

## Original Article

# Effect of low-dosage vitamin A and riboflavin on iron-folate supplementation in anaemic pregnant women

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A double-blind, placebo, controlled trial was conducted in Banyudono subdistrict, Boyolali regency, Central Java province, Indonesia. The aim of the study was to determine whether adding low-dosage vitamin A and riboflavin can enhance the effect of iron-folate supplementation in anaemic pregnant women. From July to November 2000, 202 pregnant women were screened for anaemia (haemoglobin <11.0 g/dL). One hundred and three pregnant women (51%) were found to be anaemic and were then allocated alternately into four groups. Over a period of 60 days, group IF ( $n = 29$ ) received iron-folate tablets (200 mg FeSO<sub>4</sub> and 250 µg folic acid) + 5 mg glucose; group IFR ( $n = 22$ ) received iron-folate tablets + 5 mg riboflavin; group IFA ( $n = 29$ ) received iron-folate tablets + 2.75 mg retinyl palmitate (equal to 5000 IU vitamin A); and group IFRA ( $n = 23$ ) received iron-folate tablets + 5 mg riboflavin + 2.75 mg retinyl palmitate. At the end of the study 19 pregnant women (18.4%) were excluded from the analysis because of various reasons. Statistical analysis was based on 84 women (81.5%): group IF,  $n = 25$ ; group IFR,  $n = 22$ ; group IFA,  $n = 18$ ; and group IFRA,  $n = 19$ . Haemoglobin measurements were carried out using the Technicon H1\* (cyanmethaemoglobin method). All groups showed a significant increase in haemoglobin concentration ( $P < 0.05$ ), except group IFA ( $P > 0.05$ ), with the highest increment being in group IFR. Multiple comparisons only showed significant differences between group IFR and group IFA ( $P < 0.05$ ). It can be concluded that iron-folate supplementation can increase haemoglobin concentrations in anaemic pregnant women. Adding riboflavin tends to enhance the effect of iron-folate supplementation, but this is not the case with adding vitamin A.

**Key words:** anaemic pregnant women, iron-folate, low-dosage vitamin A, riboflavin.

## Introduction

Iron deficiency anaemia (IDA) in pregnant women has detrimental effects on pregnancy outcome.<sup>1</sup> IDA is highly prevalent among Indonesian pregnant women.<sup>2,3</sup> Although an iron supplementation program for pregnant women has been provided since 1974, our recent study in the Banyudono subdistrict, Boyolali regency, Central Java province, Indonesia, showed that the prevalence of anaemia among pregnant women is still very high at around 70% (B Hanim, Suhanantyo, W Atmaka, Padmaningrum, unpubl. data, 1999).<sup>4</sup>

A recent National Household Health Survey<sup>5</sup> (1995) revealed a substantial reduction in the prevalence of IDA among pregnant women, from 63.5% in 1992 to 52.4% in 1995. However, it is still considered unacceptably high. The result of the Mother Care Project evaluation showed that mass iron-folate supplementation via health centre distribution was not very effective.<sup>6</sup> Low compliance was the main cause of the failure.<sup>7</sup> Nutrient–nutrient interactions may also affect iron status in pregnant women. In Gambia, Powers *et al.* showed that adding riboflavin to iron supplementation improved haematological parameters in pregnant women.<sup>8</sup> Similarly, Suharno *et al.* showed that adding vitamin A to iron supplementation increased haemoglobin concentration in pregnant women in West Java, Indonesia.<sup>9</sup>

Multiple micronutrient deficiencies are common in developing countries.<sup>10</sup> Our study in Polokarto subdistrict, Karanganyar regency, Central Java province, Indonesia, showed a low riboflavin intake among pregnant women.<sup>11</sup> Meat and dairy products are rarely consumed in rural areas of Indonesia. Although Boyolali regency is known as the ‘milk capital’ of Central Java, pregnant women in Banyudono subdistrict rarely consume milk because it is too expensive for most people in the village. The same is also true for meat. In East Java, IDA in adult women occurs together with vitamin A deficiency.<sup>12</sup> In Indonesia, high dosage vitamin A capsules are given only to children under five years of age and lactating mothers, not to pregnant women, even though studies show that many pregnant women are marginally vitamin A deficient.<sup>9</sup> The present study examines the effect of adding riboflavin and low-dosage vitamin A

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Accepted 22 February 2002

to iron-folate supplementation among pregnant women in a rural area of Indonesia.

## Materials and methods

### Research setting

The study took place in the rural area of Banyudono sub-district, Boyolali regency, Central Java province, Indonesia. This subdistrict is located approximately 100 m above sea level and 20 km from Solo City. It has 44 000 inhabitants with two health centres: Banyudono I and Banyudono II. Banyudono I covers 26 000 inhabitants and Banyudono II covers 18 000 inhabitants. The population density is 1746 people/km<sup>2</sup>. Arable agriculture and textiles are the main sources of income in this area. Boyolali is also known as the 'milk capital' as it is the largest producer of milk for the canned milk industry in Java. However, no milk is produced in Banyudono subdistrict itself. The majority of farmed land comprises paddy fields, with peanuts and cassava growing in poorly irrigated areas. The area is served by a network of roads and there are newspapers, electricity, radio, television, telephone, and post facilities in the area. Each year there are around 500 new pregnancies in Banyudono subdistrict.

### Subjects

In a previous study, we found that animal foods were rarely consumed by pregnant women in Banyudono subdistrict because meat and milk were too expensive. It can be predicted that pregnant women are at risk of riboflavin and iron deficiency. The main sources of iron and vitamin A are green leafy vegetables. All pregnant women who visited the Banyudono health centres' antenatal clinics from July to November 2000 were asked to participate in the study. The inclusion criteria were:

- 1 Aged less than 35 years. This cut-off was chosen arbitrarily as pregnancy complications frequently occur among older mothers.
- 2 Weeks of pregnancy between 13 and 28. Due to vitamin A toxicity to the foetus, we excluded women in the first trimester of pregnancy. The midwife checked the last date of menstruation and cross-checked with the size of the uterus.
- 3 Single pregnancy.
- 4 Apparently in good health and checked by the doctor at the antenatal clinic. Pregnant women with pre-eclampsia, congestive heart disease, tuberculosis and acute infections were excluded from the study.
- 5 Agree to participate in the study.

Of the 202 pregnant women screened, 103 were anaemic (Haemoglobin <11 g/dL), corresponding to 51% of the subjects. All pregnant women were numbered and listed. They were then allocated alternately into groups according to their numbers. Group IF ( $n = 29$ ) received iron-folate tablets + 5 mg glucose (placebo); group IFR ( $n = 22$ ) received iron-folate tablets + 5 mg riboflavin; group IFA ( $n = 29$ ) received iron-folate tablets + 2.75 mg retinyl palmitate (equal to 5000 IU vitamin A); and group IFRA ( $n = 23$ )

received iron-folate tablets + 5 mg riboflavin + 2.75 mg retinyl palmitate. These were administered seven days a week for 60 days. All supplements had the same colour, shape and size in capsule form and were given in a double-blind manner. To ensure compliance, two midwives visited the subjects at their home every week and checked the capsules consumed. Neither the midwives nor the researchers knew which capsules they had given to the subjects. Only the Head of the Health Centre kept the list and opened it at the end of the study, just before the analysis.

### Study protocol

A trained laboratory assistant took the blood samples. Venous blood samples were taken from the subjects at baseline and 60 days later at 08:00–09:00 h. Blood samples (3 mL) were collected in a vacutainer containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and kept in a cool-box, before being transferred to an accredited clinical laboratory. Haemoglobin was measured directly. The remaining blood was centrifuged to separate plasma from packed cells. The packed cells were then washed with normal saline and centrifuged. The supernatants were disposed of. An equal volume of aquadest was added to the washed cells to make the blood haemolysate. The blood haemolysates were stored frozen at  $-20^{\circ}\text{C}$  for measurement of the erythrocyte glutathione reductase activity coefficient (EGRAC). Weight, height, mid-upper arm circumference (MUAC) and dietary intake were recorded at baseline by the same researcher.

### Haemoglobin measurement

Haemoglobin measurements were obtained by analysing 100  $\mu\text{L}$  blood sample in a calibrated Technicon H1\* (Technicon Instruments, NY, USA) using the cyanmet-haemoglobin method. Haemoglobin was expressed in g/dL.

### Measurement of riboflavin status

Riboflavin status was measured using the assay for erythrocyte glutathione reductase (Egr; EC 1.6.4.2) activity as described by Powers *et al.*<sup>13</sup> EGRAC was measured with a Cobas Bio Autoanalyser at the Centre for Human Nutrition, University of Sheffield, UK. For technical reasons, only blood samples from group IFR were measured. The cut-off point for riboflavin deficiency is EGRAC <1.4.<sup>13</sup>

### Dietary assessment

An estimated record technique with a 24 h recall was used to assess dietary intake of the subjects. One of the researchers undertook the interview and analysed the nutrient intake using the Food Processor II Software Package (Esha Research, Salem, OR, USA) and Comp-Eat (Lifetime Nutritional Services, London, UK) for riboflavin intake.

### Anthropometric measurements

Weight was recorded on a calibrated mechanical bathroom scale to the nearest 0.1 kg (Krupps, Ireland) after zeroing for each measurement. Pregnant women were lightly clothed

and had removed their footwear. Height was recorded using a microtoise (Statometer™, Indonesia) and measured to the nearest 0.1 cm. Each woman stood with her buttocks, heels and back against the wall and her head in the Frankfurt plane. MUAC was measured using a plastic tape to the nearest 0.1 cm. Data was compared to Indonesian standards to ascertain nutritional status.

### Statistical analysis

Results were expressed as the mean  $\pm$  standard deviation. To see the difference between baseline and post-treatment in each group, a paired *t*-test was used. For multiple comparisons among groups an ANOVA and a least significant difference test was used. All statistical analysis was performed using SPSS for Windows, release 10.0 (Chicago, IL, USA).

### Ethical considerations

The study was approved by the Central Java Province Regional Development Board. Subjects were informed about the nature of the study and agreed to participate in the study.

### Results

One hundred and three subjects were recruited initially, but only 84 (81.5%) were included in the analysis for various reasons. Nineteen women did not have their second blood samples taken due to premature labour (nine subjects), stillbirth (one subject), migration (one subject), refusal to give blood (one subject), nausea and vomiting (two subjects) and incorrect dates given for last menstruation but with normal deliveries (five subjects). Table 1 shows the baseline characteristics of the subjects. There were no significant

differences in haemoglobin concentration among the groups at baseline. Of the 84 pregnant women recruited, five subjects were classified as wasted (4.85%). However, group IFA and group IFRA had significantly lower weights and heights ( $P < 0.05$ ) compared to the other groups. The vast majority of the subjects were on their first and second pregnancies. Sixty days after supplementation, haemoglobin concentration was measured again (Table 2). All groups showed increased haemoglobin concentrations, except group IFA ( $P > 0.05$ ). Table 3 shows the mean differences in haemoglobin concentration between the groups. Multiple comparisons only showed significant differences between group IFR and group IFA ( $P < 0.05$ ). EGRAC measurement of group IFR showed low riboflavin status at baseline ( $1.82 \pm 0.27$ ), which increased significantly after riboflavin supplementation for 60 days ( $1.28 \pm 0.33$ ;  $P < 0.001$ ). Table 4 shows nutrient intakes derived from a 24 h recall. Group IF showed a higher intake in all selected nutrients compared with the other groups. In general, the subjects showed low intakes of iron, riboflavin, protein and energy, but had high intakes of vitamin C and vitamin A.

### Discussion

Iron deficiency anaemia is recognised as a major public health problem in Indonesia. The Household Health Survey of 1995 revealed that the prevalence of IDA in pregnant women is 50.9%.<sup>14</sup> The aetiology of IDA is mainly of dietary origin. Hookworm infestation, malaria and haemoglobinopathies are very rare in the study area.<sup>15</sup> The government of Indonesia has set up a target to reduce the prevalence of IDA to 10% by the year 2018.<sup>2</sup> To achieve the target, it is not

**Table 1.** Baseline characteristics of pregnant women in this study

Variable	Group IF ( <i>n</i> = 25)	Group IFR ( <i>n</i> = 22)	Group IFA ( <i>n</i> = 18)	Group IFRA ( <i>n</i> = 19)
Age (years)	26.63 (5.67)	28.41 (5.59)	25.93 (4.54)	26.14 (6.27)
Weight (kg)	52.60 (3.46)	52.43 (3.22)	50.34 (4.81)*	49.45 (5.16)*
Height (cm)	152.99 (3.46)	154.16 (3.80)	151.79 (4.74)*	150.12 (4.58)*
MUAC (cm)	25.32 (1.52)	24.66 (1.37)	25.12 (1.68)	24.08 (2.54)*
Hb (g/dL)	10.43 (0.50)	10.20 (0.59)	10.41 (0.38)	10.37 (0.43)
Gestation age (week)	21.57 (3.82)*	24.77 (2.86)*	22.83 (6.64)	21.77 (4.53)
No. pregnancies	2.00 (0.91)	2.41 (1.37)	1.86 (1.09)	1.73 (1.16)
Education (year)	9.13 (3.95)	9.59 (4.47)	9.52 (3.41)	10.00 (2.78)

Values are presented as mean  $\pm$  SD. \*Significant at the level of  $P < 0.05$ . IF, iron-folate + 5 mg glucose; IFA, iron-folate + 2.75 mg retinyl palmitate (equivalent to 5000 IU vitamin A); IFR, iron-folate + 5 mg riboflavin; IFRA, iron-folate + 5 mg riboflavin + 2.75 mg retinyl palmitate.

**Table 2.** Haemoglobin concentrations before and after supplementation

Group	<i>n</i>	Haemoglobin (g/dL)		Increment	<i>P</i> -value
		Before	After		
IF	25	10.40 $\pm$ 0.54	10.89 $\pm$ 0.95	0.492 $\pm$ 0.983	0.020*
IFR	22	10.20 $\pm$ 0.59	11.02 $\pm$ 0.95	0.818 $\pm$ 0.673	0.000*
IFA	18	10.39 $\pm$ 0.44	10.59 $\pm$ 0.93	0.194 $\pm$ 0.733	0.276
IFRA	19	10.39 $\pm$ 0.41	10.86 $\pm$ 0.75	0.463 $\pm$ 0.750	0.015*

Values are presented as mean  $\pm$  SD. \*Statistically significant (paired *t*-test):  $P < 0.05$ . IF, iron-folate + 5 mg glucose; IFA, iron-folate + 2.75 mg retinyl palmitate (equivalent to 5000 IU vitamin A); IFR, iron-folate + 5 mg riboflavin; IFRA, iron-folate + 5 mg riboflavin + 2.75 mg retinyl palmitate.

**Table 3.** Mean differences in haemoglobin concentration after supplementation

Group (I)	Group (II)	Mean difference (I – II)	SE	P-value
IF	IFR	-0.3262	0.2356	0.170
	IFA	0.2976	0.2491	0.236
	IFRA	0.0288	0.2453	0.907
IFR	IF	0.3262	0.2356	0.170
	IFA	0.6237*	0.2561	0.017
	IFRA	0.3550	0.2524	0.163
IFA	IF	-0.2976	0.2491	0.236
	IFR	-0.6237*	0.2561	0.017
	IFRA	-0.2687	0.2651	0.314
IFRA	IF	-0.0288	0.2453	0.907
	IFR	-0.3550	0.2524	0.163
	IFA	0.2687	0.2651	0.314

\*Significant at the level of  $P < 0.05$ . IF, iron-folate + 5 mg glucose; IFA, iron-folate + 2.75 mg retinyl palmitate (equivalent to 5000 IU vitamin A); IFR, iron-folate + 5 mg riboflavin; IFRA, iron-folate + 5 mg riboflavin + 2.75 mg retinyl palmitate.

**Table 4.** Nutrient intake based on 24-h recall of all diet groups

Nutrient	Group IF ( $n = 25$ )	Group IFR ( $n = 22$ )	Group IFA ( $n = 18$ )	Group IFRA ( $n = 19$ )
Energy (kcal)	2045 ± 445.0*	1749 ± 457.0	1468 ± 349.0	1599 ± 415.0
Protein (g)	66.0 ± 18.1*	48.1 ± 15.2	50.5 ± 16.9	55.8 ± 22.5
Iron (mg)	20.8 ± 5.8*	12.7 ± 4.7	10.1 ± 3.7	13.9 ± 8.8
Vitamin C (mg)	263.4 ± 150.5*	153.9 ± 70.1	126.4 ± 69.1	120.2 ± 70.0
Vitamin A (RE)	1585.7 ± 1333.0*	755.5 ± 422.1	505.5 ± 297.0	1208.5 ± 1172.6
Vitamin B <sub>2</sub> (mg)	1.6 ± 1.2*	0.8 ± 0.4	0.5 ± 0.3	0.6 ± 0.4

Values are presented as mean ± SD. \*Significant at the level of  $P < 0.05$ . IF, iron-folate + 5 mg glucose; IFA, iron-folate + 2.75 mg retinyl palmitate (equivalent to 5000 IU vitamin A); IFR, iron-folate + 5 mg riboflavin; IFRA, iron-folate + 5 mg riboflavin + 2.75 mg retinyl palmitate; RE, retinal equivalents.

realistic to rely on iron supplementation alone. Although the government started an iron supplementation program in 1974, IDA prevalence is still unacceptably high at present.

In the case of Java, where rice is the staple and a very small amount of animal foods is eaten, a little variation was observed. The main sources of protein, vitamins and minerals are green leafy vegetables.<sup>11</sup> With such a diet, it can be predicted that pregnant women in rural areas of Java are at risk of iron and riboflavin deficiencies. Results of the dietary recall of the present study confirmed this prediction (Table 4).

Indonesian recommended dietary allowances (IRDA) for pregnant women aged 20–45 years with mean weight 54 kg and height 156 cm<sup>17</sup> are as follows: energy, 2485 kCal; protein, 50 g; vitamin C, 70 mg; vitamin A, 500 retinol equivalents (RE); iron, 46 mg; riboflavin, 1.4 mg; and folic acid, 300 µg. As we did not measure serum retinol, the vitamin A status of the subjects was unknown. Although we found that the subjects' dietary vitamin A intakes exceeded the IRDA, almost all of it came from plant sources. However, this probably explains the phenomenon of why adding vitamin A to group IFA and IFRA did not enhance the effect of iron supplementation; the subjects were probably not vitamin A deficient. In addition, the nutritional status of these groups was lower than the other groups. The vast

majority of the subjects in this study did not meet the IRDA, especially for iron (10.10–20.84 mg/day) and energy (1467–2045 kCal/day). This finding indicates that the main cause of IDA is inadequate dietary iron intake. The intake of vitamin C (which is good for iron absorption) exceeded the IRDA, due to consumption of citrus fruits and dark green leafy vegetables. However, rice-based diets and tea drinking may reduce its effect.<sup>17</sup> The low socioeconomic conditions of the subjects seemed to exacerbate the problems. A report by Hellen Keller International in 1998 revealed a reduced intake of animal foods due to an increase in the prices of basic commodities and reduced purchasing power of the population after the economic crisis in Indonesia began in July 1997.<sup>18</sup>

Several studies have been carried out on multiple micro-nutrient supplementation in pregnant women with encouraging results.<sup>8–10</sup> Powers *et al.* showed that adding riboflavin to iron supplement improved haematological parameters among pregnant women in Gambia,<sup>8</sup> while Suharno *et al.* demonstrated a better response with iron + vitamin A than iron alone in pregnant women in West Java.<sup>9</sup>

The interrelationship of nutrients affects iron metabolism.<sup>8,9,13</sup> According to Powers,<sup>20</sup> riboflavin influences iron metabolism via iron absorption, mobilisation from iron stores and iron utilisation for haemoglobin synthesis in the

bone marrow, whereas vitamin A can mobilise iron from its store. The present study shows significant increases in haemoglobin concentration in groups IF, IFR and IFRA, but not in group IFA (Table 2). Although iron-folate supplementation for 60 days can increase haemoglobin concentration (group IF), it seems that adding riboflavin tends to enhance the effect of iron supplementation to a greater degree, observable for group IFR as the greatest increment in haemoglobin concentration. The EGRAC measurement (group IFR only) showed a low riboflavin status at baseline ( $1.82 \pm 0.27$ ), but increased significantly after riboflavin supplementation for 60 days ( $1.28 \pm 0.33$ ;  $P < 0.001$ ). It is worth noting that those pregnant women (group IFR) at baseline can be classified as riboflavin deficient (EGRAC  $> 1.4$ ) and replenished after supplementation (EGRAC  $< 1.4$ ). Riboflavin deficiency is highly prevalent in developing countries and in developed countries as well.<sup>8,20</sup> Riboflavin intake in rural areas of Central Java is low<sup>12</sup> due to low consumption of animal foods and milk. In general, the subjects in this study had low riboflavin intakes (Table 4). Therefore, adding riboflavin to iron-folate supplementation in pregnant women should be considered to enhance its effect on haemoglobin level. Unexpectedly, however, adding low-dosage vitamin A (group IFA and group IFRA) did not enhance the effect of iron-folate supplementation on haemoglobin level. It is difficult to explain why this happened, especially because, unfortunately, we did not measure the vitamin A status of the subjects. However, from dietary intakes we can speculate that this was because the subjects were not deficient in vitamin A, or because they had lower nutritional status, or both (Table 1). It is also difficult to ensure that the vitamin A capsules were consumed. One can examine the faeces to check whether iron tablets were consumed,<sup>21</sup> but there are no such measures for vitamin A supplementation.

In conclusion, the results of this study indicate that adding riboflavin to iron-folate supplementation tends to enhance its effect on haemoglobin concentration among anaemic pregnant women, especially when the subjects are riboflavin deficient. A further study is needed to clarify whether adding vitamin A to iron-folate supplementation has beneficial effects in terms of haemoglobin concentration among anaemic pregnant women.

**Acknowledgements.** This study was supported in part by the Community Health Nutrition-3 Project 2000, Department of Education, Republic of Indonesia. The first author would like to thank the British Council Jakarta for its travel grant and Dr Hilary J Powers, Director of the Centre for Human Nutrition, University of Sheffield, UK, for her kindness in training the author in assessing riboflavin status using the Cobas Bio Autoanalyser. The authors also would like to thank the Prodia Clinical Laboratory, Solo, Indonesia for measuring haemoglobin using Technicon H1\* and Dr Sri Prabandini, Head of Banyudono I Health Centre, for her kind assistance.

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