-frequent supplementation schedule may have positive effects on programs. First, compliance is partially influenced by the side effects, which are dose-related (13). It is likely that if supplements are to be taken twice per week, or perhaps even less frequently, compliance would be better. Second, the cost involved in supplementation programs would be reduced if supplements could be taken less frequently. Another positive effect of less-frequent supplementation would be that the adverse interactive effect of large amounts of iron on the absorption of other micronutrients, such as zinc, would be reduced (24). Therefore even if the absolute increase in hematological indexes in compliant individuals was slightly higher with daily supplementation, the positive effects in public health terms at the population level may be larger with less-frequent supplementation.

On the basis of the results of this study the following recommendations for further research can be made. Supplementation at less-frequent intervals, such as once per week, should be investigated. The efficacy of intermittent supplementation in other anemic subjects, such as pregnant women, should also be studied. Furthermore, the optimal amount and the type of supplement should be investigated. The current recommendation for pregnant women who are anemic or at risk is 120 mg Fe/d (23). With biweekly supplementation of a different dose, or another type of iron supplement, such as through a gastric delivery system (25), might be better. The efficiency of supplementation depends also on helminthic infection of individuals. The necessity of combining iron supplements with anthelmintic drugs needs to be determined on the basis of the type and severity of worm infection in a given geographical area. Finally, the use of intermittent supplementation would need to be investigated in applied programs in terms of general efficacy, subject compliance, supplement distribution, and cooperation of health personnel.

We gratefully acknowledge the advice of MA Husaini during the proposal development and the support of R Shrimpton, and appreciate the valuable comments of N Solomons.

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