Milk fortified with iron or iron supplementation to improve nutritional status of pregnant women: An intervention trial from rural Vietnam

P. Thuy Hoa, Nguyen Cong Khan, Christine van Beusekom, Rainer Gross, Wolney L. Conde, and Ha Dui Khoi

Abstract

Anemia is still the major nutritional problem among pregnant women in Southeast Asia. The objective of this study was to measure hemoglobin status and reduction of underweight in a group of pregnant women who received iron-fortified or nonfortified milk, and another group who received iron supplements (tablets) or placebo. The 44 women in the iron-fortified milk group received 15 mg of iron per day per 400 ml of milk, and 41 women received placebo. The 40 women in the iron supplement group received 60 mg of iron per day, and 43 women received nonfortified milk. During this intervention trial, all women were supervised from the 14th to the 18th week of gestation until delivery. Blood was sampled at 0, 5, 10, and 16 weeks of intervention. After the 16th week of intervention, the changes in hemoglobin (ΔHb) concentrations in both treatment groups (the iron-fortified milk and the iron tablet groups) were not significantly different (ΔHb: −0.5 ± 0.9 and −0.3 ± 0.9 g/L, respectively), but the changes were significantly greater in the nonfortified milk and placebo groups (ΔHb: −1.2 ± 0.9 and −1.1 ± 0.8 g/L, respectively; $p < .01$). The change in transferrin saturation (ΔTS) in the iron-fortified milk group (ΔTS: 3.4 ± 12.9%) was greater than that in the placebo and nonfortified milk groups (ΔTS: −10.1 ± 9.8% and −11.6 ± 10.7 %, respectively) ($p < .01$). The weight gain of the subjects during intervention did not differ significantly in the fortified and nonfortified milk groups (Δweight: 5.0 ± 2.0 and 5.8 ± 2.1 kg, respectively), but was higher than in the iron tablet group (Δweight: 4.6 ± 3.1 kg; $p < .05$) and the placebo group (Δweight: 3.8 ± 2.5 kg; $p < .001$). Iron supplementation and fortification were seen to be effective in promoting weight gain in pregnant Vietnamese women. For women who are underweight, the administration of iron-fortified milk has additional benefits to those of supplementation, most likely due to additional energy and nutrient inputs.

Key words: Anemia, efficacy, iron-fortified milk, iron supplementation, pregnancy, weight gain

Introduction

Worldwide, anemia affects more than two billion people [1]. Pregnant women are at special risk, and the prevalence of anemia in this vulnerable group in Southeast Asia has been reported to be as high as 60% to 70% [1]. In Vietnam, the prevalence of anemia among pregnant women was reported to be 52.7%, with iron deficiency being the major cause [2]. As a result, the risk of reproductive failures such as miscarriage, stillbirths, premature birth, low birth weight, and maternal mortality is increased [3]. Many countries implement iron-deficiency control programs to increase the iron intake during pregnancy by distributing iron tablets. However, despite these efforts, no further reduction of anemia can be observed [1]. In Vietnam, iron supplementation for pregnant women was introduced in selected districts in 1991 and has been slowly expanded to most parts of the country. Nevertheless, compliance is still low because of factors such as poor motivation of the health staff and the pregnant women, the poor taste of the tablets, and negative side effects [4].

Iron supplementation is regarded as a short-term intervention [1]. In addition to supplementation, fortification and changes in food consumption are seen as medium-and long-term solutions for the control of

P. Thuy Hoa, Nguyen Cong Khan, Christine van Beusekom, Wolney L. Conde, and Ha Dui Khoi are affiliated with the National Institute of Nutrition, Ministry of Health, Hanoi, Vietnam, the Nutrition Research Institute of Friesland Dairy Foods, Leeuwarden, The Netherlands, the Department of Nutrition, Faculty of Public Health, University of São Paulo, São Paulo, Brazil, and the Deutsche Gesellschaft für Technische Zusammenarbeit, Eschborn, Germany. Rainer Gross is affiliated with the Nutrition Section, Programme Division, UNICEF, New York.

Please direct queries to the corresponding author: P.T. Hoa, 48 Tang Bat Ho Street, National Institute of Nutrition, Hanoi, Vietnam; e-mail: thuyhoanin@yahoo.com

iron-deficiency anemia. In Vietnam, many pregnant women suffer not only from micronutrient deficiencies but also from low energy and protein intake [5, 6]. Despite this, at the time of the study there was no existing iron supplementation or fortification program at the national level. As a result, the question arose as to whether fortification of food with iron reduces anemia and provides other benefits, in addition to those of iron supplementation, to the undernourished mother and her newborn during and after pregnancy. A study was carried out to compare the efficacy of two approaches to enhancing the iron nutritional status of pregnant women: iron supplementation with tablets (the traditional approach) and the use of iron-fortified cow's milk (the novel approach).

Subjects and methods

Population

The study was conducted between 1996 and 1997 in 12 communes in Dong Hung District, Thai Binh Province, in the rural delta area of the Red River in northern Vietnam. Agriculture is the main occupation of the adult household members of the study population. The criteria for the selection of this area for study were homogeneity of socioeconomic and ecological conditions, acceptance by the community, and the absence of an existing iron-supplementation program. In the health center of each commune operated by the national Ministry of Health, women between the 14th and 18th weeks of pregnancy were asked whether they were willing to participate in the study. The eligibility criteria were age 20 to 32 years; no more than two prior pregnancies; no stillbirths, premature births, or hemorrhage in previous pregnancies; no manifestations of chronic or infectious diseases, including hookworm infection; hemoglobin (Hb) > 70 g/L; and no planned travel or plans to move out of the area during the study period. Mothers who did not sign an informed consent did not participate in the study. At the end of the recruitment process, a total of 202 women were chosen to participate in the study.

Ethical considerations

The data collected have been used for study purposes only. The women were informed about the purpose of the study and the research institution before agreeing to participate. Assurance was given that cooperation was voluntary. The ethical committee of the National Institute of Nutrition approved the research protocol.

Study design

The study had four intervention groups. For practical reasons, it was possible to implement only one type of intervention per commune (block randomly adjusted). Each group started with 44 subjects, based on a between-group difference in hemoglobin of 5 g/L, with a significance level of $p = .05$, a power of 0.9, and a dropout rate of 20%. Participants were randomly assigned to treatment and control groups.

The women in the four groups received daily interventions. Group 1 received 400 ml of milk fortified with iron (IM); group 2 received the same volume of nonfortified milk (M); group 3 received one daily iron-folic acid supplement in pill form (IS); and group 4 received one placebo tablet (P).

Table 1 shows the contribution of energy and selected nutrients from the milk or tablets. Group 1 received 15 mg of iron daily as ferrous fumarate from the fortified milk. All milk powder (iron-fortified or nonfortified) was also enriched with vitamin C and folic acid. The iron-fortified and nonfortified milk had the same white color and identical smell and flavor. Both types of milk powder were specially produced and packaged for the study (Friesland Dairy Foods Company, Leeuwarden, Netherlands). Group 3 received a tablet daily containing 200 mg of ferrous sulfate (60 mg of elemental iron) and 250 μg of folic acid according to World Health Organization (WHO) recommendations [7]. The iron supplement and the placebo tablet had the same red color and shape. The two treatments could not be distinguished by sight. The tablets were provided by UNICEF and produced by Weiders Farmas Rytiske A/S, Norway. The pharmaceutical factory No II "Dopharma" of the Ministry of Health, Hanoi, Vietnam, produced

TABLE 1. Daily nutritional contribution according to the type of intervention in the four groups

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Contribution	With iron	Without iron	Iron	Placebo
Energy (kcal)	120	120	0	0
Protein (g)	6.8	6.8	0	0
Elemental iron (mg)	15	0	60	0
Folic acid (μg)	200	200	250	0
Vitamin C (mg)	17.5	17.5	0	0

the placebo for the single blind study.

Since Vietnamese women rarely consume cow's milk, it was necessary to start the study with an adaptation phase of one week. During this week, 101 subjects received gradually increasing amounts of milk: 100 ml for the first 2 days, 200 ml on the 3rd day, 300 ml on the 4th day, and 400 ml at on the 5th and 6th days. Non-milk-drinking Southeast Asian communities suffer widely from lactose intolerance [8, 9]. The daily gradual increase of milk was intended to help achieve cultural acceptance; however, for practical reasons it was not possible to plan a long enough exposure to realize a biological effect, i.e., a shift in the flora of the colon. Fortunately, most women did not have symptoms even when given the full amount of milk from the beginning.

Study organization

Each survey team consisted of four study workers who were trained before the beginning of the study. The task of each study worker was to prepare and distribute the milk or tablets, to interview the women, and to motivate them to take the distributed commodities regularly. Each survey team was appointed to cover four or five mothers. Between 2 and 3 p.m. every day, the subjects arrived at the home of their study worker. The study workers prepared the milk shortly before the time of administration. The women then drank the milk or took the tablet with water in the presence of the study worker, to ensure compliance.

During the first month of intervention, the designated supervisors of the research team visited the study workers on a weekly basis at random. The supervisors verified the information collected by the study workers at random (e.g., intake of milk or tablets, side effects) by asking the women in the study. The collected information was then compared with the results from the form completed by the study worker. On the first round, one error was found in 4.1% of the forms. All errors discovered were corrected. Based on the errors found and on inquiries, the supervisors assisted the study workers in organizational and methodological matters. From the second month on, the supervisors coached and monitored the study workers on a monthly basis.

Measurements

At baseline and at weeks 5, 10, and 16 of the study, 3 ml of venous blood was collected from each woman before she received the milk or supplement. The cyanmethemoglobin method was used to determine hemoglobin concentration [10] immediately after blood collection. After the hemoglobin determination, the serum was stored at –20°C for about 5–6 days. Serum iron (SI) was determined according to the recommendations of Gibson [11]. Total iron-binding capacity (TIBC) was determined according to Ramsay's recommendations [12]. Both biochemical analyses were carried out in the laboratory of the National Institute of Nutrition of the Ministry of Health. All analyses were performed in duplicate. Serum transferrin saturation (TS) was calculated according to Gibson's recommendations [11]. Anemia and iron-deficiency anemia were assessed by using the WHO classifications [10].

Height and weight measurements were performed at baseline following the recommendations of Gibson [11]. Weight was also measured at weeks 5, 10, and 16 and before delivery. Body weight was measured to the nearest 0.1 kg by an electronic weighing scale (SECA 770 alpha, SECA, Hamburg, Germany) with the woman wearing light clothing. Body height was measured to the nearest 0.1 cm by a microtoise (UNICEF, Copenhagen, Denmark). Pregestational weight was recorded from the subjects' health cards.

The women were asked to collect stool samples in small plastic containers, which were distributed at the beginning of the study. Within a week after collection, the samples were analyzed for hookworms according to the Kato-Katz method [13].

Food intake was assessed by 24-hour recall, repeated on three consecutive weekdays at the initiation of the study, as described by Gibson [11]. The Vietnamese food-composition table was the basis for the calculation of energy and nutrient intakes [14].

Statistical analysis

Data were entered by using SPSS for Windows software, Version 7.5 (SPSS, Chicago, IL, USA). The following statistical analyses were performed: analysis of variance (ANOVA) or analysis of covariance (ANCOVA), with hemoglobin concentration adjusted to the initial values, to analyze between-group differences in nutritional status; binary logistic regression to model the relationship between deteriorated iron status and adequate weight gain during pregnancy, adjusted for initial hemoglobin and prepregnancy weight (as a socioeconomic marker); and multinomial logistic regression for unordered multiple traits, adjusted for initial hemoglobin and prepregnancy weight, to model the relationship between adequate or inadequate iron status and weight gain during pregnancy [15]. The hypothesis under study was tested by independent t-tests to compare the four intervention groups with regard to the distribution of baseline variables that could influence changes in iron status and weight gain (e.g., baseline anthropometric data, iron status data, pregnancy indicators, nutrient intake, age, and family income); and by paired sample t-tests to assess in each group changes in iron status and weight during the follow-up period. The three first statistical analyses were implemented by SPSS, Version 7.5; the last two

tests were done with the Stata Version 6.0 software package (Stata, College Station, TX, USA).

Results

Table 2 shows the number of women excluded from data collection according to intervention group, along with the reasons for exclusion. Of the 202 women initially enrolled, a complete data set was obtained for 168. No significant differences between the excluded and the studied individuals were seen.

Table 3 compares the groups at baseline with regard to selected anthropometric data, pregnancy indicators, and nutrient intake data. No statistically significant differences were found among the groups.

Table 4 shows changes in iron status (hemoglobin concentration and TS) and weights during the trial. At the initiation of the study, there were no significant differences among the groups in the three measured indicators of nutritional status. At the end of the intervention, the hemoglobin concentrations of all four groups had decreased significantly ($p < .001$, paired t-test). However, the final hemoglobin concentrations and the changes among groups differed significantly ($p < .001$, ANCOVA adjusted for initial hemoglobin). The decrease in hemoglobin concentration in the iron-fortified milk and supplement groups was significantly less (–0.5 ± 0.9 and –0.3 ± 0.9 g/L, respectively) than in the unfortified milk and placebo groups (–1.2 ± 0.9 and –1.1 ± 0.8 g/L, respectively).

At baseline, the TS values showed no significant differences among groups (ANOVA). As with hemoglobin concentration, there was a significant difference among groups in TS changes ($p < .001$, ANOVA). The TS value decreased in the iron-fortified milk group and increased slightly in the supplement group (–2.7 ± 9.4% and 3.4 ± 12.9%, respectively), but the nonfortified milk

TABLE 2. Number of subjects and reasons for dropout according to intervention group

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Reason	With iron	Without iron	Iron	Placebo
Change of residence	0	2	0	1
Illness	2	1	1	1
Miscarriage	0	2	0	0
Premature delivery	1	3	4	2
Delivered before blood was taken	1	0	1	1
Refused taking of blood	2	2	2	1
Hemolysis	0	1	1	2
Total	6	11	9	8

TABLE 3. Comparison of selected anthropometric data, pregnancy indicators, and nutrient intake data between groups at baseline[a]

	Groups receiving milk (400 ml)		Groups receiving iron supplement or placebo	
Characteristic	With iron ($n = 44$)	Without iron ($n = 41$)	Iron ($n = 40$)	Placebo ($n = 43$)
Age (yr)	25.0 ± 3.7	25.8 ± 4.3	25.5 ± 3.8	25.3 ± 3.7
Pregestational weight (kg)	44.0 ± 3.9	44.0 ± 4.4	43.6 ± 3.4	43.8 ± 3.4
Weight at beginning of study (kg)	45.4 ± 5.0	45.2 ± 4.0	45.1 ± 3.6	46.0 ± 4.3
Height (cm)	153.2 ± 5.4	153.0 ± 5.1	152.9 ± 3.9	152.3 ± 4.5
Pregestational BMI (kg/m^2)	18.8 ± 1.4	18.7 ± 1.3	18.7 ± 1.7	19.2 ± 1.7
No. of children	0.5 ± 0.5	0.6 ± 0.5	0.5 ± 0.5	0.5 ± 0.5
No. of pregnancies	1.5 ± 0.6	1.7 ± 0.7	1.8 ± 0.8	1.7 ± 0.8
Duration of gestation (wk)	15.6 ± 1.4	15.6 ± 1.5	16.2 ± 1.6	16.6 ± 1.6
Energy intake (kcal/day)	2,188 ± 856	2,027 ± 812	2,124 ± 729	2,071 ± 664
Protein (g)[b]	36.2 ± 15.3	30.9 ± 11.8	35.6 ± 14.1	35.1 ± 13.3
Food iron intake (mg/day)	10.3 ± 4.4	9.9 ± 3.9	10.1 ± 3.7	9.7 ± 3.6
Food vitamin C intake (mg/d)	50.4 ± 33.6	47.8 ± 45.1	42.7 ± 31.2	41.1 ± 29.6

BMI, Body-mass index.

a. Values are means ± SD. There are no significant differences between groups ($p > .05$).

b. Protein was calculated with estimation of NPU (net protein utilization) = 60

TABLE 4. Hemoglobin, transferrin saturation, and maternal weight (mean ± SD) of the four intervention groups during pregnancy at baseline and after 16 weeks of intervention

	Hemoglobin (g/L)			Transferrin saturation (%)			Weight (kg)		
Group	Baseline[a]	Week 16[b]	Change[b,c]	Baseline[a]	Week 16[d]	Change[c,e]	Baseline[a]	Week 16	Change[b,e]
IM	117.4 ± 6.7	112.1 ± 8.4[f]	−0.5 ± 0.9	26.3 ± 7.8	23.7 ± 6.7	−2.7 ± 9.4	45.4 ± 4.9	50.4 ± 4.6[g]	5.0 ± 2.0
M	117.5 ± 10.6	105.2 ± 11.3[g]	−1.2 ± 0.9	26.1 ± 8.0	16.0 ± 5.8	−10.1 ± 9.8	45.2 ± 4.4	50.9 ± 5.5[g]	5.8 ± 2.1
IS	116.3 ± 8.9	113.3 ± 8.8[g]	−0.3 ± 0.9	24.6 ± 10.2	28.0 ± 9.0	3.4 ± 12.9	45.1 ± 3.6	49.7 ± 4.0[g]	4.6 ± 3.1
P	115.5 ± 7.5	104.1 ± 10.0[g]	−1.1 ± 0.8	26.0 ± 11.1	14.4 ± 7.1[g]	−11.6 ± 10.7	46.1 ± 4.2	49.9 ± 4.9[g]	3.8 ± 2.5

IM, Iron-fortified milk; M, nonfortified milk; IS, iron supplement; P, placebo.
Differences between groups:
a. Not significant (paired sample *t*-test)
b. $p < .001$ (ANOVA).
c. $p < .001$ (ANCOVA adjusted for initial hemoglobin).
d. Difference between baseline and 16th week.
e. $p < .01$ (ANOVA).
f. $p < .05$ (paired *t*-test).
g. $p < .001$ (paired *t*-test).

and the placebo groups both showed marked decreases in TS concentration (−10.1 ± 9.8% and −11.6 ± 10.7%, respectively).

The mean weights of the women in the four groups, which did not differ at baseline (ANOVA), increased significantly, as expected, with the advance of pregnancy beyond 16 weeks ($p < .001$, paired *t*-test). The weight increases differed significantly among groups ($p = .004$, ANOVA). The highest weight increases were found in the nonfortified- and fortified-milk groups (5.0 ± 2.0 and 5.8 ± 2.1 kg, respectively), followed by the supplemented group (4.6 ± 3.1 kg) and the placebo group (3.8 ± 2.5 kg). **Figure 1** shows the risk of developing anemia during pregnancy according to the four types of intervention, taking into consideration the initial hemoglobin levels. In all groups, the risk increases with reduced initial hemoglobin concentration. However, supplementation and fortification reduce the risk of anemia drastically, even if women are already anemic at the beginning of pregnancy. **Figure 2** shows the probability of adequate weight gain after intervention (at least 20% of prepregnancy weight) adjusted for prepregnancy weight. The probability of an adequate weight gain increases rapidly in women with a prepregnancy weight below 50 kg. However, the probability of an adequate weight gain despite a lower prepregnancy weight is highest in the fortified-milk group, followed by the nonfortified-milk group.

Discussion

Undernutrition and micronutrient deficiencies are still widespread among Vietnamese women. In 1994, it was estimated that about 30% of reproductive age women in rural area suffered from chronic energy deficiency (body-mass index < 18.5 kg/cm^2) [16]. In mothers with children under five years of age, this rate exceeded 40% [5]. The anthropometric data collected from the pregnant women in this study showed the same pattern

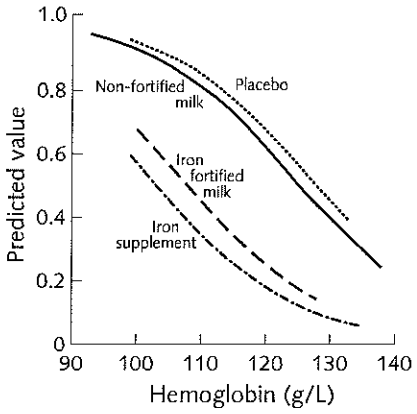

FIG. 1. Probability of anemia (hemoglobin < 110 g/L) adjusted for initial hemoglobin after intervention, according to the four different types of intervention

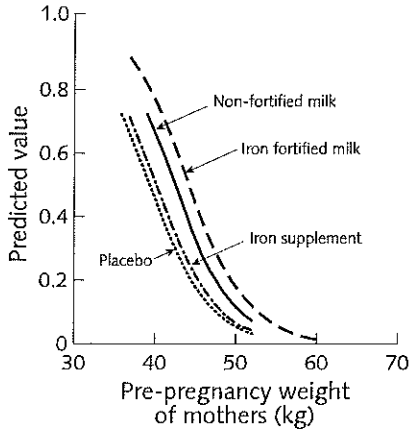

FIG. 2. Probability of adequate weight gain (> 20% of pre-pregnancy weight) adjusted for prepregnancy weight after intervention, according to the four types of intervention

of high prevalences of undernutrition (**table 3**).

At the baseline, the prevalences of anemia (Hb < 110 g/L) and of iron deficiency (TS < 16%) were 19% and 16%, respectively. The anemia prevalence was lower than that observed in Indonesian women in the first and second trimesters of pregnancy [17, 18]. During pregnancy, women have increasing requirements for iron. Iron is needed for the increasing maternal red cell mass (the demand is equal to 500–600 mg of iron) and for the growing fetus and placenta (with a demand of 350–450 mg of iron). When the normal physiological loss of iron from skin, stool, and urine is added to these demands, there is a cumulative need for iron during pregnancy of 1,100 to 1,400 mg, or 4 to 5 mg daily [10]. However, the need for iron is not evenly distributed throughout pregnancy. There is no increase or only a slight increase in the iron requirements in the first half of pregnancy, followed by a marked increase in the second half, leading to a daily demand for iron uptake in the third trimester as high as 8 to 10 mg daily.

In Vietnam, the average diet contains about 9 to 10 mg of iron daily, with an estimated net absorption rate of 5% to 10% [15]. The findings of this study confirm these low iron intakes in pregnant women (**table 3**). Therefore, the low iron intake from food and the increased iron requirements at the later stage of pregnancy result in a decreasing hemoglobin concentration in the blood (**table 3**).

According to the findings in this study, iron depletion can be compensated for or slowed down by iron supplementation and fortification, depending on the iron status at the beginning of the pregnancy. **Figure 2** suggests that the probability of anemia is slightly lower in the supplemented than in the fortified-milk group. However, the difference is relatively low, considering that the fortified group received only 25% of the additional daily iron dose as compared with the tablet-supplemented group. Therefore, the question remains whether women really need the recommended daily dose of 60 mg of iron for the control of anemia.

A weight gain of 9 to 12 kg during pregnancy has been recommended for pregnant women with an adequate pregestational weight [19, 20]. In Vietnam, mean weight gain of pregnant women in rural areas has been reported as 6.6 kg [14]. These published data are consistent with the mean weight gain of the placebo group (6.1 kg) in this study. Taking the weight gain recommendations into consideration, less than a quarter of the women (22.8%) achieved this goal in the present longitudinal observations. However, the prevalence of insufficient weight gain during pregnancy differed significantly between groups. About one-third of the women in the iron-fortified milk and the nonfortified milk groups achieved the recommended weight increase. In the iron supplementation group, one-fifth of the women (20%) met the weight gain recommendation, whereas only 9% of the women in the placebo group did so. As shown in **fig. 2**, the probability of adequate weight gain during pregnancy depends not only on diet, but also on socioeconomic factors and the prepregnancy weight of the woman. In particular, low-weight pregnant women benefit from fortified food.

Conclusions

The administration of milk fortified with 15 mg of iron per day and iron supplementation with 60 mg of iron per day in tablet form improved the iron status of pregnant Vietnamese women in this study. These findings suggest that doses even lower than 60 mg of iron per day are sufficient to prevent a drastic increase in anemia rates as pregnancy advances. However, if a woman enters pregnancy with anemia, or even with empty iron stores, supplementation and fortification, even under controlled conditions, seem to be insufficient to eliminate iron deficiency during gestation. This underscores the importance of preventing anemia among women of childbearing age before pregnancy.

Since the women suffered not only from low iron intake (and possibly also from other micronutrient deficiencies), but also from low energy consumption, the additional energy and nutrients obtained from the milk contributed to the weight gain of the mothers in the fortified-milk group during pregnancy. The distribution of fortified milk to pregnant women is far more expensive than the distribution of iron supplements alone. However, in the Vietnamese situation, in which mothers have a high prevalence of acute undernutrition, supplementation alone was insufficient to address weight gains during pregnancy. Rather, the increased availability of fortified food commodities such as milk at the household level should be considered.

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