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Nutritional Health of Indonesian Adolescent Girls:

the role of riboflavin and vitamin A on iron status

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Abstract

In developing countries, adolescent girls often have anemia and micronutrient deficiencies. The objective of this thesis was to investigate the role of riboflavin or vitamin A as a determinant of anemia and iron deficiency in Indonesian adolescent girls. Several cross-sectional studies, involving a total of 856 adolescent schoolgirls, were carried out in Jakarta and Tangerang. Most studies, except for the one in East Jakarta, failed to show any relationship between riboflavin and hemoglobin or plasma ferritin concentration. A clear relationship between vitamin A status and hemoglobin concentration was observed only when all survey results were combined, whilst a relationship between vitamin A status and plasma ferritin concentration was observed in Central Jakarta and all survey results combined.

Anemia in the majority of the girls was related to iron deficiency. This was substantiated by our findings that iron supplementation for 16 weeks resulted in a reduction of anemia prevalence as compared to placebo group and conversely, that the prevalence of anemia increased at 16 weeks after cessation of iron supplementation.

In a randomized controlled trial involving 258 anemic adolescent schoolgirls, daily supplementation with riboflavin or vitamin A in addition to iron for eight weeks failed to improve or only marginally improved concentration of hemoglobin or plasma ferritin beyond that achieved by supplementation with iron alone. However, addition of riboflavin may have improved the iron status of girls with more severe anemia, and vitamin A supplementation resulted in a 19% (95% CI: 1% to 39%) increase of plasma ferritin concentration. Despite the marginal treatment effects, supplementation with riboflavin or vitamin A markedly improved biochemical indicators of riboflavin and vitamin A status, respectively.

The prevalence of anemia (9-57%) in our studies indicates that anemia is a problem of public health significance. Dietary intakes of riboflavin, vitamin A and iron were poor. The prevalence range of riboflavin, vitamin A and iron deficiency was 21-96%, 7-25% and 20-58%, respectively. The prevalence of girls with iron status below a minimal target (as indicated by serum ferritin concentration < 30 µg/L) and with inadequate vitamin A stores (as indicated by serum retinol concentration <1.05 µmol/L) ranged between 50-82% and 40-73%, respectively. The high prevalence of vitamin A and riboflavin deficiencies are a cause of public health concern; however, our findings alone provide insufficient ground to justify supplementation with riboflavin and vitamin A for intervention programs aimed at alleviating anemia. Further studies are needed to determine the functional consequences of low riboflavin and vitamin A status on the health of adolescent girls. In addition, prospective studies on the efficacy of micronutrient intervention in adolescent girls on the pregnancy performance and outcome are proposed.

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PhD thesis by Drupadi HS Dillon, 2005.

Contents

Chapter

1

General Introduction

1. The burden of anemia in adolescent girls

Anemia is the most common nutritional disorder in developing countries, affecting mostly children below five years of age and pregnant women. Anemia during pregnancy has received much attention on account of the associated risk of adverse pregnancy outcomes. However, anemia is also a concern in non-pregnant women of reproductive age, including post-menarchal adolescent girls.

In developing countries, the prevalence of anemia among adolescent girls ranges from 17 to 89% [1-12], as compared to 7 to 22% in developed countries [13-15]. Adolescent girls usually have a higher prevalence of anemia than their male counterparts [8,9,11,16-19].

Low socio-economic status, low intake of bioavailable iron, infectious disease such as malaria, hookworm, and schistosomiasis, along with menstruation are factors deemed to be responsible for the high prevalence of anemia among adolescent girls in developing countries[11,12,20,21]. In addition, age, late adolescence and low intake of vitamin A are contributing factors to anemia in adolescent girls [8,11,16,22].

Although anemia is often caused by iron deficiency, deficiencies of other micronutrients: vitamin A, vitamin E, riboflavin, vitamin B_6, folic acid, vitamin B_{12}, vitamin C, and copper (Figure 1) may also be involved. It is hypothesized that vitamin A, riboflavin, vitamin C and copper are nutrients involved in iron metabolism, while the other micronutrients are involved in other aspects of hematopoiesis [23,24].

Anemia is related to a range of disadvantages. In adolescent girls, it may reduce school achievement and physical activity [13,22]. Lower hemoglobin concentration was observed to be associated with a lower body mass index in Nigerian adolescent girls[25] and in Tanzanian adolescent boys [21]. Height-for-age or weight-for-age was also associated with hemoglobin concentration in school children aged 5-12 years in Bangladesh, after adjustment for age, sex and socio-economic status [26].

2. Micronutrient goals in adolescence.

Adolescent girls, about 600 million in all, comprise about 10% of the world population, with about 80% residing in developing countries [20]. During adolescence, girls gain up to 50% of their adult weight, approximately 20% of their adult height and 50% of their adult skeletal mass [20]. The growth spurt and the establishment of reproductive capacity, physiological preparations for motherhood, that occurs during adolescence, place great demand for nutrients in adolescent girls. A good nutritional status during adolescence lays the foundation for a healthy adult life and prepares adolescent girls for an optimal motherhood.

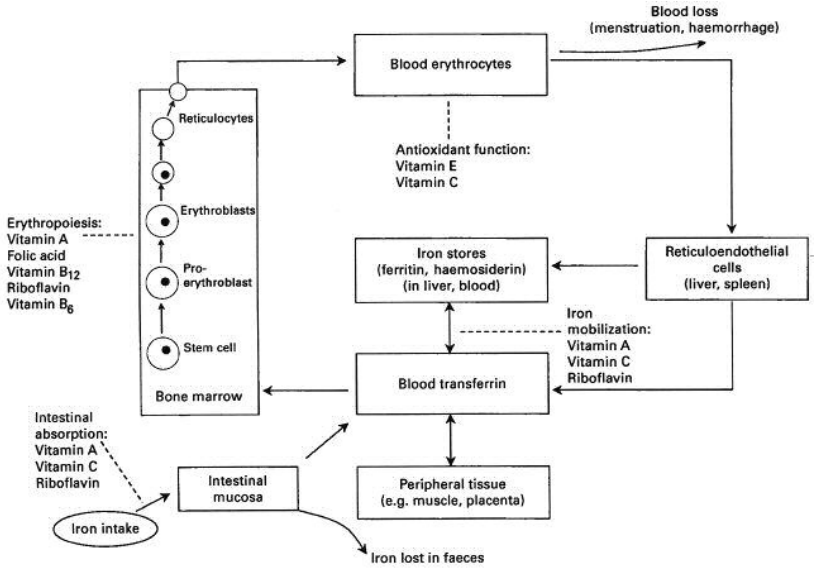

FIGURE 1. Vitamin roles in iron metabolism and erythropoiesis[23]

Many studies, both in developed and developing countries have shown, however, that the nutrient intake of adolescent girls is inadequate [27,36].

Three micronutrients that were the subjects of the studies presented in this thesis – riboflavin, vitamin A and iron – will be discussed in greater detail below.

2.1 Riboflavin

In addition to its role in iron metabolism, riboflavin as a coenzyme plays an important role in the provision of energy, metabolism of ß-oxidation of fatty acid, niacin, vitamin B_6 and homocysteine [37]. The existing reports on the functional consequences of riboflavin deficiency are mostly from animal models and as yet are not fully understood. Inadequate intake of riboflavin can lead to disturbances in energy production [37] and might interfere with the growth process during adolescence.

The dietary recommendation for riboflavin intake ranges from 1.0 to 1.2 mg/day for Indonesian adolescent girls aged between 10-19 years [38]. There are currently no data available on the riboflavin intake of Indonesian adolescent girls. However, riboflavin intake was observed to be below recommendation values in 20% and 65% of adolescent girls in the United Kingdom and in India, respectively [31,33].

The erythrocyte glutathione reductase activity coefficient (EGRAC) is commonly used as an indicator of riboflavin deficiency. The cut-off point to define deficiency is not yet well established; however, a several investigators use EGRAC ≤1.4 as normal and a value >1.4 as an indication of deficiency.

Male adolescents appear to have a better riboflavin status than their female counterparts [39]. In developed and developing countries, the prevalence of riboflavin deficiency among adolescent girls ranges from 27% to 95% [37,40,41]. Data on the riboflavin status of Indonesian adolescent girls is not available.

2.2 Vitamin A

In addition to its possible role in iron metabolism, vitamin A is essential during growth as cellular differentiation, the maturation process of reproductive organs, bone formation, and development of the immune function all need vitamin A. It has been suggested that vitamin A plays an important role in sexual maturation [42], visual function and susceptibility to infection [43].

Several studies have highlighted the functional health consequences of vitamin A deficiency, although there are fewer data available in adolescent girls than for children aged below five years and pregnant and lactating women. After adjusting for age, sex and socio-economic factors, children aged 5-12 years with a higher serum retinol concentration had better height-for-age or weight-for-age than their peers [26,44]. It has also been shown that school children aged 9-12 years receiving vitamin A supplements had larger increases of their height and weight than the placebo group [9].

The few reports that are available on vitamin A deficiency among adolescent girls in developing countries indicate that it may range from 7% to 32% [1,4,5,11]. The dietary recommendation for vitamin A intake is 500 RE/day for Indonesian adolescent girls aged between 10-19 years [38]. As in the case of riboflavin status, there is no published data available on the vitamin A intake of Indonesian adolescent girls. The prevalence of adolescent girls with vitamin A intake below the recommended level has been reported to range from 8% to 18% in both developed and developing countries [31,33,34,36].

Serum retinol concentrations of < 0.7 µmol/L, 0.7-1.05 µmol/L and ≥1.05 µmol/L indicate vitamin A deficiency, low vitamin A status, and adequate stores of vitamin A, respectively [46]. In the United Kingdom, vitamin A deficiency was found in less than one percent of young British aged 4-18 years; 81% of them had adequate stores of vitamin A [47].

Serum retinol concentration increases with age [5,44,48,49], but less so in females than in males [50], and less so in developing than in developed countries. Prevalence

values reported for vitamin A deficiency may be overestimates because most studies have based their results only on serum or plasma retinol concentration, without taking into account the effects of infection-induced inflammation. Inflammation, which is more common in developing countries, may lower serum retinol concentration independently of vitamin A status [50-53].

2.3 Iron

In addition to its oxygen-binding role in hemoglobin, iron is involved in many other biochemical processes in the human body. It is essential for muscle metabolism and energy use, skeletal and muscle growth, immune function and nuclear metabolism and gene transcription [42,54,55].

Only few data are available on the functional consequences of iron deficiency in adolescent girls. Adolescent girls supplemented with iron showed better verbal learning and memory than their peers receiving placebo [56]. Iron supplementation increased height and weight more than placebo in school children 9-12 years of age [9], while supplementation with iron and folic acid enhanced growth more than placebo group in Indian adolescent girls [57].

Iron requirements of non-menstrual adolescent girls are estimated to range between 1.22 -1.46 mg/day, and 1.39-2.54 mg/d in post-menarchal girls [15].

Approximately half of the adolescent girls in the developed countries are reported to have an intake below the recommended value [33,36]. The dietary recommended intake of iron ranges between 14-25 mg/day for Indonesian adolescent girls aged 10-19 years [38]. No published data are available on the iron intake of Indonesian adolescent girls. A study has shown that the intake of iron among adolescent girls in developing countries is less than their peers in developed countries [35]. Thirty six percent of Indian adolescent girls were found to be consuming less than 70% of the recommended daily allowance of iron [31].

Serum ferritin concentration < 12 µg/L indicates iron deficiency [14], whilst a value of ≥ 30 µg/L is considered as the minimal target iron store [58,59]. The prevalence of iron deficiency ranged from 14 to 80% among adolescent girls in developing countries [1,3,4,7,12,13,22,33,60,61]. As with the prevalence of vitamin A deficiency, however, these values may be underestimates because infection-induced inflammation increases serum or plasma ferritin concentration independently of iron status [52,62].

3. Effects of riboflavin and vitamin A on iron metabolism

Riboflavin may influence hemoglobin concentration or iron status through several mechanisms. Riboflavin deficiency is associated with erythroid hypoplasia and

reticulocytopenia [37,63]. The impaired erythropoiesis is believed to be due, at least in part, to disturbances in iron absorption and metabolism [37,64]. In vitro and animal studies have shown that riboflavin in its biologically active forms can play a role in the reduction and thereby the release of intracellular ferritin iron [65-67]. This reducing process is necessary to mobilize ferritin iron from storage tissues, especially in the bone marrow. In erythroblasts, it is essential to release iron from transferrin after its receptor-mediated uptake so that it is made available for subsequent incorporation into hemoglobin. In infants, riboflavin deficiency can lead to disturbances in the gastrointestinal structure such that the duration of the functional maturity of enterocytes is reduced [64,68]. Both the impaired release of iron from enterocytes and the reduced life span of enterocytes may contribute to the loss of absorbed iron. Several intervention studies have shown that concentration of hemoglobin or plasma ferritin increased by supplementation with riboflavin and iron as compared to iron supplementation alone [69-72]. Other studies, however, failed to substantiate such results [73,74]. There are only a few studies on the relationship between riboflavin and anemia or iron status, thus more epidemiological and intervention studies are called for. This thesis tries to address this particular concern.

Animal, epidemiological and intervention studies have indicated that vitamin A deficiency may cause anemia [75-79]. Although the exact mechanisms are yet to be elucidated, the evidence suggests that vitamin A can modulate iron metabolism [80]. Other possible mechanisms were hypothesized: no effect of vitamin A on erythropoietin production [81], nor erythrocyte turnover [82], nor positive effect on non-heme iron absorption [83]. The beneficial effect of vitamin A on hemoglobin or iron status has been shown in several supplementation studies providing vitamin A alone or in addition to iron [3,9,85-88]. Other studies, however, have failed to reveal such an effect [11,72,89,90].

4. The importance of nutritional health for safe motherhood

In Indonesia, 27% of girls get married before 16 years of age. The national average age of marriage for girls is 19 years, and marriage is mostly followed soon by first pregnancy [91].

The few available data on Indonesian adolescent girls aged 12-18 years of age indicate that the prevalence of anemia ranges from 21% to 26%, while the prevalence of iron deficiency and vitamin A deficiency is 37% and 31%, respectively [4,10].

During this period of rapid growth, girls with micronutrient deficiencies might not be able to achieve optimal growth, especially in their reproductive capacity. Furthermore, should such a condition persist, they would be entering pregnancy plagued by anemia and micronutrients deficiencies, with all the attendant risks. Thus,

it would appear crucial that Indonesian adolescent girls should already have achieved good nutritional status before the age of 19 years. It is feared that failure to achieve this goal possibly would impose additional health burdens upon the future generation. The burden of anemia and micronutrient deficiencies will be discussed in more detail in the following sections.

4.1 Anemia

Anemia and micronutrient deficiencies during pregnancy can affect the pregnant women themselves and their pregnancy outcome. Early pregnancy anemia leads to increased risk of preterm birth and low birth weight [92-95]. An iron store at conception corresponding to serum ferritin concentration ≥ 30 µg/L is a minimal target to prevent subsequent anemia in pregnancy [58,59].

Data in the developing countries indicate that the prevalence of anemia among pregnant women is 52% [96]. In Indonesia, anemia occurs in 51% in pregnant women (unpublished national data, the Ministry of Health, 2002).

4.2 Riboflavin

In communities where riboflavin is deficient, a progressive decline in riboflavin status near parturition is common [97]. The prevalence of riboflavin deficiency during pregnancy was 98% in The Gambia and 20% in the United Kingdom [97]. Riboflavin in addition to iron supplements given to pregnant women increased hemoglobin concentration to higher levels than with either placebo or iron alone [72,74]. The studies, however, did not report the prevalence of riboflavin deficiency and its consequences on pregnancy performance and outcome.

4.3 Vitamin A

Vitamin A deficiency increases vulnerability of women during pregnancy, a time of increased vitamin A requirement [98]. Serum concentration < 0.7 µmol/L during pregnancy has been associated with preterm delivery [99]. It would appear that girls, who fail to attain adequate vitamin A stores during adolescence (as indicated by serum or plasma retinol concentration ≥ 1.05 µmol/L), are likely to be at increased risk of adverse health consequences associated with vitamin A deficiency when becoming pregnant.

It is estimated that more than 7.2 million pregnant women in the developing countries are vitamin A deficient [100], and vitamin A deficiency is considered to be a major public health problem [20]. The prevalence of vitamin A deficiency in Indonesian pregnant women ranges between 16-31% [78,101,102]. Serum retinol concentration was

found to be lower in pregnant women than in their non-pregnant peers [103].

There are several studies assessing the efficacy of vitamin A supplementation to pregnant women. A study in Indonesia showed that additional vitamin A to weekly iron supplementation during pregnancy can lead to increased vitamin A concentration in breast milk [104], increased serum retinol concentration in young infants [105] compared to iron supplements alone. Infants of mothers with adequate vitamin A status near term had better mental development than those of mothers with vitamin A deficiency [106]. In Nepal, supplementation with vitamin A or ß-carotene during pregnancy reduced maternal mortality by 40% [107], shortened the length of delivery and reduced symptoms of night blindness [108]. It has been suggested that vitamin A supplementation during the first trimester, when the formation of placenta occurs, may explain the reduced maternal mortality observed in the previous Nepal study [109]. If this were indeed the case, then vitamin A status in the first trimester of pregnancy would have been very important. It would appear that adequate store of vitamin A at conception are important to contain maternal mortality.

4.4 Iron

Iron requirement is doubled during pregnancy. Particularly in the second trimester, iron demand increases due to the expansion of hemoglobin mass and the demand placed by both the fetus and placenta [59]. As a consequence, iron sufficiency is difficult to maintain unless iron stores are adequate at the onset of pregnancy. Thus, it appears essential that women have sufficient store corresponding to a serum ferritin concentration of 30 µg/L in the absence of inflammation, because 1 µg ferritin/L corresponds to approximately 10 mg storage iron [58]. Pregnant women in developing countries, such as Indonesia, are unlikely to achieve this level due to poor intake of bioavailable iron [110].

The prevalence of iron deficiency is estimated to be 2-5 times more than iron deficiency anemia [96]. Iron deficiency among Indonesian pregnant women ranges between 45-71% [102,111].

In Indonesia it was found that iron supplementation during pregnancy, resulted in a higher increase of hemoglobin concentration and lower prevalence of anemia compared to the placebo group [88].

5. Programs to control nutritional anemia

Anemia during pregnancy is of severe public health significance in Indonesia. Moreover, challenges in combating iron deficiency are far greater and more complex in developing countries than in developed countries, due to the broader spectrum of

aggravating conditions such as poverty, poor-diets, low education and negligence, and endemic infections. Limited human and financial resources, and absence of credible monitoring systems contribute to the complexity of attaining successful outcomes.

Programs addressing anemia and iron deficiency by iron supplementation for pregnant women have been ongoing for decades. The major impediments on program effectiveness are coverage and compliance. In Indonesia for example, the effectiveness of programs is limited by insufficient tablet supply, inadequate coverage and low compliance [112,113].

Despite these programs, Indonesia has experienced little appreciable improvement over the last three decades. It was because of this, that the Government of Indonesia launched two additional nationwide programs in 1996: iron supplements aimed at children aged under 5 years and women in their reproductive age, especially factory workers. There is no such program focusing on adolescent girls, despite the fact that as adolescent girls experience menarche, they also belong to the group of females of reproductive age. Unfortunately, the program aimed at factory workers has not been adopted by most of the parties involved, and no monitoring system is yet in place.

Despite emerging data on the relationship between vitamin A and anemia, the existing program only focuses on overcoming anemia by giving iron and folic acid supplements; no other micronutrients are added in the regimen.

6. Adolescent girls as a target for programs to control micronutrient deficiencies

As reviewed above, adolescent girls with anemia probably have inadequate iron stores to prevent anemia during pregnancy. Similarly, adolescent girls with vitamin A deficiency probably have insufficient stores of vitamin A at conception to prevent deficiency during pregnancy. This underlines the importance of good micronutrients status during adolescence. Unfortunately, adolescent girls have not been accorded adequate attention. As an example, a training program on adolescents' nutritional health for the South-East Asia region was only introduced in 1999, more than two decades after initiation of the first anemia alleviation program for pregnant women.

In the Indonesian national education system, secondary schooling is included in a compulsory 9 years of basic education, so that the majority of Indonesian adolescent girls are at school approximately up to the age of 15-18 years. When they complete this compulsory education, it is a matter of few years for them to reach 19 when they are likely to have their first pregnancy. Adolescent girls attending school are readily accessible, while trying to reach them at a later stage would become increasingly difficult. Implementation, monitoring, as well as evaluation of micronutrients

supplementation programs is relatively easy in schools. Furthermore, it may well resolve the problems of tablet distribution, coverage and compliance, which constitute the major challenges currently confronting health authorities in implementing iron supplementation programs for pregnant women. Thus, efforts to reduce the high prevalence of anemia, iron and vitamin A deficiency during pregnancy need to aim at adolescent girls who are on the brink of motherhood.

7. Objectives and outline of the thesis.

Concern with finding ways and means to prevent anemia in Indonesian pregnant women, this thesis aimed at assessing deficiencies of riboflavin and vitamin A as determinants of anemia and iron status in adolescent girls.

The immediate objectives are as follows:

1. To assess the prevalence of anemia and deficiencies of riboflavin, vitamin A and iron;
2. To asses the relationship between status of riboflavin or vitamin A and iron status;
3. To measure the efficacy of supplementation with riboflavin, vitamin A and iron on anemia and indicators of iron status.

8. Study sites and design considerations

Indonesia is the biggest archipelagic country in the world, and has a population of more than 220 millions. It has only two seasons: a dry season from April to October, and a rainy season from October to April.

The studies were conducted in Central and East Jakarta, and the nearby locality of Tangerang. These locations were relatively close to laboratory facilities, where blood samples could be processed with dispatch. This was important because blood must be processed as soon as possible after specimen collection for accurate determination of a number of biochemical indicators, especially EGRAC.

Jakarta was declared malaria-free in 1962. Central Jakarta is the most-densely populated area of Jakarta, and is inhabited by people from a wide social economic spectrum. The urban slum of Central Jakarta is inhabited with a preponderance of low-income households. Most of them are small vendors, small-scale factory workers or low-rank employees. East Jakarta is contiguous to Central Jakarta, and is inhabited by a mix of low and middle-class families. Tangerang, located about 50 km west of Jakarta, is a rural area, inhabited by families whose income-earners include low rank employees, low-income farmers, small vendors and factory workers.

This study was directed towards adolescent girls aged 11-18 years, who were attending state secondary schools. Such schools usually provide education for children from low-income families. None of the schools in this study had ever been targeted for either deworming or iron supplementation programs.

Schools serve as better gathering places of subjects, because 60% of Indonesian adolescent girls attend schools (unpublished data, the Ministry of Education, 2004). Schools are convenient for implementing the intervention studies, as securing coverage and compliance becomes much easier.

We carried out cross-sectional studies to estimate the prevalence of micronutrient deficiencies, and to obtain insight into their relation to anemia. The cross-sectional studies were implemented in the following order: East Jakarta in April 1995, Tangerang in August 1999 and in December 2000, and Central Jakarta in August 2002. The intervention studies aimed to investigate into the causal effects of micronutrient supplementation on anemia and iron status. The intervention studies were conducted as follows: 16 weeks of iron-placebo supplementation in Tangerang from August to December 1998; and 8 weeks of iron-vitamin A-riboflavin in Central Jakarta from August to October 2002.

9. Ethical considerations

Before inviting girls to participate, the researcher explained the objectives and procedures of the study to all schoolgirls. In addition, each girl received a written explanation outlining the objectives and procedures of the study along with an informed consent form for her parents. Girls, who decided to participate were asked to return the completed form with both the girl's and her parent's signatures. The study protocol had received prior approval of the Ethical Committee of the Faculty of Medicine, University of Indonesia.

The chapters in the thesis address the following issues:

Chapter 2: to measure the effect of weekly iron supplementation on iron status, and to assess the result of cessation of iron supplementation on iron status;
Chapter 3: to assess the relationship between riboflavin status and iron status;
Chapter 4: to assess to relationship between status of riboflavin or vitamin A and iron status;
Chapter 5: to measure the effect of daily supplementation with riboflavin and vitamin A additional to iron on iron status.

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Chapter

2

Iron status of Indonesian adolescent schoolgirls after 16 weeks cessation of weekly iron supplementation: a randomized-controlled trial

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ABSTRACT To reduce the high prevalence of anemia during pregnancy, adolescence may be an ideal time to implement programs to improve iron status of potential mothers. A double-blind randomized placebo-controlled trial, involving 202 post-menarchal girls aged 11-17 years in Tangerang, Indonesia, was carried out to measure the effect of weekly iron supplementation on iron status in adolescent schoolgirls, after 16 weeks of supplementation, and at 16 weeks after cessation of supplementation.

Girls were randomly allocated to weekly supplementation for 16 weeks with either iron or its placebo. Concentrations of hemoglobin and serum ferritin were assessed at baseline, at 16 weeks, and at 16 weeks after cessation of the supplements.

At baseline, the prevalence of anemia, iron deficiency, iron deficiency anemia, and girls with minimal target iron status as indicated by serum ferritin concentration ≥ 30 µg/L was 54%, 21%, 15%, and 37%, respectively. At 16 weeks, the iron group had a lower prevalence of anemia (by 22%, 95%CI: 9-35%, *P < 0.001*), iron deficiency (by 12%, 95%CI: 2-22%, *P = 0.008*) and iron deficiency anemia (by 10%, 95%CI: 2-18%, *P = 0.006*), and a higher prevalence of girls with minimal target iron status (by 28%, 95%CI: 15-41%, *P < 0.001)* compared to the placebo group. After 16 weeks of cessation, the prevalence of anemia and iron deficiency anemia did not or only marginally differ between the two groups, and 60% of girls who received iron supplements had reached minimal target iron status.

Sixteen weeks of weekly iron supplementation is insufficient to reach minimal target iron stores in adolescent schoolgirls. We suggest giving iron supplements at least weekly to adolescent girls throughout the school years.

INTRODUCTION

Despite efforts by the Indonesian Government to control anemia, surveys have shown the prevalence of anemia among pregnant women to be 51%, while it was 30-50% among women of reproductive age [1,2]. This indicates that anemia remains a severe public health problem in Indonesia. However, data on anemia of Indonesian adolescent girls is scarce. Anemia among Indonesian adolescent schoolgirls was prevalent, ranges between 20-30%, and is higher than their male counter parts [3,4].

In Indonesia, data show that 27% girls get married before 16 years of age. Girls on average marry at around 19 years of age, and a first pregnancy commonly follows soon thereafter [5]. Iron requirements almost double during pregnancy: especially for women in developing countries, it is very difficult to meet this demand unless iron stores are adequate at the start of pregnancy [6]. The consequences of anemia during pregnancy include an increased risk of preterm birth and low birth weight [7-10]. This indicates that women must have sufficient iron stores at conception [6]. Thus one possible way to reduce anemia in pregnancy could be to control nutritional anemia among adolescent girls.

In 1996, the Government of Indonesia launched a nationwide program with the aims to improve health, to build iron stores in women at reproductive age and to increase productivity of female factory workers by supplementing iron tablets for 4 months every year. The efficacy of such a regimen of iron supplementation has not been studied in Indonesia. Post-menarchal adolescent girls attending schools are readily accessible and could become an important target group for this new initiative.

This study aimed to measure the effect of weekly iron supplementation on iron status in Indonesian adolescent schoolgirls after 16 weeks of supplementation, and at 16 weeks after cessation of the supplementation (hence, at 32 weeks from baseline).

MATERIALS AND METHODS

Subjects

This study was conducted in a public junior high school in a rural area of Cikupa, Tangerang, Indonesia, where there was no on-going program for deworming or iron supplementation of school children. The school housed 227 post-menarchal girls.

We included girls with no self-reported blood abnormalities or chronic diseases, and who were willing to participate in the study. At one week before randomization, they received one single dose of 500 mg mebendazole under supervision by the researcher (DD) to control worm infections.

The intervention and supplements

A colleague blindly and randomly allocated each girl to either placebo or iron group using tables with random numbers, after eligibility had been fully established. For each girl, an independent staff packed the tablets for subsequent distribution in separate plastic bags with the name of the girl and the appropriate school class.

Each iron tablet contained 60 mg iron as $FeSO_4.7H_2O$ and 0.25 mg folic acid. Placebo tablets were indistinguishable in shape and color from tablets with iron. Both tablets were produced by PT Indo Farma, Jakarta, Indonesia. Tablets were given during mid-morning school break for morning classes and before school started for afternoon class, so that in both occasions, girls took the tablets with some food. Girls swallowed one tablet every week for 16 weeks, under supervision of the researcher (DD); those who were absent received their tablet the following day under supervision by a teacher who assisted actively during the implementation of the study. During the study period, girls were asked not to consume any other vitamin or mineral supplements or change their dietary habits.

Girls, who were still anemic after the study had been completed, received one iron tablet weekly for 16 weeks under the teacher's supervision.

Assessment methods

A structured questionnaire was used to obtain data on socio-economic, health and menarche status at baseline, and any possible self-administration of iron tablets during the 16 week cessation of the supplementation.

Height was measured to the nearest 0.1 cm, using a microtoise (CMS Ltd, London, UK), and weight was assessed to the nearest 0.1 kg, using digital weight scales (Model 770, SECA, Hamburg, Germany).

Approximately 2.5 mL of venous blood was drawn from girls in the non-fasting state. Immediately thereafter, 1 mL of blood was transferred to an EDTA tube and the rest was kept in another tube without anticoagulants. All blood samples were kept in a cool box during transport to the laboratory.

Hemoglobin concentration was measured on the EDTA blood within 5 hours of collection by the cyanmethaemoglobin method, using controls and reagents from the manufacturer (Haemoglobin Merckotest no.1.03317.0001, Merck, Darmstadt, Germany), and using a photometer (Eppendorf PCP 6121, Hamburg, Germany).

Blood samples collected in tubes without anticoagulants were centrifuged within 2 hours after blood collection for 15 minutes (5000 x g) at 10-15°C to obtain serum for subsequent measurement of ferritin concentration. The sera were kept in freezer at -20°C before laboratory analysis, which took place within 36 weeks. Serum ferritin concentration was measured in an IMx instrument (Abbott Laboratories, Abbott Park, Ill, USA) using reagents, calibration and control sera from the same manufacturer (IMx reagent pack no.2219-20, IMx Ferritin Mode 1 calibrator no.2219-01, IMx Ferritin controls no.2219-10). In addition to using control sera, reproducibility and accuracy were checked by measuring 10% of the samples in duplicate. The coefficient of variation of all measurements was <10%. All biochemical analyses were performed at the SEAMEO-TROPMED Laboratory, Jakarta.

Collection of both anthropometric and blood samples were repeated at 16 weeks after supplementation, and at 16 weeks after cessation of the supplementation (at 32 weeks from baseline).

At 32 weeks from baseline, girls were asked to bring fresh stool samples in standard plastic containers. Within 4 hours of collection, the samples were transferred to a special stool storage refrigerator. Worm infestation was assessed by Kato-Katz method and based on stool egg count [11]. These tests were performed within 48 hours after collection, at the Department of Parasitology, Faculty of Medicine, University of Indonesia.

Data analysis

Epi-Info 2002 (CDC, Atlanta, GA 30341-3717, USA) was used to calculate the body mass index (BMI) Z-score.

We used the following definitions: anemia: hemoglobin concentration <120 g/L [12]; iron deficiency: serum ferritin concentration <12 µg/L [13]; iron deficiency anemia: coexisting anemia and iron deficiency. We considered serum ferritin concentration ≥ 30 µg/L in the absence of inflammation as a minimal target iron status [6,14].

Severity of worm infestation was defined based on criteria by the World Health Organization [15].

We analyzed the data using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). Data that was not normally distributed as assessed by visual inspection of distributions and Kolmogorov-Smirnov tests were log-transformed as appropriate. The Mann-Whitney U-test was used to assess group differences in variables that were not normally distributed. Treatment effects were evaluated using linear regression techniques by assessing group differences in indicators measured at 16 weeks and at 32 weeks, with and without adjustment for baseline binary variables, namely anemia (present or absent), iron deficiency (serum ferritin concentration <12 µg/L or ≥ 12 µg/L), household size (2-4, 5-6, 7-8, ≥ 8 members) and self-administered iron supplementation during the cessation of supplementation (yes or no).

RESULTS

Of 227 post-menarchal girls attending the school, 202 were randomized, and 191 completed the study (Figure 1). Data on serum ferritin concentration was not available for 8 girls at baseline and 16 weeks after supplementation (due to either hemolysis or insufficient sera). At baseline, 44% of the girls reported that their menstrual periods had not been regular in the previous four months. The girl's age ranged between 11.8-16.5 years. The girls participating in the trial consumed at least 90% of the scheduled tablets. Thirty four (18%) girls reported to have taken iron supplements during 16 weeks cessation of the supplementation. Only 132 girls submitted stools: 23% of them had light ascariasis or trichuriasis, and we detected no girls with hookworm.

At baseline, the prevalence of anemia, iron deficiency, iron deficiency anemia, and girls with minimal target iron status was 54%, 21%, 15%, and 37%, respectively. The prevalence of girls with BMI Z-score < -2.00 SD was 4%. Age, age at menarche, time since menarche, BMI Z-score and hemoglobin concentration were similar between treatment groups (Table 1).

Figure 2 shows the prevalence of anemia, iron deficiency, iron deficiency anemia and girls with minimal target iron status by supplementation groups at 16 weeks. At 16 weeks, the prevalence of anemia, iron deficiency, iron deficiency anemia in the iron group was 22% (95% CI: 9-35%; *P<0.001)*, 12% (95% CI: 2-22%; *P=0.008*), 10% (95% CI: 2-18%; *P=0.006*) lower, respectively, compared to the placebo group, while the prevalence of girls with minimal target iron status was 28% (95% CI: 15-41%; *P<0.001)* higher. We found no evidence of group differences at 16 weeks after

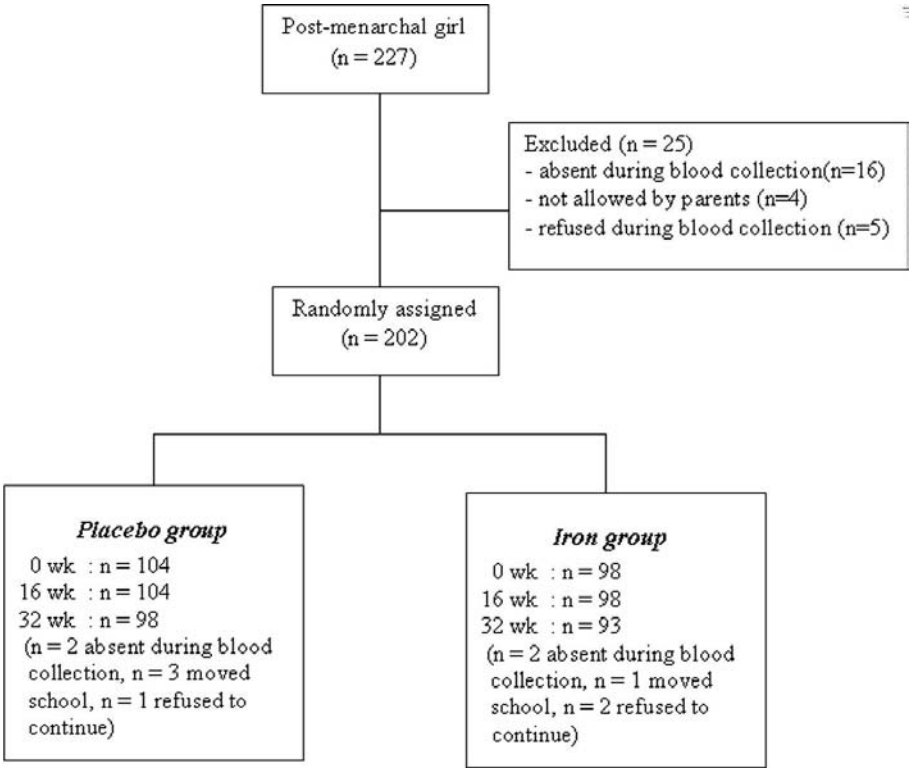

FIGURE 1. Flow diagram of Indonesian adolescent schoolgirls through randomization.

cessation of iron supplementation in the prevalence of anemia and iron deficiency anemia; however, the iron group had 11% (95% CI: 1-21%, *P = 0.05*) lower prevalence of iron deficiency and 26% (95% CI: 12-40%, *P<0.001*) higher prevalence of girls with minimal target iron status compared to the placebo group.

Table 2 shows the effects of weekly iron supplementation. At 16 weeks, concentrations of hemoglobin and serum ferritin were higher in the iron group than in the placebo group (2.9 g/L and 21%, respectively; adjusted for baseline factors). At 32 weeks, however, the corresponding values were 1.2 g/L and -12%, respectively.

There was no evidence that the effect of iron supplementation on hemoglobin concentration at 16 weeks (2.3 g/L; 95% CI: -2.2 to 6.8 g/L) or 32 weeks (1.1 g/L; 95% CI: - 4.1 to 6.2 g/L) depended on baseline anemic status. Neither did serum ferritin concentration at 16 weeks (17%; 95% CI: -22 to 77%) or at 32 weeks (-15%; 95% CI: - 46 to 34%) depend on baseline iron deficiency.

Girls in the iron group, who were anemic at baseline, 29% remained anemic after iron supplementation for 16 weeks. Seven percent of girls with iron deficiency anemia at baseline and who received iron supplements, remained anemic at 16 weeks, although their serum ferritin concentrations increased to values of ≥ 12 µg/L.

TABLE 1.

Baseline characteristics of Indonesian adolescent schoolgirls, by intervention group

	Intervention group	
	Placebo	Iron
n	104	98
Age, *year*	14.2 ± 0.9	14.1 ± 0.9
Age at menarche, *year*	12.2 ± 0.9	12.0 ± 1.0
Time since menarche, *month*	21.7 (14.2; 30.4)	21.8 (13.4; 36.9)
Body mass index Z-score	- 0.35 ± 0.75	- 0.40 ± 0.86
Hemoglobin concentration, *g/L*	116.9 ± 11.1	118.9 ± 12.2
Serum ferritin concentration, *µg/L* [1]	19.8 (11.6; 37.5)	25.8 (15.9; 41.4)

Values are mean ± SD or median (25^{th}; 75^{th} percentile).
[1]Placebo group (n = 101) and Iron group (n = 93).

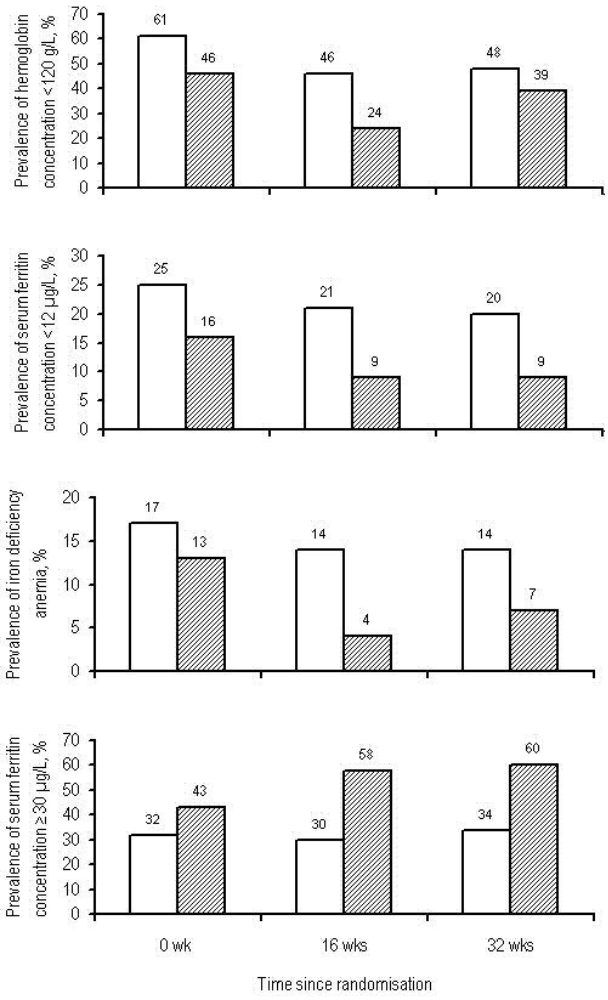

FIGURE 2. Prevalence of anemia, iron deficiency and minimal target iron status of Indonesian adolescent schoolgirls in placebo (□) and iron group (▨) at different times during the study period.

TABLE 2

Effects of iron supplementation on concentrations of hemoglobin and serum ferritin in Indonesian adolescent schoolgirls at baseline, after 16 weeks and 32 weeks, by treatment group

	Placebo group	Iron group	Crude treatment effect [a]	Adjusted treatment effect
Hemoglobin concentration, *g/L*				
Baseline	116.9 ± 11.1	118.9 ± 12.2	Not applicable	Not applicable
At 16 wk	119.7 ± 9.7	125.0 ± 9.3	5.3 [2.5 to 8.0]	3.5 [1.3 to 5.8] [b]
At 32 wk	119.2 ± 10.5	122.1 ± 10.5	2.9 [- 0.1 to 5.9]	0.9 [- 1.6 to 3.4] [c]
Serum ferritin concentration, *µg/L*				
Baseline	19.8 (11.6; 37.5)	25.8 (15.9; 41.4)	Not applicable	Not applicable
At 16 wk	21.5 (12.9; 33.6)	38.1 (22.4; 55.8)	76% [42% to 118%]	58% [33% to 86%] [b]
At 32 wk	24.6 (14.2; 36.6)	35.7 (18.9; 47.4)	42% [14% to 78%]	28% [7% to 55%] [c]

Mean ± SD or median (25^{th}; 75^{th} percentile)

[a] Treatment effects [95% confidence interval] were calculated as the difference in hemoglobin concentration between iron and placebo group, or as the percentage change in serum ferritin concentration in the iron group relative to the placebo group.

[b] As above ([a]) and adjusted for baseline factors (anemia or not anemia; serum ferritin concentration < 12 µg/L or ≥ 12 µg/L); household size (2-4, 5-6, 7-8, ≥ 8 members).

[c] As above ([b]) and additionally adjusted for self-administered supplements during 16 weeks cessation of iron supplementation (yes or no).

DISCUSSION

In this study, the iron status of adolescent schoolgirls improved after 16 weeks of weekly iron supplementation, but these gains had largely disappeared at 16 weeks after cessation of supplementation.

Few participants dropped-out, and compliance was excellent. Infection-induced inflammation can result in increased serum ferritin concentration independently of iron status [16,17]. At baseline, however, girls received mebendazole to control worm infestation, and worm infestations measured at the end of the intervention were light. Thus, although we did not measure indicators of inflammation, we are confident that the group differences in serum ferritin concentration measured at 16 weeks and 32 weeks after randomization indicated differences in iron status due to iron supplementation.

Similar to findings from other studies [18-21], we found that iron supplementation increased concentrations of hemoglobin and serum ferritin in adolescent schoolgirls. Furthermore, similar to other studies [22,23], we found that 16 weeks cessation of iron supplementation caused a decline in iron status; although its effect on serum ferritin concentration could still be noticed, the magnitude was less than half of the initial improvement (Table 2). The study on Indonesian adolescent girls [22], which compared weekly versus daily supplementation with iron, folic acid, vitamin C and vitamin A for 12 weeks, showed that 6 months after cessation of the supplementation, the decrease in plasma ferritin concentration in the daily group was larger compared to the weekly group, leading to a similar plasma ferritin concentration between the two regimens. Hemoglobin concentration continued to increase in Malaysian girls who received weekly supplementation with iron and folic acid for 22 weeks compared to the level at 12 weeks, while plasma ferritin concentration did not decline [18].

In yet another study among Indonesian adolescent schoolgirls [20], it was found that weekly iron supplementation (60 mg elemental iron/week) was more efficacious in improving hemoglobin concentration than daily supplementation (60 mg elemental iron/day) for 4 days provided during menstruation.

It has been recommended that women enter pregnancy with iron stores of ≥ 300 mg [6], which corresponds to serum ferritin concentrations ≥ 30 µg/L[14]. In the iron group, we observed that the prevalence of girls with minimal target iron status had increased, whereas in the placebo iron group this prevalence persisted at levels found at baseline (Figure 2). This indicates that girls without iron supplementation can hardly reach that target. The high prevalence of anemia in these girls at 16 weeks after cessation indicates that our regimen of iron supplementation for 16 weeks was insufficient to control anemia in these girls.

The iron requirement of adolescent girls who have already experienced menarche is between 1.39 - 2.54 mg/d [24]. Dietary recall data indicate that the iron intake of at least half of adolescent girls in both developed and developing countries are less than the recommendation daily allowance [25,26]. Although data on the iron intake by Indonesian adolescent girls is not available, we suspect that the iron intake of the girls is also low.

Our findings suggest that a regimen of weekly iron supplementation should be given for periods longer than 16 weeks. In Indonesia, basic education is compulsory for 9 years, and covers children aged 6-17 years. Approximately 60% of Indonesian adolescent girls attend schools (unpublished data, the Ministry of Education, 2004), and most of these girls attend school up to the age of 15-17 years. Weekly iron supplementation throughout the school years could be integrated with several on-going health activities in state schools. It appears that such a program would provide an opportunity to help 6.5 million adolescent girls attending schools in Indonesia (unpublished data, the Ministry of Education, 2004) preparing for safe motherhood.

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Chapter

3

Riboflavin status in relation to hemoglobin concentration and iron stores in Indonesian adolescent schoolgirls

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ABSTRACT Anemia is mostly caused by iron deficiency, however, other micronutrient, riboflavin, is hypothesized to have related to anemia and iron status. A cross-sectional study was conducted to investigate erythrocyte glutathione reductase activation coefficient (EGRAC), an indicator of riboflavin status, in relation to concentrations of hemoglobin and plasma ferritin in a population of adolescent girls with a high prevalence of anemia.

The study was carried out in a school of urban East Jakarta, Indonesia. One hundred seven post-menarchal adolescent schoolgirls aged 15–18 years from middle socio-economic class, participated in the study.

The prevalence of anemia, iron deficiency, iron deficiency anemia, and riboflavin deficiency was 45%, 25%, 25%, and 21%, respectively. No hookworm egg was detected, but 9% of girls had light ascariasis and 14% had light trichuriasis. Only 55% and 1% of girls met their dietary requirements for riboflavin and iron, respectively. Anemic girls had lower riboflavin status and lower plasma ferritin concentration compared to non-anemic girls. EGRAC was negatively related to concentrations of hemoglobin and plasma ferritin.

Anemia and inadequate iron stores are a major public health problem in Indonesian adolescent girls. Riboflavin status is negatively related to concentrations of hemoglobin and plasma ferritin. A randomised controlled trial is required to assess the extent to which supplementation with riboflavin in addition to iron can overcome this problem.

INTRODUCTION

Although anemia is most commonly associated with iron deficiency, studies in humans have shown that riboflavin deficiency can contribute to anemia and reduced iron status. Thus concentrations of hemoglobin and plasma ferritin were increased when riboflavin was supplemented in addition to iron compared to iron supplementation alone [1-4]. However, other studies could not show clear beneficial effects of riboflavin supplementation on hemoglobin concentration [5,6].

The prevalence of riboflavin deficiency as reported from developing countries ranges from 42 to 96% [3,7-9] as compared to 16 to 29% in developed countries [5,8,10,11,]. Male adolescents appeared to have a better riboflavin status than their female counterparts [10]. There is no data for Indonesian adolescent girls.

Animal products especially milk are good sources of riboflavin. Low riboflavin status was observed in individuals with low intakes of milk or meat [8,13]. Among adolescents in New York, more milk consumption was associated with a declining prevalence of riboflavin deficiency [11]. In the UK, it has been shown that among young people aged 4-18 years, milk consumption declines with age, and that 95% of girls aged 15-18 years were riboflavin deficient [14]. In Indonesia, where intake of animal products and milk is low, it would seem likely that riboflavin deficiency is highly prevalent among adolescent girls.

Anemia and iron deficiency are the main nutritional problems during adolescence [15]. Studies have shown a much higher prevalence of anaemia associated with iron deficiency among both adolescent girls and young female adults compared to their male counterparts [16-18].

We hypothesized that both riboflavin and iron deficiency are common among Indonesian adolescent girls, and that riboflavin deficiency is related to anaemia or iron deficiency. This study was aimed to investigate riboflavin status in relation to concentrations of hemoglobin and plasma ferritin of adolescent schoolgirls in Jakarta.

MATERIALS AND METHODS

Subjects

This cross-sectional study was conducted in a public senior high school in a suburb area of East Jakarta. The municipal education authority appointed the school as the site for the study. This school housed 264 post-menarchal girls.

Assessment methods

Data on socio-economic status were obtained from school registers, and information on health and menarche were obtained using a structured questionnaire.

Dietary intake was assessed on 2 consecutive days, without weekend days, using a 24-hour recall method.

Girls were measured barefoot while wearing school uniforms of similar weight. Height was measured to the nearest 0.1 cm, using a microtoise (CMS Ltd, London, UK), and weight was assessed to the nearest 0.1 kg, using digital weight scales (Model 770, SECA, Hamburg, Germany).

Approximately 2.5 mL venous blood was drawn from girls in the non-fasting state. Immediately thereafter, 1 mL of blood was transferred to an EDTA tube and the remaining blood was transferred to a heparinised tube. All tubes were wrapped in black paper to protect blood samples from light exposure, and they were kept in a cool box during transport to the laboratory.

The heparinized blood samples were centrifuged for 15 minutes (5000 x g) at 10-15^{o}C to obtain plasma for ferritin concentration measurement. The packed red blood cells were washed 3 times with sterile physiological saline solution and stored for determination of the erythrocyte glutathione reductase activation coefficient (EGRAC) as an indicator of riboflavin status. Both plasma and washed red blood cells were kept at -20C for subsequent biochemical analysis, which was conducted within 30 days after blood collection.

Hemoglobin concentration was measured in the EDTA blood sample within 5 hours of collection by the cyanmethemoglobin method with reagents from the manufacturer (Hemoglobin Minoton, Germany), using a coultercounter (Minos STE 8P, Roche, Germany).

Plasma ferritin concentration was determined in an IMx instrument (Abbott Laboratories, Abbott Park, Ill, USA), using reagents, calibration and control sera from the manufacturer (IMx reagent pack no. 2219-20, IMx Ferritin Mode 1 calibrator no. 2219-01, IMx Ferritin controls no.2219-10). EGRAC was assessed using a modified Glatzle method [19]. The analyses were performed at SEAMEO-TROPMED

Laboratory, Jakarta. In addition to using control and calibrator sera, reproducibility and accuracy were checked by analysing 10% of the concentrations of hemoglobin and plasma ferritin in duplicate.

Each girl was provided with a standard plastic container and asked to bring a fresh stool sample. Assessment of worm infestation, based on stool egg counts by Stoll method [20], was performed within 48 hours after collection, at the Department of Parasitology, Faculty of Medicine, University of Indonesia.

Data analysis

Socio-economic class was defined based on World Bank classification [21]: low (≤$675/capita/year), middle ($676-8,355/capita/year), and high (≥ $8,356/capita/year).

The mean daily energy and nutrient intake were calculated using the Indonap Program with Indonesian food composition data [22] or Malaysian food composition data [23] for foods not listed in the Indonesian food composition data. Intake of nutrients was compared to Indonesian Recommended Daily Allowances[24].

We used the following definitions: anemia: hemoglobin concentration <120 g/L [25]; iron deficiency: plasma ferritin concentration <12 µg/L [26]; iron deficiency anemia: co-existing anemia and iron deficiencyy; riboflavin deficiency: EGRAC >1.4[27]. We considered plasma ferritin concentration ≥ 30µg/L in the absence of inflammation as a minimal target for iron status [28,29].

Severity of worm infestation was classified according to criteria from the World Health Organization classification [30] into light, average, and severe infection.

Data, that were not normally distributed as assessed by visual inspection of distributions and Kolmogorov-Smirnov test, were log-transformed as appropriate. Independent t-test or Mann-Whitney U-test was applied to assess differences in nutritional status and intakes of nutrients between anemic and non-anemic girls. Fisher's exact test was performed to examine relationships between binary variables. Linear regression analyses were performed to assess the associations between riboflavin status and concentrations of hemoglobin or plasma ferritin, with and without adjustment for worm infestations and socio-economic strata as potential confounders.

We analyzed the data using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL 60606, USA).

RESULTS

Of 257 girls who agreed to participate, we randomly selected 114, and obtained complete data for 107. Most girls (77%) came from middle socio-economic class, and their age ranged 15-18 years. All participating girls stated not to have been hospitalized within 3 months, nor to have experienced diarrhoea or fever within 2 weeks before the interview. No hookworm egg was detected, but 9% of the girls had light ascariasis and 14% had light trichuriasis.

The prevalence of anemia, iron deficiency, iron-deficiency anemia and riboflavin deficiency was 45%, 25%, 25% and 21%, respectively, while 50% of the girls had plasma ferritin concentrations ≥ 30 µg/L. In girls with riboflavin deficiency, the corresponding values were 45.8%, 81.5% and 81.5%. None of the girls with riboflavin sufficiency had anemia, iron deficiency or iron-deficiency anemia. The data on food intake indicated that only 55% and 1% of girls met their dietary requirements for riboflavin and iron, respectively.

Table 1 shows characteristics of the girls by anemia status. Anemic girls had similar height, weight and intake of energy, total protein, iron and riboflavin as their non-anemic counterparts. The EGRAC was higher ($P<0.001$) and plasma ferritin concentration was lower ($P<0.001$) in anemic girls as compared to non-anemic girls.

Figure 1 shows the relationships between EGRAC with concentrations of hemoglobin or plasma ferritin. Univariate linear regression analysis showed that EGRAC was negatively related to hemoglobin concentration and plasma ferritin concentration: an increase of 1 unit EGRAC corresponded to a 33.5 /L (95% CI: 27.8–39.2 g/L) decrease in hemoglobin concentration, and 92% (95% CI: 89-94%) decrease of plasma ferritin concentration. Adjustment for ascariasis, trichuriasis and socio-economic status resulted in similar estimates. The 5-, 25-, 75-, and 95-percentiles of EGRAC were 0.94, 1.07, 1.31 and 1.97, respectively. Thus the relationships as described in the preceding paragraph indicate that an increase in EGRAC corresponding to the interquartile range (0.24) would be related to a decrease in hemoglobin concentration of 8 g/L and a decrease in plasma ferritin concentration of 46%.

TABLE 1.
Indicators of nutritional status and intake of energy and nutrients
of Indonesian adolescent schoolgirls in East Jakarta, by anemia status

	Anemic	Non-anemic
n	48	59
Weight, *kg*	46.3 ± 5.1 (0.7)	48.6 ± 6.8 (0.9)
Height, *cm*	154.6 ± 4.3 (0.6)	154.1 ± 4.5 (0.6)
Plasma ferritin concentration, *µg/L*	10.4 ± 18.3	39.8 ± 20.6
EGRAC [1]	1.37 ± 0.32	1.12 ± 0.12
Intake/day		
Energy, *MJ*	8.1 ± 1.6	8.0 ± 1.4
Total protein, *g*	51.3 ± 10.4	51.2 ± 10.3
Iron, *mg*	11.6 ± 4.1	10.4 ± 3.3
Riboflavin, *mg*	1.0 ± 3.7	0.9 ± 6.4

Values are mean ± SD (SE) or median ± SD

[1] EGRAC: erythrocyte glutathione reductase activation coefficient

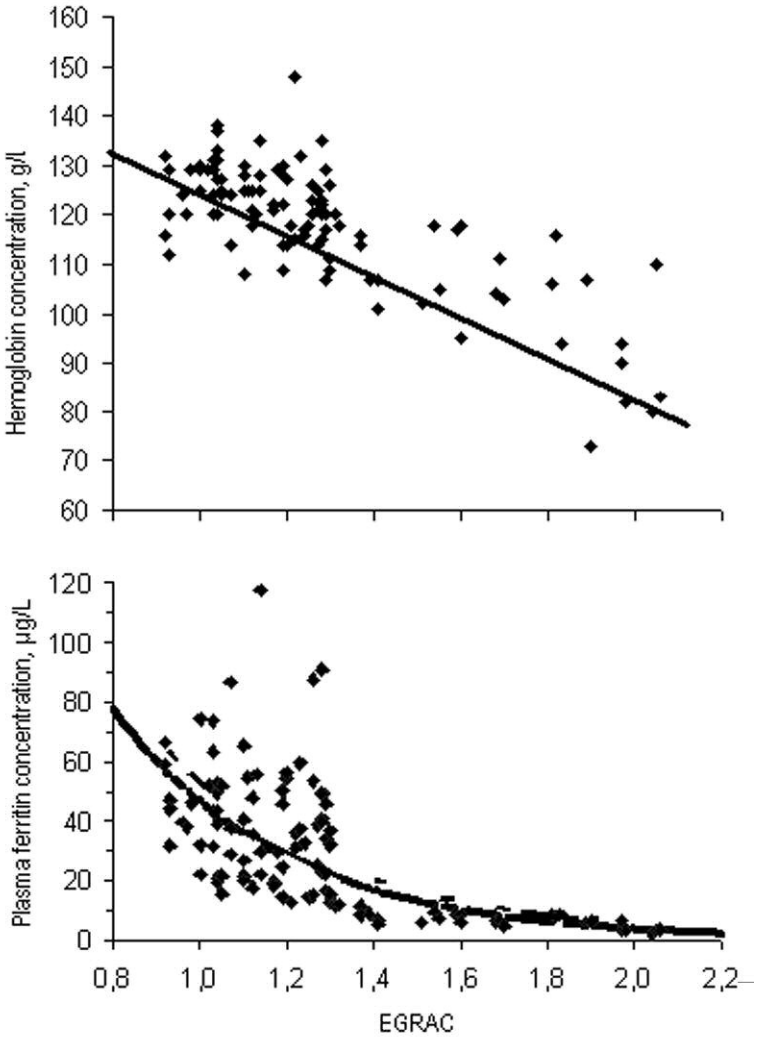

FIGURE 1. Erythrocyte glutathione reductase activity coefficient (EGRAC) in relation to hemoglobin concentration and plasma ferritin concentration in Indonesian adolescent schoolgirls.

DISCUSSION

This study showed that in Indonesian adolescent schoolgirls in Jakarta, riboflavin status was positively related to concentrations of hemoglobin and plasma ferritin. There was no evidence that our results were confounded by worm infestations or socio-economic status. Worm infestations were observed in only few girls and low in intensity, and are unlikely to have influenced our results.

In this study, we did not measure indicators of inflammation, such as plasma C-reactive protein concentration. However, very few girls had worm infections, and other infections are probably uncommon in this population. Additionally, in two earlier studies among adolescent schoolgirls, we found no evidence of a relationship between plasma C-reactive protein concentration and EGRAC (DD, unpublished data), although others have described such an association in rheumatoid arthritis [30]. Thus, we consider it to be unlikely that infection-induced inflammation led to substantial confounding in the relationship between EGRAC and serum ferritin in this study (Figure 1, bottom).

Various mechanisms have been proposed on how riboflavin status can influence hemoglobin concentration or iron status. Riboflavin deficiency is associated with erythroid hypoplasia and reticulocytopenia [14,31]. The impaired erythropoiesis is probably due at least in part to disturbances in iron absorption and metabolism [14,32]. In vitro and animal studies have shown that riboflavin in its biologically active forms can play a role in the reduction and thereby release of intracellular ferritin iron [33-35]. Thus it appears that riboflavin is needed to release iron from ferritin in enterocytes to transferrin in plasma, and to mobilise iron from stores in the liver, marrow and spleen. Additionally, in infants, riboflavin deficiency can lead to disturbances in the gastrointestinal structure so that the duration of the functional maturity of enterocytes is reduced [33,36]. Both the impaired release of iron from enterocytes and the reduced life span of enterocytes may contribute to the loss of absorbed iron.

In this study, we observed that the prevalence of anemia was 45%, whilst a prevalence > 40% is considered as severe public health significance [25]. Plasma ferritin concentration reacts as an acute phase protein and is increased in inflammation independently of iron status [37]. Because we had no data on inflammation, we cannot rule out that we underestimated the prevalence of iron deficiency (25%) and iron deficiency anemia (25%), and overestimated the prevalence of girls who reached minimal target iron status (50%). However, as we argued above, the effect of inflammation in our study was probably marginal.

We anticipated and found that the intakes of iron and riboflavin were low, as also reported in other studies [9,14,38]. Earlier studies had shown that the intake of

energy and nutrients in adolescent girls is low, which is associated with undernutrition and nutritional anemia [11,39-42].

We conclude that anemia and inadequate iron stores are a major public health problem in Indonesian adolescent schoolgirls, and that a randomised controlled trial is required to assess the extent to which supplementation with riboflavin in addition to iron can alleviate this problem.

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Chapter

4

Concentrations of hemoglobin and plasma ferritin are not related to riboflavin status but to vitamin A status in Indonesian adolescent schoolgirls

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ABSTRACT Anemia is mostly caused by iron deficiency, while several vitamins are related to anemia. Riboflavin and vitamin A are postulated to have relationships with anemia or iron deficiency, however, data was scarce on adolescent girls. Several cross-sectional studies on Indonesia adolescent schoolgirls were carried out to assess the relationship between status of riboflavin and iron and between status of vitamin A and iron. The age range of the post-menarchal girls was 10.9-16.7 years. The prevalence of anemia, deficiencies of iron, riboflavin and vitamin A ranged 9-57%, 20-57%, 59-96% and 7-21%, respectively. The prevalence of girls with iron stores below a minimal target (as indicated by plasma ferritin concentration < 30 µg/L) and with inadequate vitamin A stores (as indicated by plasma retinol concentration < 1.05 µmol/L) ranged 63-72% and 40-67%, respectively. Status of riboflavin was not related to concentration of hemoglobin or plasma ferritin, however, vitamin A deficiency was related to low concentration of hemoglobin and plasma ferritin concentration.

INTRODUCTION

Anemia is commonly associated with iron deficiency; however, deficiencies of other micronutrients such as vitamin A and riboflavin have also been shown to be associated with anemia and disturbances of iron metabolism.

Intervention studies have shown that riboflavin supplementation can reduce anemia or increase iron status in pregnant and lactating women, men and school children [1-5]. However, no data are available on the effect of riboflavin supplementation in adolescents.

Cross-sectional studies [6-13] have shown that vitamin A status is related to anemia or iron metabolism, whilst intervention studies have shown that vitamin A supplementation can reduce the prevalence of anemia [14-18]. Most of the above studies included young children or pregnant women, and only three studies involved adolescent girls [12,13]. In other studies that included adolescent subjects, such relationships could not be shown [19-21].

There are few data on the vitamin A status and iron status of Indonesian adolescents, and no data are available on the prevalence of riboflavin deficiency. a study in east Java, Indonesia has shown that the prevalence of vitamin A deficiency was similar in adolescent schoolgirls and their male counterparts [21]. In east Jakarta, however, the prevalence of anemia and iron deficiency was higher in schoolgirls than schoolboys [22].

We used data from several cross-sectional studies in post-menarchal adolescent schoolgirls in Indonesia to assess the relationships between status of riboflavin and iron, and between status of vitamin A and iron. Iron status was assessed by concentrations of hemoglobin and plasma ferritin.

MATERIALS AND METHODS

Subjects

We conducted three cross-sectional studies in arbitrarily selected state secondary schools that had not been involved in any deworming or iron supplementation programs. The following schools were included: one school in rural Tangerang, where data were obtained in August 1999 during the dry season, and in December 2000 during the rainy season (henceforth referred to as Tangerang-1 and Tangerang-2, respectively); and five schools in an urban slum of central Jakarta, where data were

obtained in August 2002 during the dry season (henceforth referred to as Central Jakarta). Girls from the Tangerang school, who were enrolled in the 1999 study, were not included in the 2000 study.

Girls enrolled in the state schools usually come from families of middle-low socio-economic status. The school registers indicated that girls from both Central Jakarta and Tangerang came from families of low rank employees, factory workers or small vendors, while girls from Tangerang also came from small farm households.

The schools involved in Tangerang-1, Tangerang-2 and Central Jakarta housed 284, 204, 1223 post-menarchal girls, respectively.

For the Central Jakarta schools, we used baseline data for girls who participated in a randomized controlled trial (chapter 5). These girls had been screened for anemia using a battery-operated portable hemoglobinometer (HemoCue, Ängelholm, Sweden). Girls in the Tangerang school had not undergone such screening.

We excluded from the analysis one girl in Tangerang-1 with an indication of abnormally high iron stores (plasma ferritin concentration of 572 µg/L) and all girls with inflammation as indicated by plasma C-reactive protein concentrations > 5 mg/L (5%, 15% and 3% of girls studied in Tangerang-1, Tangerang-2 and Central Jakarta, respectively). All girls reported to have no history of blood diseases or chronic diseases, and had not been treated for any health problems.

After all assessments were completed, all anemic girls of Tangerang school received one iron tablet weekly for 16 weeks under the supervision of their teacher.

Assessment methods

A structured questionnaire was used to obtain information on health, menarche and the use of vitamin or mineral supplements.

Height was measured to the nearest 0.1 cm, using a microtoise (CMS Ltd, London, UK), while weight was assessed to the nearest 0.1 kg using a digital weighing scale (Model 890, SECA, Hamburg, Germany). Girls were measured barefoot while wearing school uniforms of similar weight from their respective schools. Equipment was calibrated before use. In each study, the same observer performed the measurements in duplicate. Epi-Info 2002 (CDC, Atlanta, GA 30341-3717, USA) was used to calculate the body mass index (BMI) Z-score.

Approximately 4 mL of non-fasting cubital venous blood was drawn into tube without anticoagulant, and protected from light. Soon after blood collection, 0.5 mL of blood was transferred to an EDTA tube, and the remaining blood was transferred to a heparinized tube and kept in a cold box during transport to the laboratory.

Hemoglobin concentration was measured in the EDTA blood sample within 5 hours of venous blood collection by the cyanmethemoglobin method with standard solutions and reagents from the manufacturer (Hemoglobin Merckotest Merck, Darmstadt, Germany), using a photometer (Eppendorf PCP 6121, Hamburg, Germany). Within 3 hours after blood collection, the heparinized blood samples were centrifuged for 15 minutes (5000 x g) at 5-10ºC. The packed red blood cells were washed 3 times with sterile physiological saline for the measurement of erythrocyte glutathione reductase activation coefficient (EGRAC) as an indicator of riboflavin status. Both plasma and washed packed red blood cells were kept at -70ºC for measurements within 4 months after collection.

Plasma ferritin concentration was measured as follows: in Tangerang-1 study it was measured using an IMx instrument (Abbott Laboratories, Abbott Park, Ill, USA), with reagents, calibration and control sera from the manufacturer; in Tangerang-2 and Central Jakarta studies, it was measured by a sandwich enzyme-linked immunosorbent assay (ELISA) [23], using a Labsystems Multiskan Ascent instrument (Helsinki, Finland) with antibody ferritin and anti-feritin-horse radish perioxidase (code A0133 and P 0145, Dako, Glostrup, Denmark). Ten sera that had been arbitrarily selected were measured in duplicate to compare results from the two different methods, resulting in a coefficient of variation of <10%.

Plasma concentration of C-reactive protein (CRP) was measured by sandwich ELISA method [23], with antibody CRP and anti-CRP-HRP (no.A0073 and no.P0027, Dako). The EGRAC was measured using a modified method [24] in erythrocytes collected in Tangerang-2 and Central Jakarta, but not in Tangerang-1. Plasma retinol concentration was measured as an indicator of vitamin A status by high-pressure liquid chromatography (Waters 515, Milford, MA 071757, USA), using an external serum reference (no.968a, NIST, Gaithersburg, MD 20899, USA).

In addition to using control sera, the reproducibility and accuracy were checked by running pooled sera and 10% of samples in duplicate, during each measurement. The coefficient of variation of all measurement was <10%. All laboratory analyses were performed at the SEAMEO-TROPMED Regional Center for Community Nutrition, University of Indonesia, Jakarta, Indonesia.

Data analysis

We analyzed the data using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL 60606, USA), and used the following definitions: inflammation: plasma CRP concentration

>5 mg/L; wasting: BMI Z-score ≤ -2.00 SD; anemia: hemoglobin concentration <120 g/L [25]; iron deficiency: plasma ferritin concentration <12 µg/L [26]; vitamin A deficiency and low vitamin A status: plasma retinol concentrations <0.70 µmol/L and 0.70-1.05 µmol/L, respectively [27]; riboflavin deficiency: EGRAC >1.4 [87]. We considered plasma ferritin concentration ≥ 30 µg/L in the absence of inflammation as a minimal target for iron status [29,30], and a plasma retinol concentration of ≥ 1.05 µmol/L as an indication of adequate stores of vitamin A [27].

Data which were not normally distributed as assessed by visual inspection of distributions and the Kolmogorov-Smirnov test, were log-transformed as appropriate. Group differences were examined by independent t-tests and linear regression analyses for variables that were normally distributed; in all other cases, the Kruskal-Wallis test was used.

In a multivariate linear regression analysis, we further examined independent relationships between hemoglobin concentration and iron status (plasma ferritin concentration <12 µg/L and ≥ 12 µg/L), vitamin A status (plasma retinol concentration < 0.70 µmol/L, 0.70-1.05 µmol/L and ≥ 1.05 µmol/L) and riboflavin status (EGRAC ≤ 1.4 or >1.4), use of supplements by the girls, while adjusting for study site. Similar analyses were used to assess such relationships with plasma ferritin concentration as the independent variable.

RESULTS

We recruited 749 girls: 269 girls of Tangerang-1, 173 girls of Tangerang-2, and 307 girls of Central Jakarta. The plasma ferritin concentrations of 6 girls in Tangerang-1 and 1 girl in Tangerang-2 were not measured because the amount of volume was insufficient for the laboratory analyses. The age range of all girls involved in this study was between 11.3 to 17.1 years.

The percentage of girls reported to usually take supplements with vitamins or mineral was 10%, 6%, 21% in Tangerang-1, Tangerang-2, Central Jakarta, respectively.

Table 1 describes the study population: concentrations of plasma ferritin and retinol of girls were highest in Tangerang-1. Girls in Tangerang-2 had the highest chronological age, age at menarche, longest time since menarche, hemoglobin concentration, and EGRAC. Central Jakarta girls had the lowest concentrations of hemoglobin, plasma retinol and ferritin.

TABLE 1
Characteristics of Indonesian adolescent schoolgirls by study site

	Study site		
	Tangerang-1 (1999)	Tangerang-2 (2001)	Central Jakarta (2002)
n	269	173	307
Age, *year*	13.2 ± 0.9	14.9 ± 0.8	13.6 ± 1.0
Age at menarche, *year*	12.1 ± 1.0	12.9 ± 1.1	12.3 ± 1.1
Time since menarche, *month*	10.5 (6.5; 18.0)	25.3 (16.0; 32.0)	13.4 (7.5; 23.7)
Body mass index Z-score	-0.28 ± 0.87	-0.46 ± 0.85	-0.23 ± 0.93
Hemoglobin concentration, *g/L*	124.5 ± 11.7	135.4 ± 11.5	116.3 ± 12.3
EGRAC[1]	Not measured	1.82 (1.61; 2.05)	1.47 (1.32; 1.63)
Plasma ferritin concentration, *µg/L*	25.2 (13.6; 37.6)	18.9 (12.8; 30.8)	11.3 (5.4; 25.1)
Plasma retinol concentration, *µmol/L*	1.10 (0.97; 1.29)	1.03 (0.88; 1.21)	0.93 (0.74; 1.12)

Mean ± SD or median (25^{th}; 75^{th} percentile).
[1] EGRAC: erythrocyte glutathione reductase activity coefficient

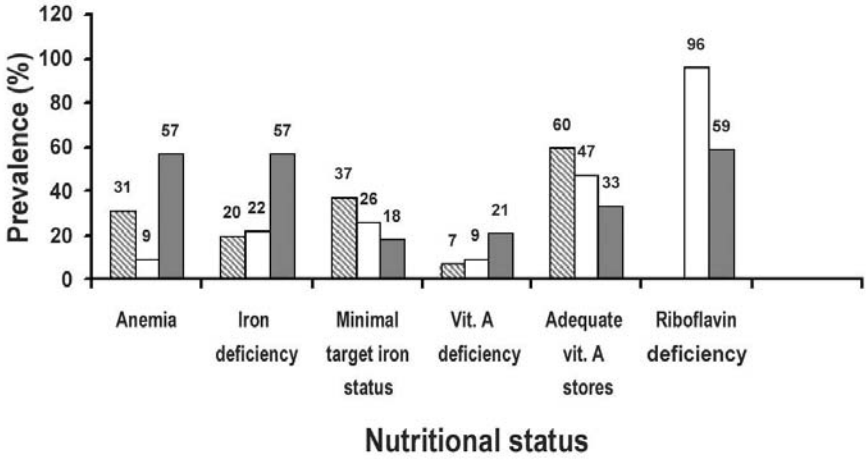

FIGURE 1. Prevalence of different nutritional status of Indonesian adolescent schoolgirls by study site.
(▨) Tangerang-1 (□) Tangerang-2 (■) Central Jakarta

Figure 1 shows the prevalence of different nutritional status of participating girls in the three surveys. Girls in the Central Jakarta survey generally had poorer status for iron and vitamin A than their peers in the Tangerang surveys. The prevalence of riboflavin deficiency was very high in both Tangerang-2 and Central Jakarta.

Table 2 shows hemoglobin concentrations by micronutrient status. In girls from Tangerang-2, we found no evidence that riboflavin deficiency as indicated by EGRAC > 1.40 was associated with decreased hemoglobin concentration. Except in girls of Tangerang-1, hemoglobin concentration appeared marginally lower in girls with vitamin A deficiency (plasma retinol concentration < 0.70 µmol/L). Hemoglobin concentrations were consistently lower in girls with plasma ferritin concentrations <12 µg/L than in their peers with plasma ferritin concentrations ≥ 12 µg/L.

There was no evidence that hemoglobin concentration was related to riboflavin status across study sites or when all studies were combined, with or without adjustment for iron and retinol status (Table 3). With the exception of Tangerang-1, where vitamin A status was best (Table 1), low status and deficiency of vitamin A appeared consistently associated with lower hemoglobin concentration (Table 3). When data from all surveys were combined, hemoglobin concentration was lower in vitamin A deficiency compared to the non-deficient vitamin A status. Hemoglobin concentration was consistently higher in girls with plasma ferritin concentration ≥ 12 µg/L than in their peers with plasma ferritin concentration <12 µg/L across studies.

No evidence was found that plasma ferritin concentration was related to riboflavin status across study sites or when data of all surveys were combined, with or without adjustment for vitamin A status (Table 4).

In both Tangerang studies, plasma ferritin concentration was not related to vitamin A status, with or without adjustment for riboflavin status. However, in Central Jakarta, where vitamin A status was lowest (Table 1), plasma ferritin concentration was lower in girls with vitamin A deficiency, with or without adjustment for riboflavin status. When all studies were combined, plasma ferritin concentration was associated with vitamin A status, with or without adjustment for riboflavin status (*P = 0.01*)

TABLE 2

Hemoglobin concentration of Indonesian adolescent schoolgirls by different micronutrient status

	Study site					
	Tangerang-1 (1999)		Tangerang-2 (2001)		Central Jakarta (2002)	
	n	Estimate	n	Estimate	n	Estimate
EGRAC [1]						
≤ 1.40		Not measured	7	127.7 ± 18.6	127	116.9 ± 12.7
> 1.40		Not measured	166	135.7 ± 11.1	180	115.8 ± 12.0
Difference		Not measured		8.0 [-16.8 to 0.7]		1.1 [-1.7 to 3.9]
Plasma retinol concentration						
≥ 0.70 µmol/L	251	124.4 ± 11.6	157	135.8 ± 11.1	244	116.8 ± 12.0
< 0.70 µmol/L	18	126.1 ± 13.4	16	131.4 ± 15.2	63	114.2 ± 13.3
Difference, µmol/L		-1.7 [-7.3 to 4.0]		4.4 [-1.6 to 10.3]		2.6 [-0.8 to 6.0]
Plasma ferritin concentration						
≥ 12 µg/L	211	125.7 ± 11.3	134	136.6 ± 11.1	147	121.7 ± 9.5
< 12 µg/L	53	119.7 ± 12.4	38	130.9 ± 12.2	160	111.3 ± 12.5
Difference, µg/L		6.0 [2.6 to 9.6]		5.7 [1.6 to 9.8]		10.4 [7.9 to 12.8]

Mean ± SD [95% confidence interval]

[1] EGRAC: erythrocyte glutathione reductase activity coefficient

TABLE 3

Relationship between hemoglobin concentration and riboflavin or vitamin A status in Indonesian adolescent schoolgirls

	Study site						All [5]	
	Tangerang-1 (1999)		Tangerang-2 (2001)		Central Jakarta (2002)			
	n	Estimate	n	Estimate	n	Estimate	n	Estimate
Univariate analysis								
EGRAC 1								
≤ 1.4		Not measured	7	Reference	127	Reference	134	Reference
> 1.4		Not measured	166	8.0 [-0.7 to 16.8]	180	-1.1 [-3.9 to 1.7]	346	-0.3 [-3.0 to 2.3]
Plasma retinol concentration								
≥ 1.05 µmol/L	161	Reference	82	Reference	101	Reference	344	Reference
0.7 - 1.05 µmol/L	90	-1.7 [-4.7 to 1.4]	75	-0.3 [-3.9 to 3.4]	143	-2.0 [-5.2 to 1.1]	308	-1.4 [-3.3 to 0.5]
< 0.7 µmol/L	18	1.1 [-4.7 to 6.8]	16	-4.5 [-10.7 to1.7]	63	-3.8 [-7.7 to 0.1]	97	-2.8 [-5.5 to 0.0]
Plasma ferritin concentration								
≥ 12 µg/L	211	Reference	134	Reference	147	Reference	492	Reference
< 12 µg/L	53	-6.1 [-9.6 to -2.6]	38	-5.7 [-9.8 to -1.6]	160	-10.4 [-12.9 to -7.9]	251	-8.2 [-10.0 to -6.4]
Multivariate analysis								
EGRAC [1, 2]								
≤ 1.4		Not measured	7	Reference	127	Reference	134	Reference
> 1.4		Not measured	166	6.8 [-1.9 to 15.5]	180	-1.0 [-3.6 to 1.5]	346	-0.4 [-2.9 to 2.0]
Plasma retinol concentration3								
≥1.05 µmol/L	161	Reference	82	Reference	101	Reference	344	Reference
0.7 - 1.05 µmol/L	90	-1.2 [-4.3 to 1.8]	75	-0.6 [-4.2 to 3.0]	143	-1.7 [-4.5 to 1.2]	308	-1.2 [-3.4 to 1.1]
< 0.7 µmol/L	18	1.0 [-4.7 to 6.7]	16	-3.9 [-0.0 to 2.2]	63	-3.0 [-6.5 to 0.6]	97	-3.0 [-6.1 to 0.0]
Plasma ferritin concentration4								
≥ 12 µg/L	211	Reference	134	Reference	147	Reference	492	Reference
< 12 µg/L	53	-5.9 [-9.4 to -2.4]	38	-5.2 [-9.3 to -1.1]	160	-10.2 [-12.7 to -7.7]	251	-8.9 [-11.1 to -6.8]

Mean [95% confidence interval]

[1] EGRAC: erythrocyte glutathione reductase activity coefficient

[2] Adjusted for plasma retinol concentration (≥1.05 µmol/L, 0.7-1.05 µmol/L, < 0.7 µmol/L), plasma ferritin concentration (≥12 µg/L, < 12 µg/L), take supplements (yes or no).

[3] Adjusted for EGRAC (≤1.4, >1.4), plasma ferritin concentration (≥12 µg/L, < 12 µg/L), take supplements (yes or no).

[4] Adjusted for EGRAC (≤1.4, >1.4), plasma retinol concentration (≥1.05 µmol/L, 0.7-1.05 µmol/L, < 0.7 µmol/L), take supplements (yes or no).

[5] Additional adjusted for study site.

TABLE 4
Relationship between plasma ferritin concentration and status of riboflavin or vitamin A in Indonesian adolescent schoolgirls

	Study site						All [4]	
	Tangerang-1 (1999)		Tangerang-2 (2001)		Central Jakarta (2002)			
	n	Estimate	n	Estimate	n	Estimate	n	Estimate
Univariate analysis								
EGRAC [1]								
≤ 1.4		Not measured	7	Reference	127	Reference	134	Reference
> 1.4		Not measured	166	32 [- 21 to 121]	180	- 4 [- 25 to 22]	346	-2 [- 20 to 21]
Plasma retinol concentration								
≥ 1.05 µmol/L	161	Reference	82	Reference	101	Reference	344	Reference
0.7 - 1.05 µmol/L	90	-16 [- 31 to 1]	75	-12 [- 27 to 10]	143	- 23 [- 41 to 15]	308	- 17 [- 28 to -5]
< 0.7 µmol/L	18	-21 [- 45 to 10]	16	-17 [- 36 to 21]	63	- 36 [-54 to -10]	97	- 29 [- 42 to -13]
Multivariate analysis								
EGRAC [1,2]								
≤ 1.4		Not measured	7	Reference	127	Reference	134	Reference
> 1.4		Not measured	166	36 [-19 to 129]	180	- 6 [- 26 to 20]	346	- 3 [- 21 to 20]
Plasma retinol concentration3								
≥ 1.05 µmol/L		Not measured	82	Reference	101	Reference	344	Reference
0.7 - 1.05 µmol/L		Not measured	75	-13 [- 30 to 10]	143	- 23 [- 41 to 15]	308	-18 [- 32 to - 2]
< 0.7 µmol/L		Not measured	16	-17 [- 37 to 23]	63	- 36 [- 54 to -10]	97	- 31 [- 46 to -11]

Values indicated the percentage change in plasma ferritin concentration relative to the reference category, and the corresponding [95% CIs]
[1] EGRAC: erythrocyte glutathione reductase activity coefficient
[2]Adjusted for plasma retinol concentration (≥1.05 µmol/L, 0.7-1.05 µmol/L, < 0.7 µmol/L), take supplements (yes or no).
[3]Adjusted for EGRAC (≤ 1.4, > 1.4), take supplements (yes or no).
[4]Additional adjusted for study site.

DISCUSSION

Despite the high prevalence of riboflavin deficiency in our surveys, we found no evidence that this was related to decreased concentration of hemoglobin or plasma ferritin. We only included girls without indication of inflammation (plasma CRP > 5 mg/L) in this study, and we also considered the independent relationships between status of vitamin A and riboflavin with iron status.

We are not aware of other cross-sectional studies that assessed the relationships between riboflavin status and concentrations of hemoglobin or plasma ferritin. By contrast, intervention studies, that have shown that supplementation with riboflavin alone or in combination with iron, can increase iron status in pregnant and lactating women, men and school children [1-5,31]. Observational studies such as ours are vulnerable to confounding, however, and a randomized controlled trial would be needed to assess the effect of riboflavin supplementation on iron status in adolescent girls.

In Tangerang-1, where girls had better iron status compared to their peers in Tangerang-2 and Central Jakarta, we failed to show a relationship between hemoglobin concentration and vitamin A status. In individual survey in Tangerang-2 and Central Jakarta, and when data of all surveys were combined, although lower hemoglobin concentration was found in girls with vitamin A deficiency as compared with their peers with normal vitamin A status, the evidence was weak because of the low precision in the measurement that was achieved with this sample size.

In agreement with our findings, other studies in adolescent girls with vitamin A deficiency showed that hemoglobin concentration was related to plasma retinol concentration [12,13].

In all surveys, plasma ferritin concentration was consistently lower in girls with low vitamin A status, and to an even greater extent in girls with vitamin A deficiency (Table 4). However, in individual survey it could not be ruled out that the relationship observed was due to chance; sufficient precision was obtained only when combining survey data. Our findings corroborate with those from several intervention studies, which have shown that adolescents supplemented with vitamin A additional to iron had increased concentrations of hemoglobin or plasma ferritin when compared to their peers receiving iron alone [17,18].

The high prevalence of deficiencies of riboflavin and vitamin A observed in our study should raise public health concern. Riboflavin plays an important role as a coenzyme in the provision of energy, metabolism of ß-oxidation of fatty acids, niacin, vitamin B_6 and homocysteine. Inadequate intake of riboflavin possibly leads to disturbances in energy production [32] and interfere with growth.

At least 40% of the girls studied had inadequate vitamin A stores. Vitamin A plays important roles visual function, susceptibility to infection [33] and possibly in sexual maturation [34]. Furthermore, at least 63% of the girls had insufficient iron stores, which may adversely affect growth and verbal learning [16,35,36].

We conclude that low vitamin A status and vitamin A deficiency are related to reduced iron status. Deficiencies of riboflavin, vitamin A and iron, and girls without minimal target iron status or inadequate stores of vitamin A are prevalent in the population studied.

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Chapter

5

Supplementation with vitamin A or riboflavin additional to iron has no or only marginal effects on hemoglobin concentration or iron stores in Indonesian adolescent schoolgirls:

a randomized-controlled trial

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ABSTRACT Vitamin A and riboflavin status have been reported to be associated with anemia and iron deficiency. Anemic adolescent girls may therefore benefit from supplementation with vitamin A or riboflavin in addition to iron. This study aimed to measure the effects of supplementing vitamin A or riboflavin, in addition to iron, on hemoglobin concentration and iron stores as indicated by plasma ferritin concentration.

A double-blind randomized controlled supplementation trial involving 258 anemic adolescent schoolgirls aged 11-17 years was conducted in Jakarta. In a 2x2 factorial design, girls were allocated to four daily treatment groups: placebo, vitamin A, riboflavin, and vitamin A plus riboflavin. The study was designed to evaluate the effects on hemoglobin concentration, and plasma ferritin concentration as an indicator of iron stores. Concentrations of hemoglobin, plasma ferritin, tranferrin receptor, and retinol, and erythrocyte glutathione reductase activity coefficient were assessed before and after supplementation for 8 weeks.

The prevalence of anemia declined to 21% in placebo group, 24% in vitamin A group, 19% in riboflavin group, and 18% in vitamin A plus riboflavin group. The prevalence of iron, vitamin A and riboflavin deficiencies declined from 56% to 4%, 25% to 15% and 61% to 33%, respectively. No girls were infected with hookworm, 11% had either mild ascariasis or mild trichuriasis.

Despite the distinct improvement in iron, vitamin A and riboflavin status shown in this study, supplementation with vitamin A or riboflavin, either alone or in combination, did not or only marginally improve hemoglobin and iron store above that achieved with iron supplementation alone.

INTRODUCTION

In Southeast Asia, the prevalence of anemia has been reported to be 46% among non-pregnant women and 52% among pregnant women [1]. Iron deficiency is considered to be the main cause of anemia. In developing countries, the risk is increased by deficiencies of other micronutrient such as vitamin A.

Due to increased demand for iron by the mother and the fetus during pregnancy, iron sufficiency is difficult to achieve unless iron stores are adequate at the start of pregnancy [2]. The consequences of anemia include reduced work productivity, increased risk of preterm birth, and low birth weight [3-6]. Thus, it is essential that women have sufficient stores of iron at conception.

Studies in different parts of the world indicate that adolescent girls are prone to anemia and deficiencies of several micronutrients, especially iron [7-16]. Studies also showed that anemia, iron and riboflavin deficiencies become more prevalent as girls become older [9,14,16,17]. Anemia and iron deficiency affect school achievement, verbal learning and physical activity among adolescent girls [9,18,19].

In Indonesia, about half of adolescent girls marry at 19 years of age, and most will have their first baby very soon thereafter [20]; this will affect their iron status and that of their offspring. Therefore, adolescence may be an ideal time to implement programs to improve iron status of potential mothers especially as girls attending school are readily accessible.

Studies have shown that supplementation with vitamin A in addition to iron improves hemoglobin concentration and iron status [21-26]. It has been postulated that vitamin A plays a role in the mobilization of iron from stores to become available for erythropoiesis [27], thus contributing to the increase of hemoglobin and iron status. Other studies have shown the effects of riboflavin in improving hemoglobin levels and iron status [28-32]. It has been postulated that riboflavin aids in the release of iron from ferritin so that it becomes available for erythropoiesis [33], thereby improving hemoglobin and iron status.

This study aimed to measure the effects of supplementing vitamin A or riboflavin, in addition to iron, on hemoglobin concentration and iron stores as indicated by plasma ferritin concentration.

MATERIALS AND METHODS

Subjects

This study was conducted from August to October 2002 in 5 state junior high schools of an urban slum area of central Jakarta, Indonesia. In this area, malaria and hookworm infections are rare (Margono SS, personal communication, 2002), and no deworming or iron supplementation programs have been implemented. Girls aged 11-17 years with no self-reported blood abnormalities or chronic diseases were invited to participate in the study.

The hemoglobin levels of girls agreeing to participate were screened first using a battery operated portable hemoglobinometer (HemoCue AB, Angelholm, Sweden). Girls with anemia (hemoglobin level was <120 g/L) underwent second hemoglobin screening within 2 weeks using a Coulter apparatus. The intervention started within a week in girls whose anemia was confirmed at the second screening. Girls who were excluded after the second screening received weekly iron supplementation for 8 weeks.

We estimated that a sample size of 64 girls per group would provide 90% probability of detecting group differences in hemoglobin concentration of 7.1 g/L, assuming $\alpha = 0.05$, a standard deviation of hemoglobin concentration within groups of 10.6 g/L [12] and 10% drop-out during the intervention period. The hemoglobin difference of 7.1 g/L was achieved by adolescent schoolgirls in Jakarta after 8 weeks of daily supplementation with iron in combination with vitamin A, C and folic acid [12].

The intervention

An independent staff blindly and randomly allocated each of 258 eligible girls to one of 4 groups (Figure 1), all of which received iron. In addition, girls received placebo for both retinyl acetate and riboflavin in the placebo group; retinyl acetate and placebo riboflavin in the vitamin A group; placebo retinyl acetate and riboflavin in the riboflavin group; and retinyl acetate and riboflavin tablets in the vitamin A plus riboflavin group. Supplementation was provided daily at school on 6 working days each week for 8 weeks. All girls swallowed their tablets under strict supervision of one of the three field researchers (DD, HJM, MBB). Girls, who were absent, received double tablets the following day. Tablets were given during the mid-morning school break for morning classes and before school started for afternoon classes within 2 hours after breakfast or lunch. Girls who had not eaten in the previous 2 hours were given a biscuit before consuming the tablets, to limit potential dropouts due to side effects.

During the intervention period, the girls were not allowed to consume any other vitamin or mineral supplements, antihelminth drugs, nor change their dietary habits; this message was repeated during tablet distribution from time to time. The researchers, laboratory staff and girls were all blinded to treatment assignments until all laboratory analyses had been completed.

The supplements

PT Kimia Farma (Bandung, Indonesia) produced the supplements for the study. The iron tablets were dark red in color; the vitamin A tablets were yellow, and the riboflavin tablets were pink: placebo tablets were indistinguishable in shape and color from their active counterparts.

Some tablets were arbitrary selected, and concentrations of iron, retinyl acetate, riboflavin and placebo tablets were determined by TNO Food and Nutrition Research (Zeist, The Netherlands). This analysis showed that the iron, vitamin A and riboflavin tablets contained 60 mg iron as $FeSO_4.7H_2O$; 2.424 mg retinyl acetate; 6.1 mg riboflavin, respectively, and confirmed that the placebo tablets were free of active ingredients. An independent person packed the tablets that to be distributed to the girls, in separate plastic bags each week during the intervention.

Assessment methods

At baseline, a structured questionnaire was used to obtain information on menarche and the use of antihelminth drugs and vitamin/mineral supplements.

Height was measured to the nearest 0.1 cm, using a microtoise (CMS Ltd, London, UK), while weight was assessed to the nearest 0.1 kg using a digital weighing scale (Model 890, SECA, Hamburg, Germany). During measurements, girls wore standard school uniform without footwear. Equipment was calibrated before use. The same observer performed the anthropometric assessments in duplicate before and at the end of the study.

During first screening of hemoglobin concentration, finger prick capillary blood was used and the HemoCue machine was checked daily, using a reference cuvette to secure its performance within an acceptable range.

At the beginning and end of the study, about 4 mL of non-fasting cubital venous blood was drawn into tube without anticoagulant, protected from light. Soon after blood collection, 0.5 mL of blood was transferred to an EDTA tube and the remaining was transferred to a heparinized tube and kept in a cool box before transporting to the laboratory.

Hemoglobin was analyzed on the EDTA blood sample within 5 hours of collection by the cyanmethemoglobin method with standard solutions and reagents from the manufacturer (Hemoglobin Merckotest Merck, Darmstadt, Germany), using a photometer (Eppendorf PCP 6121, Hamburg, Germany). Within 3 hours after blood collection, the heparinized blood samples were centrifuged for 15 minutes (5000 x g) at 5-10ºC. The packed red blood cells were washed 3 times with sterile physiological saline and stored for the analysis of erythrocyte glutathione reductase activation coefficient (EGRAC). Both plasma and washed packed red blood cell were kept at -70ºC for analysis within 4 months.

Plasma concentrations of ferritin, C-reactive protein (CRP), transferrin receptor (TfR) were determined by sandwich assay [34], using antibody ferritin and anti-feritin-HRP (code A0133 and P 0145, Dako, Glostrup, Denmark), antibody CRP and anti-CRP-HRP (no.A0073 and no.P0027, Dako), and antibody TfR and anti-sTfR-HRP (clone 23 D10 no.4Tr26 and clone13E4 no.4 Tr26, Hytest, Turku, Finland). Control sera were used for CRP and ferritin (Liquicheck, Biorad, Hercules, CA, USA) and TfR (Ramco Lab., Vernon Houston, TX 77006, USA). EGRAC was assayed by a modified method [35] using ELISA (Labsystems Multiskan Ascent, Helsinki, Finland). Retinol concentration was assayed by HPLC method (Waters 515, Milford, MA 071757, USA), using an external serum reference (no.968a, NIST, Gaithersburg, MD 20899, USA).

In addition to using control and calibration sera, the reproducibility and accuracy were ensured by running pooled sera during every analysis, and analyzing 10% of samples in duplicate. The coefficient of variation of all analyses was <10%. All laboratory analyses were performed at the SEAMEO-TROPMED Regional Center for Community Nutrition, University of Indonesia, Jakarta, Indonesia, under the supervision of a laboratory consultant from University of Hohenheim, Germany. All sera were analyzed after the completion of the intervention study.

Two weeks before the supplementation period ended, each girl was asked to bring a fresh stool sample in a standard plastic container. Within 4 hours after collection, the samples were transferred to a special stool storage refrigerator at the Department of Parasitology, Faculty of Medicine, University of Indonesia. Assessment of worm infestation was based on eggs count by Kato-Katz method and the presence of hookworm larvae was tested by Harada-Mori method [36]. All parasitological analyses were carried out at the department, and were performed the following day after collection or the subsequent Monday when stools were collected on the previous Saturday.

At 4-6 weeks after the start of supplementation, girls were instructed to keep

a detailed 24-hour diary of their food intake of the previous day. We showed standard portion to the girls to check the amount of their reported intakes, and these reports were corrected as needed.

Data analysis

Data analyses were performed on data of 258 girls, or on available biochemical data of 252 girls who completed the study. Epi-Info 2002 (CDC, Atlanta, GA 30341-3717, USA) was used to calculate the body mass index Z-score (BMI Z-score).

Cut-off value used was >5 mg/L for plasma CRP concentration, and to define deficiencies were < 120 g/L for hemoglobin concentration[37], plasma ferritin concentration <12 g/L [38], > 8.3mg/L for plasma TfR concentration (according to specifications by the manufacturer), < 0.70 µmol/L for plasma retinol concentration [39] and > 1.4 for EGRAC [40]. Iron deficiency anemia was defined by hemoglobin concentration < 120 g/L and plasma ferritin concentration < 12 µg/L. We considered plasma ferritin concentration ≥ 30 µg/L in the absence of inflammation as target iron status [2,41], and a plasma retinol concentration of ≥ 1.05 µmol/L as an adequate store of vitamin A [42,43].

Severity of worm infestation was classified according to criteria from the World Health Organization classification [44] into no infection, light, average or severe infestation.

Dietary intake was analyzed using the Nutrisurvey package [45]. Nutrients intakes were compared to Indonesian Recommended Daily Allowances of 19 mg, 500 RE, 1.2 mg for iron, vitamin A, and riboflavin, respectively [46].

Data that were not normally distributed as assessed by visual inspection of distribution and Kolmogorov-Smirnov test, were log-transformed as appropriate. Treatment effects were evaluated by assessing group differences in indicators measured at the end of the intervention, using multivariate linear regression techniques, with and without adjustment for potential confounding by baseline factors such as menarche (not yet menarche, menarche occurred 0-12 months, >12 months before the start of the study), concentration of hemoglobin (<100 g/L, 100-109.9 g/L, 110-119.9 g/L), concentrations of plasma ferritin (< 5 µg/L, 5-11.99 µg/L, 12-29.99 µg/L, ≥ 30 µg/L), transferrin receptor (≤ 8.3 mg/L, > 8.3-14.99 mg/L, ≥ 15 mg/L), retinol (<0.70 µmol/L, 0.70-1.05 µmol/L, ≥ 1.05 µmol/L), riboflavin status determined by EGRAC (≤ 1.40, > 1.40), and BMI Z-score (< -1.21, -1.21 to < -0.51, -0.51 to 0.18, > 0.18).

In an exploratory analysis, we assessed the relationships between biochemical indicators measured at baseline, categorized as above, using the Kruskal-Wallis test. The Mann-Whitney test was applied to test correlations amongst biochemical indicators. Because we found no evidence that the effect of vitamin A on hemoglobin concentration

was influenced by riboflavin, we also assessed the effect of vitamin A in a pooled analysis, comparing all girls with or without vitamin A with regards to their concentrations of hemoglobin and plasma ferritin at the end of the intervention. Similarly we conducted a pooled analysis to assess the effect of riboflavin supplementation.

RESULTS

Of 2,023 adolescent girls attending junior high schools in the study area, 258 were enrolled in the study, of whom 28% non-menarchal and 72% post-menarchal girls. Six girls failed to complete the study for various reasons (Figure 1). Study groups were similar in their baseline characteristics (Table 1). Only 8% of the girls had a BMI Z-score < -2.00 SD.

Stools were obtained from 238 girls: no hookworm was detected, and only 11% subjects were infested with very low egg counts of either Ascaris or Trichuris. At baseline, 251 girls reported not having taken any antihelminth drug within one year before the intervention. The remaining seven girls indicated having taken antihelminth drugs: one girl did not submit stool, two had low counts of Trichuris eggs, and four showed no egg in their stools.

Most girls (92%) took >95% of the 48 presented tablets. At 4-6 weeks after the start of supplementation, the proportions of girls who reported intakes of vitamin A, riboflavin and iron less than the Indonesian recommended daily dietary allowances were 75%, 88%, and 95% respectively.

At the end of the study, the prevalence of anemia was reduced to 21%: 21% in the placebo group, 24% in the vitamin A group, 19% in the riboflavin group and 18% in the vitamin A plus riboflavin group. Iron status as assessed by the prevalence of anemia, plasma concentrations of ferritin and transferrin receptor improved dramatically in all groups. However, prevalence of iron deficiency as indicated by plasma transferrin receptor concentration > 8.3 mg/L was much higher (< 32%) compared to the status indicated by plasma ferritin concentration (< 8.5%) (Figure 2). The prevalence of deficiencies of vitamin A or riboflavin was reduced to 6% and 14% in groups receiving vitamin A or riboflavin, respectively. By contrast, in groups receiving placebos for these vitamins, the prevalence values persisted at levels observed at baseline (Figure 2). The prevalence of low vitamin A status in groups receiving iron, iron plus riboflavin, iron plus vitamin A, and iron plus vitamin A plus riboflavin was 67%, 68%, 46% and 52%, respectively.

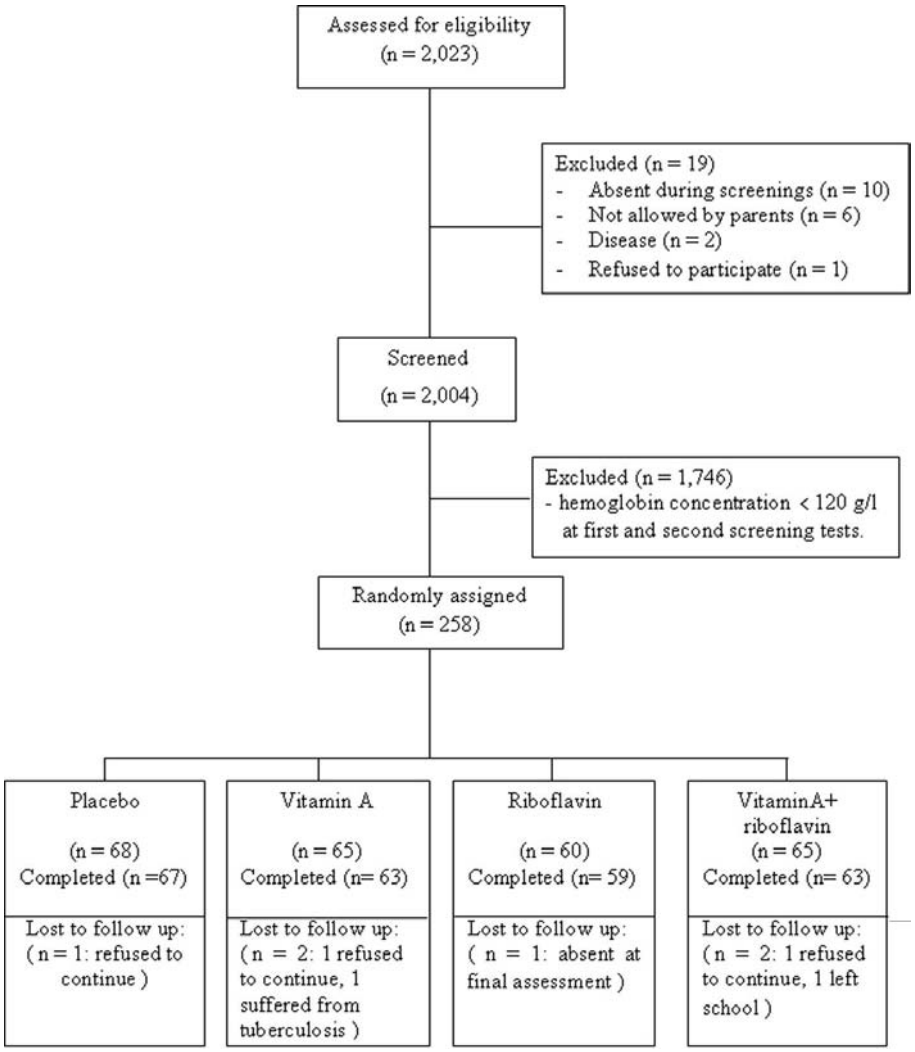

FIGURE 1. Flow chart of participating Indonesian adolescent girls through each stage of randomization

TABLE 1

Characteristics of Indonesian adolescent schoolgirls at baseline.

	All	Intervention group			
		Placebo	Vitamin A	Riboflavin	Vitamin A + Riboflavin
n	252	67	63	59	63
Age, *y*[1]	13.4 ± 1.1	13.4 ± 1.1	13.4 ± 1.1	13.2 ± 1.1	13.5 ± 1.1
Body mass index Z-score[1]	-0.53 ± 0.97	-0.68 ± 0.96	-0.58 ± 0.89	-0.40 ± 1.20	-0.45 ± 0.86
Hemoglobin concentration, *g/L*[2]	111.6	108.9	111.5	112.2	113.1
	(104.4; 116.6)	(104.0; 115.9)	(103.0; 116.2)	(104.4; 117.7)	(105.2; 117.3)
< 100 g/L, n (%)	42 (16.7)	12 (17.9)	12 (19.1)	9 (15.3)	9 (14.3)
100 – 109.9 g/L, n (%)	66 (26.2)	24 (35.8)	13 (20.6)	15 (25.4)	14 (22.2)
110 – 119.9 g/L, n (%)	144 (57.1)	31 (46.3)	38 (60.3)	35 (59.3)	40 (63.5)
Plasma ferritin concentration, *µg/L*[2]	9.4	8.6	9.4	9.5	11.3
	(4.7; 23.9)	(4.7; 21.7)	(4.5; 22.6)	(4.4; 29.4)	(6.1; 22.1)
Plasma transferrin receptor concentration, *mg/L*[2]	12.2	12.4	14.2	10.9	11.0
	(7.9; 17.2)	(8.2; 17.9)	(7.4; 18.5)	(8.2; 16.6)	(7.7; 16.7)
Plasma retinol concentration, *µmol/L*[2]	0.86	0.88	0.92	0.82	0.83
	(0.69; 1.05)	(0.67; 1.02)	(0.75; 1.15)	(0.69; 1.00)	(0.68; 1.05)
EGRAC* [2]	1.48	1.53	1.48	1.42	1.50
	(1.29; 1.65)	(1.34; 1.68)	(1.24; 1.65)	(1.26; 1.58)	(1.30; 1.71)
Menarche status					
Not yet occurred, n (%)	70 (27.8)	18 (26.9)	14 (22.3)	21 (35.6)	17 (27.0)
0 – 12 months ago, n (%)	73 (29.0)	23 (34.3)	21 (33.3)	11 (18.6)	18 (28.6)
> 12 months ago, n (%)	109 (43.2)	26 (38.8)	28 (44.4)	27 (45.8)	28 (44.4)

[1] Mean ± standard deviation.
[2] Median (25^{th}; 75^{th} percentiles).
* EGRAC = erythrocyte glutathione reductase activity coefficient.

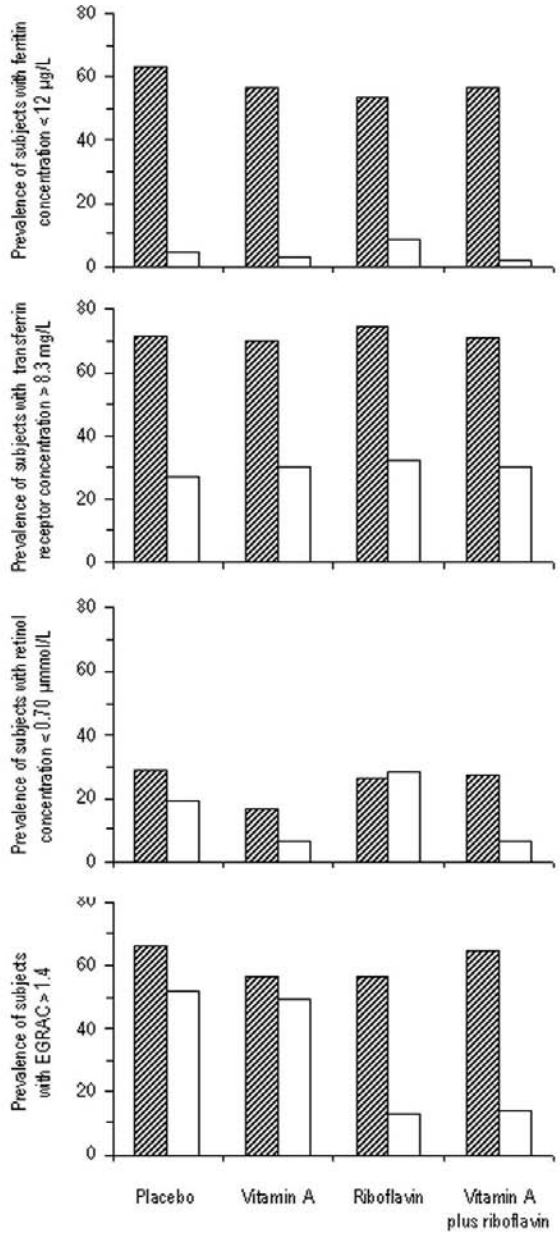

FIGURE 2. Prevalence of deficiency of iron, vitamin A and riboflavin in the four groups of Indonesian adolescent schoolgirls at the beginning and end of the study.
(▨) before and (□) after supplementation

TABLE 2

Effects of interventions on hemoglobin, plasma ferritin and transferrin receptor concentrations in Indonesian adolescent schoolgirls after 8 week supplementation.

	Intervention group			
	Placebo	Vitamin A	Riboflavin	Vitamin A + Riboflavin
n	67	63	59	63
Hemoglobin concentration, *g/L*				
After intervention[1]	129.8 ± 13.8	130.7 ± 11.5	131.2 ± 13.1	128.3 ± 11.5
Change from baseline[2]	22.5 (9.5; 30.5)	20.7 (10.7; 31.7)	15.5 (8.2; 30.2)	17.3 (6.2; 27.9)
Crude difference from placebo group[3]		0.9 [-3.4 to 5.2]	1.4 [-3.1 to 5.8]	-1.5 [-5.8 to 2.9]
Adjusted difference from placebo group[4]		1.1 [-3.3 to 5.6]	1.6 [-2.9 to 6.3]	-1.5 [-5.8 to 2.9]
Plasma ferritin concentration, *µg/L*				
After intervention[2]	34.3 (19.3; 48.8)	37.5 (25.4; 66.6)	30.8 (20.1; 56.0)	39.0 (27.9; 51.7)
Change from baseline[2]	19.6 (12.3; 29.5)	24.8 (12.1; 42.2)	17.5 (8.0; 34.4)	23.3 (15.4; 35.0)
Crude difference from placebo group[3]		1.2 [0.9 to 1.5]	1.0 [0.8 to 1.3]	1.2 [1.0 to 1.5]
Adjusted difference from placebo group[4]		1.1 [0.9 to 1.4]	1.1 [0.9 to 1.4]	1.1 [0.9 to 1.4]
Plasma transferrin receptor concentration, *mg/L*				
After intervention[2]	6.7 (5.2; 8.4)	6.4 (4.4; 8.8.)	6.4 (4.7; 9.7)	6.9 (4.8; 8.8)
Change from baseline[2]	-4.7 (-11.1; -1.3)	-6.6 (-11.6; -0.9)	-3.4 (-10.4; -0.5)	-4.0 (-7.9; -1.3)
Crude difference from placebo group[3]		1.0 [0.8 to 1.1]	0.9 [0.8 to 1.1]	0.9 [0.8 to 1.1]
Adjusted difference from placebo group[4]		0.9 [0.8 to 1.1]	0.9 [0.8 to 1.1]	1 [0.8 to 1.2]

[1] Mean ± standard deviation.
[2] Median (25^{th}; 75^{th} percentiles).
[3] Differences between means of the intervention groups compared to placebo group [95% confidence interval] at 8 week. Effects were tested by multivariate linear regression.
[4] As above adjusted for prognostic factors at baseline: hemoglobin concentration (< 100 g/L; 100-109.9 g/L; 110-119.9 g/L); plasma concentrations of ferritin (< 5 µg/L; 5-11.99 µg/L; 12-29.99 µg/L; ≥ 30 µg/l); transferrin receptor (≤ 8.3 mg/L; > 8.3-14.99 mg/L; ≥ 15 mg/L); retinol (< 0.7 µmol/L; 0.7-0.85 µmol/L; 0.86-1.04 µmol/L; ≥ 1.05 µmol/L); erythrocyte glutathione reductase activity coefficient (≤1.40; > 1.40); body mass index Z-score (< -1.21; -1.21 to < -0.51; -0.51 to 0.18; > 0.18); menarche status (not yet occurred; menarche occurred 0-12 months; menarche occurred > 12 months at the beginning of the study).

The occurrence of iron deficiency anemia was 56% at baseline and reduced to almost zero (0.4%) at the end of the study. However, the prevalence of BMI Z score <-2.00 hardly changed (data not shown). The prevalence of girls with target iron status at baseline was 18.5% increasing to 59% after supplementation, while it was 27% increasing to 42% for ideal vitamin A status.

We observed no evidence that supplementation with vitamin A or riboflavin had effects on hemoglobin concentration and concentrations of ferritin and transferrin receptor in plasma. Adjustment for potential confounders, led to similar effect estimate (Table 2). There was no indication that the effect of supplementation with A and riboflavin in combination on hemoglobin concentration differed from the summed effect of supplementation with single vitamins (interaction effect: -3.7 g/L, 95% CI: -10.0 to 2.5 g/L), neither on plasma concentrations of ferritin nor transferrin receptor (1.0 g/L, 95% CI: 0.8 to1.4 g/L; and 1.1 g/L, 95% CI: 0.8 to1.4 mg/L respectively).

In the pooled analysis, we compared all girls with vitamin A with their peers with vitamin A placebo. Thus vitamin A supplementation resulted in 19% (95% CI: 1% to 39%) increase in plasma ferritin concentration but no or only marginal effect on hemoglobin concentration (-1 g/L; 95% CI: - 4.1 to 2.2 g/L). When similarly comparing all girls with riboflavin with their peers with riboflavin placebo, we found negligible effects on hemoglobin concentration (- 0.2 g/L; 95% CI -3.4 to 3.1 g/L) and plasma ferritin concentrations (8% increase; 95% CI: -9% to 27%).

At baseline, 8 girls had CRP concentration > 5 mg/L, whilst at the end of the intervention period, 10 girls had concentration >5 mg/L; however, exclusion of these data did not affect the effect estimates of the supplementation.

The effect of riboflavin supplementation on hemoglobin concentration was negatively related to baseline concentrations of hemoglobin ($P = 0.03$) and plasma transferrin receptor ($P = 0.04$). There was no indication that this effect depended on plasma ferritin concentration at baseline (data not shown).

When analyzing relationships among biochemical variables measured at baseline, we observed that experience of menarche or a longer period of menarche was associated with a lower iron status as indicated by concentrations of hemoglobin ($P = 0.01$), plasma ferritin ($P = 0.0001$) and plasma transferrin receptor ($P = 0.0001$). Similarly, concentration of hemoglobin was much lower among those with the lowest status of ferritin ($P = 0.0001$), transferrin receptor ($P = 0.0001$) or retinol ($P = 0.036$) plasma concentrations. There was no indication that plasma concentrations of ferritin nor transferrin receptor had any relationships with status with respect to vitamin A or riboflavin; also between hemoglobin concentration and riboflavin status.

We observed some common baseline characteristics shared by 51 girls (21%) who were still anemic at the end of the study: their plasma ferritin concentrations were higher compared to girls who became non-anemic (Mann-Whitney, $P = 0.002$), and 59% of them had hemoglobin concentration between 110-119.9 g/L that persisted despite normal plasma ferritin concentrations. Before and after supplementation, there was no indication of relationship between hemoglobin concentration and plasma

concentrations of ferritin, transferrin receptor, retinol nor EGRAC among these still anemic girls.

DISCUSSION

In this study, supplementation with vitamin A and riboflavin in addition to iron failed to improve hemoglobin concentration beyond that achieved by supplementation with iron alone. Vitamin A may have led to a small increase (19%) in plasma ferritin concentration as indicated by the results in Table 2, and in the pooled analysis. However, the interventions did lead to a marked improvement in vitamin A and riboflavin status.

There was no evidence that the effect estimates were confounded by baseline factors; additionally, few participants dropped-out and compliance was excellent. Worm infestations and inflammation occurred in only few girls and did not seem to influence the results. Therefore, we are confident that the group differences observed at the end of the intervention are due to the supplementation with vitamin A and riboflavin only. Supplementation with iron increased the concentrations of hemoglobin and plasma ferritin (Table 2), as found in many studies. Thus, although iron status of the girls probably improved as a result of iron supplementation, it did not respond to additional supplementation with vitamin A, riboflavin or both.

In our study, we selected girls with anemia because we expected that the treatment effects would be larger than in non-anemic girls. Thus, we would anticipate even smaller effects in a mixed population of anemia and non-anemic girls than observed in this study.

Most girls studied had mild anaemia. Based on our exploratory analysis, we cannot exclude the possibility that riboflavin supplementation could improve iron status in girls with more severe anemia or iron deficiency. Further studies would be needed to examine this hypothesis.

Our finding is of particular interest as previously our group showed that additional vitamin A increased hemoglobin concentration among Indonesian pregnant women [23,26], as also reported by different investigators [21,22,24,25]. Similarly, the beneficial effects of additional riboflavin to iron supplementation were reported by different investigators [28-32]. Other studies, similar to this study, could not show an effect of vitamin A or riboflavin on hemoglobin concentration [16,30,32,47-49].

Of several studies that included only anemic subjects at baseline, some were able to show beneficial effects of additional vitamin A to iron supplementation [21,23,24],

whereas others could not [32,47,48]. In the latter three studies, the anemia could have been due to causes other than vitamin A deficiency. The results from our present study were unexpected because the girls were vitamin A deficient as indicated by the response in serum retinol concentrations; also, they had a much higher prevalence of vitamin A deficiency compared to both of our previous studies among pregnant women in West Java [23,26].

Additional riboflavin to iron supplementation has been shown to improve hemoglobin concentration in anemic pregnant women [28,32], anemic men [30], and lactating women [49]. However, such effects were not found in anemic children [30] and pregnant women [49]. Unfortunately, these studies did not report whether subjects were randomized to treatment. Vitamin A and riboflavin make iron available for erythropoiesis through different mechanisms [27,33]. In this study, we observed a 19% higher plasma ferritin increment in the vitamin A group, however, the prevalence of riboflavin deficiency was 51%. Thus, although iron stores were increased, there may have been insufficient riboflavin to make this iron available for erythropoiesis. Similarly, in the riboflavin group, 29% of the girls were still vitamin A deficient, so that there may have been insufficient iron from stores available for riboflavin to play its role in reducing iron. Thus, conditions necessary for a hematopoetic response to supplementation with vitamin A or riboflavin may not only be an inadequate status with respect to both vitamins but may also require a degree of up-regulation of erythropoiesis present in anemic pregnant women which was perhaps not achieved in our study population.

An alternative explanation would be that the girls were not only deficient in vitamin A and riboflavin, but also in other micronutrients. If this were the case, a beneficial effect of supplementation with vitamin A or riboflavin may only be seen when also improving the status of other micronutrients, for example through supplementation with such micronutrients. Candidates for deficiency include vitamin B6, folic acid, vitamin B12 or copper, among which only copper has a direct role in iron metabolism, as do vitamin A and riboflavin. It could have been the case of 21% of the girls who were still anemic at the end of the intervention: unlike the responders, the hemoglobin concentrations of these non-responders at baseline were not correlated with plasma concentrations of ferritin or transferrin receptor, suggesting that their anemia was not due to iron deficiency. This observation underlines the possible role of other micronutrients deficiencies [47], and the importance of assessing other micronutrients in relation to anemia. Another possibility is that these girls had α- or ß-thalassemia trait that cause mild anemia. A published Indonesian data indicates that the prevalence of such trait was 2.2% and 4%, respectively [50].

The consequences of deficiencies of vitamin A, riboflavin and iron are not

limited to anemia only. Iron is an essential nutrient for growth and immune function; vitamin A is essential for reproductive development and sexual maturation [51]; riboflavin acts as an electron carrier and participates in energy metabolism to provide the fast-growing body with energy during adolescence. Low status of iron, vitamin A or riboflavin will give unfavorable consequences to the girls: growth and sexual maturity may be disturbed and iron deficiency anemia could easily recur. A normal plasma transferrin receptor indicates adequate iron supply to the tissues. Its elevation is associated with both enhanced erythrocyte production and tissue deficiency of iron [41]. Hence, despite the remarkable improvement in ferritin levels as a result of the supplementation regimen, 1/3 of the girls experienced either enhanced erythrocyte production due to rapid growth or more likely, tissue iron deficiency that persisted despite the interventions.

Many girls receiving vitamin A continued to have low vitamin A status at the end of the intervention. To improve vitamin A and riboflavin status in the population studied, vitamin A and riboflavin should be supplemented for a period of at least 8 weeks. Because 41% of the girls studied had not achieved their minimal target stores, we recommend that daily iron supplementation should also continue for a period longer than 8 weeks.

Teenage pregnancies are common in Indonesia, and after leaving secondary school, these girls will be difficult to reach; this indicates the urgency to make micronutrients available for them. Countries with limited budgets should start school-based supplementation programs with iron to alleviate iron deficiency and anemia; adding vitamin A and riboflavin should be considered because these micronutrients may provide other health benefits.

We conclude that among urban low-income anemic adolescent girls whose nutrient intake is poor, supplementation with iron, vitamin A and riboflavin improved status of iron, vitamin A, and riboflavin, respectively. Vitamin A or riboflavin does not meaningfully contribute to improving hemoglobin concentration, and results in no or only a marginal increase in iron stores.

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Chapter

6

General Discussion

The main objective of this thesis is to measure the role of riboflavin, vitamin A and iron in relation to anemia among Indonesian adolescent schoolgirls. We investigated the possible relationships between anemia and riboflavin, vitamin A or iron, using both cross-sectional and intervention studies. We also took into consideration potential bias when interpreting the results.

We undertook our studies in Jakarta and Tangerang, which represent urban and rural settings, respectively. Compared to other locations in Indonesia, people living in these locations have easier physical access to health, education and other public services. The locations are not considered as malaria endemic areas, which have the potential to magnify the anemia problem. We also selected adolescent girls attending state schools, which have never been targeted by any nutrition related programs. Girls not attending school are more likely coming from poorer families and can be assumed to have a poorer nutritional status. Thus we consider that our population fairly represents the majority of Indonesian adolescent girls living in a relatively favourable environment. It should be noted that even these girls did not necessarily have good micronutrient status.

Based on the findings of our two intervention studies (*chapter 2,5*), we concluded that anemia in adolescent schoolgirls was predominantly due to iron deficiency. Because of ethical considerations, we did not include a placebo group in the second intervention study (*chapter 5*). The increase of hemoglobin concentration > 10 g/L after 8 weeks of iron supplementation with or without riboflavin or vitamin A indicated that the anemia in this population was likely to have been due at least in part to iron deficiency [1].

The relative importance of vitamin A and riboflavin on iron status of adolescent girls.

When planning our trial (*chapter 5*), we expected that the hemoglobin response to supplementation with riboflavin and vitamin A would have been larger in anemic girls than in their non-anemic peers. Additionally, we considered that non-anemic girls would not or only marginally benefit from the interventions. Thus our decision to restrict participation in the trial to anemic girls was aimed at measuring the maximal effects of the supplementation. In this study, 60% of girls were borderline anemic (hemoglobin concentration 110-119.9 g/L) and the remaining girls were moderate anemic. We found that riboflavin and vitamin A led to no or only a marginal hemoglobin response, we can be confident that the effects on the concentrations of hemoglobin and ferritin in the mixed population of anemia and non-anemic girls would have been even less.

We are not aware of any studies conducted on adolescent girls to show the relationship between riboflavin and iron status. It was evident that there was a relationship between riboflavin and iron status in the East Jakarta study (*chapter 3*); in contrast no such result was observed in the other cross-sectional studies (*chapter 4*). In the intervention study (*chapter 5*), despite an improvement of riboflavin status in girls who received riboflavin supplements, we found no evidence that daily supplementation with riboflavin additional to iron improved hemoglobin concentration more than supplementation with iron alone (*chapter 5*). We cannot exclude the possibility that riboflavin supplementation could have improved iron status in girls with more severe anemia or iron deficiency. This deserves further investigations because few randomized trials have been carried out on this topic.

Several intervention studies in adolescent girls [2,3] had shown marked improvements after additional vitamin A supplementation on iron status beyond that of iron alone. However, our study (*chapter 5*) and others [4,5] did not show beneficial effects of vitamin A. In our study, despite an improvement of vitamin A status in girls who received vitamin A supplements, it obviously did not contribute to the additional increments of hemoglobin concentration. However, we observed 19% increase in plasma ferritin concentration in all girls who received additional vitamin A. In contrast to our study, all studies conducted by others [2-5] included both anemic and non-anemic girls. The two studies, which showed an effect, had only post-menarchal girls as their subjects; whereas those, which did not [4,5], included both pre- and post-menarchal girls. Additionally, the findings from our cross-sectional studies were inconsistent (*chapter 4*): we could not show a relationship between vitamin A and iron status in the Tangerang-1 study, although such a relationship was observed in Tangerang-2 and Central Jakarta studies. We cannot adequately explain the inconsistencies between our findings and those from other trials. It could be that the magnitude of the effect of supplementation with vitamin A depends on unknown, study- or site-specific factors, which deserves further investigation.

Anemia is a public health problem among adolescents especially girls [1,6]. Different combinations of iron supplementation have been proposed to overcome the problem [2-5,7]. Iron supplementation given to adolescent schoolgirls markedly improved their iron status as indicated by increments in hemoglobin and plasma ferritin concentrations, the prevalence of girls with minimal target iron status, and a reduction in plasma transferrin receptor concentrations (*chapter 2,5*). Despite these improvements, approximately 20% of girls in our study did not recover from anemia. There was an indication that their anemia was not due to iron deficiency as iron supplementation, which was able to increase plasma ferritin concentration, could not necessarily increase the hemoglobin concentration.

The failure to improve hemoglobin concentration by additional riboflavin or vitamin A to iron supplements shows the importance of considering other micronutrients to effectively improve hemoglobin concentration. Other vitamins deficiencies may have been the possible cause in limiting the hematological response. The whole range of vitamins and minerals should play in concert in the process of hemoglobin synthesis; one single micronutrient deficiency could hinder the process, leading to anemia. A study in anemic preschoolers showed lack of hemoglobin response to iron supplements, which was due to vitamin B12 deficiency [8]. Perhaps there is a need for multivitamin supplements studies to compare the effectiveness of different vitamin combinations in order to examine possible interaction or enhanced effects. In our study (*chapter 5*), however, we did not observe any interaction effect of vitamin A and riboflavin on the iron status of the girls. This may have been due to insufficient sample size to provide adequate precision in measuring such an effect.

The importance of optimal vitamin A and riboflavin status in adolescence girls.

During adolescence, girls experience growth spurt especially in reproductive organs maturation process, rendering the importance of nutrients provisions in optimal amounts, including riboflavin, vitamin A and iron.

In our studies, we did not assess the clinical or functional consequences of riboflavin, vitamin A and iron deficiencies, especially during this peak growth of adolescence. The adolescent girls were deficient of riboflavin (*chapter 3-5*), of vitamin A (*chapter 4,5*) and of iron (*chapter 2-5*). Although riboflavin or vitamin A did not contribute to increase iron status shown in our study (*chapter 5*), however, the important roles of these two vitamins and also iron during this period of the girls' life are being recognized [9-15]. In addition, the micronutrients intakes of the adolescent girls in our studies were poor (*chapter 3,5*). These problems demand special attention.

The preparation for safe motherhood

The age range of girls in our studies was 11-17 years. In Indonesia, girls on average get married at the age of 18 years, and often soon followed by their first pregnancy [16]. Nutrients stores of women when they enter pregnancy are important determinants of perinatal mortality [17]. In our studies (*chapter 2-5*), we observed a high prevalence of anemia, and two third of girls had not achieved minimal target iron status or adequate store of vitamin A. Girls with anemia, low status of riboflavin, vitamin A or iron will enter pregnancy with potential risks: there is an increased

risk of preterm birth in pregnant women with low hemoglobin level during the first trimester [18-21]. As adolescent girls in our studies will reach the age of 18 years in a matter of years, therefore, their low status of micronutients deserves special attention.

Based on our findings, we conclude that in our study population:

1. additional riboflavin to daily iron supplementation for eight weeks did not contribute to the improvement of hemoglobin or plasma ferritin concentrations.
2. additional vitamin A to daily iron supplementation for eight weeks did not contribute to the improvement of hemoglobin or plasma ferritin concentrations.
3. anemia in the majority of the girls was due to iron deficiency.
4. cessation of iron supplementation for 16 weeks decreased iron status.
5. anemia is a public health problem.
6. significant deficiency of riboflavin, vitamin A and iron do exist.
7. about two-thirds of girls did not reach minimal target iron status or adequate store of vitamin A.

Future research

Anemia in adolescent girls, most probably caused by poor micronutrient status, is of severe public health significance to Indonesia. Our studies could not specifically address the effects of additional micronutrients, such as riboflavin, in mitigating this problem. However, it is plausible that the low micronutrient status will influence other aspects of adolescent health.

We, therefore, suggest further investigation targeting adolescent girls. This research may concentrate on the following topics:

1. More research might be justified to study whether a very low riboflavin status does have functional consequences threatening health.
2. In this thesis main attention is given to improving micronutrient status in adolescent girls indicating that such an improvement should especially support and improve pregnancy performance and outcome in young girls becoming pregnant. It is therefore recommended to carry out a prospective study on the efficacy of micronutrient intervention in adolescent girls on the pregnancy performance and outcome.

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Summary

Anemia is a problem of severe public health significance in Indonesia, especially in pregnant women. The usual cause of anemia is iron deficiency; however, as is the case in many other developing countries, other micronutrients deficiencies are common and may also cause anemia. Adolescent girls, especially post-menarchal girls are more prone to anemia due to blood loss during menstruation compared to their pre-menarchal counterparts. Moreover, dietary data has shown that the nutrient intake of adolescent girls is insufficient to meet their requirements. Poor micronutrient status or deficiency could adversely influence their ongoing growth and sexual maturation process. It is important that adolescent girls maintain a good micronutrient status during this important period of pre-reproductive life to build a strong basis of women's nutritional health throughout their entire reproductive life. Failure to realize this would affect adolescent girl's current micronutrient status, and would impose additional health burdens upon future generations. Programs to overcome anemia exist in Indonesia but they do not target adolescent girls. Data on the nutritional status of adolescent girls is scarce.

This thesis aimed at assessing deficiencies of riboflavin and vitamin A as possible determinants of anemia and iron status in adolescent girls.

We carried out cross-sectional and intervention studies to assess the relationship between riboflavin, vitamin A or iron status and anemia. The study population was Indonesian adolescent girls aged 11-18 years and attending state secondary schools. The studies were conducted in both rural and urban slum settings of west Java and Jakarta, both in the rainy or dry seasons of 1995, 1998-2000 and 2002.

The first intervention study (*chapter 2*) was a placebo-controlled randomized trial to assess the efficacy of weekly supplementation with iron versus placebo for 16 weeks (*n = 202*). It showed that iron supplementation improved concentrations of hemoglobin and serum ferritin, reduced the prevalence of anemia (by 22%, 95%CI: 9-35%) and iron deficiency (by 12%, 95%CI: 2-22%) and increased the prevalence of girls with minimal target iron status (by 28%, 95%CI: 15-41%). These gains in iron status were largely lost at 16 weeks after cessation of iron supplementation.

Cross-sectional studies in assessing the relationship between riboflavin and iron status showed inconsistent results. The study in East Jakarta (*chapter 3*), involving post-menarchal adolescent girls aged 15-18 years (*n = 107*) showed a relationship with or without adjustment for worm infestation and socio-economic factor: a reduction of riboflavin status corresponding to an increase of the erythrocyte glutathione reductase activity (EGRAC) by 1 unit was related to a reduction of hemoglobin concentration by 33.5 g/L (95% CI: 27.8-39.2 g/L), and a decrease in plasma ferritin concentration by 92% (95% CI: 89-94%). However, studies in Tangerang and Central Jakarta (*chapter 4*), which involved post-menarchal adolescent girls aged 11-17 years (*n = 749*), failed to show a relationship between riboflavin status and concentration of hemoglobin or plasma ferritin.

A clear relationship between vitamin A status and hemoglobin concentration was observed only when all surveys results were combined. Relationship between vitamin A status and plasma ferritin concentration was observed when all survey results combined and in Central Jakarta (*n = 307*), with the highest prevalence of anemia (57%), deficiencies of iron (57%) and vitamin A (21%).

A second intervention study (*chapter 5)* aimed at assessing the efficacy of daily supplementation for 8 weeks with riboflavin or vitamin A, given either alone or in combination in anemic adolescent schoolgirls (*n = 258*). All girls in this study also received daily iron supplements. Supplementation with riboflavin or vitamin A resulted in no or only marginal effects on concentrations of hemoglobin and plasma ferritin. In an exploratory subgroup analysis, it was found that vitamin A supplementation increased plasma ferritin concentration by 19% (95% CI: 1-39%). Additionally, when assessing effects on concentration of hemoglobin, plasma ferritin or plasma transferrin receptor, it appeared that girls with the lowest iron status at baseline benefited more from supplementation with riboflavin additional to iron than girls who received iron only. Although iron supplementation sharply reduced iron deficiency (58% to 4%), 30% of the girls still had elevated concentrations of plasma soluble transferrin receptor, indicating residual iron deficiency. Despite the marginal effects of additional riboflavin or vitamin A upon improving iron status, supplementation with riboflavin and vitamin A markedly reduced the prevalence of deficiencies of riboflavin and vitamin A, respectively.

In the intervention studies (*chapter 2,5*), anemia persisted in 21-29% of girls receiving iron supplements.

The percentage of girls with intakes of riboflavin, vitamin A or iron reported below the Indonesian recommended daily dietary allowances was 45-88%, 75% and 95-99%, respectively (*chapter 3,5*).

In our study populations (*chapter 2-5*), the prevalence of anemia (9-57%) indicates that anemia in Indonesian adolescent schoolgirls remains a problem of major public health significance. The prevalence of riboflavin, vitamin A and iron deficiency ranged 21-96%, 7-25%, and 20-58%, respectively. The prevalence of girls with iron stores below the minimal target to prevent anemia in subsequent pregnancies ranged between 50% and 82%; the prevalence of inadequate vitamin A stores ranged between 40% and 73%.

In conclusion, anemia, deficiencies of riboflavin, vitamin A and iron, insufficient iron status and inadequate store of vitamin A are highly prevalent among Indonesian adolescent schoolgirls, and the intake of these micronutrients is poor. The major cause of anemia is iron deficiency while only a small part is due to deficiencies other than iron. Adolescent girls aiming to reach sufficient iron status before pregnancy should be encouraged to continue taking weekly iron supplements during their school years.

Supplementation with riboflavin or vitamin A additional to iron does not or only marginally improves iron status as compared to supplementation with iron alone.

The high prevalence of vitamin A and riboflavin deficiencies are a cause for concern; however, because the functional consequences of these deficiencies in adolescent girls is poorly known, our findings provide insufficient ground to justify intervention programs for riboflavin and vitamin A.

Future studies should be conducted to investigate the functional consequences of low riboflavin and vitamin A status on the health of adolescent girls, and to assess the efficacy of micronutrient supplementation in adolescent girls on pregnancy performance and outcomes.

Samenvatting

Bloedarmoede is een groot probleem voor de volksgezondheid in Indonesië, vooral in zwangere vrouwen. De gebruikelijke oorzaak is ijzergebrek; echter, zoals in zoveel ontwikkelingslanden, komt gebrek aan andere micronutriënten ook vaak voor, en dit kan ook bloedarmoede veroorzaken. Door bloedverlies hebben adolescente meisjes na de eerste menstruatie vaker bloedarmoede dan meisjes die nog niet hebben gemenstrueerd. Gegevens over de voedselconsumptie hebben bovendien laten zien dat de inname van voedingsstoffen onvoldoende in de behoeften voorziet.

Een gebrekkige status aan micronutriënten kan het zich ontwikkelende groeiproces en de geslachtelijke rijping benadelen. Het is daarom belangrijk dat adolescente meisjes al vóór de voortplanting een sterke basis opbouwen die nodig is voor een gezonde voedingstoestand gedurende hun gehele reproductieve leven. Als dit niet wordt verwezenlijkt, dan leidt dit tot een armzalige voedingsstatus tijdens de adolescentie, en belast dit de gezondheid van toekomstige generaties. Er bestaan programma's ter bestrijding van bloedarmoede in Indonesië, maar deze zijn niet gericht op adolescente meisjes. Gegevens over de voedingstoestand van adolescente meisjes zijn schaars.

Het onderzoek dat in dit proefschrift wordt beschreven, was erop gericht om te kunnen beoordelen in hoeverre gebrek aan riboflavine en vitamine A oorzaken zijn van bloedarmoede en ijzerstatus in adolescente meisjes.

Wij voerden zowel een dwarsdoorsnedenonderzoek als interventiestudies uit om de relaties tussen status voor riboflavine, vitamine A, ijzer en bloedarmoede te onderzoeken. De studiepopulatie bestond uit Indonesische adolescente meisjes in de leeftijd van 11 tot 18 jaar die rijksmiddelbare scholen bezochten. De studies werden uitgevoerd in krottenbuurten in steden en op het platteland van westelijk Java en Jakarta, zowel in de regenseizoenen en droge seizoenen van 1995, 1998-2000 en 2002.

De eerste interventiestudie (*hoofdstuk 2*) betrof een gerandomiseerd experiment om de werkzaamheid te bepalen van wekelijkse suppletie met ijzer versus placebo gedurende 16 weken (n=202). Deze studie toonde aan dat ijzersuppletie leidde tot verhoogde concentraties van hemoglobine en serumferritine, een verlaging van de prevalentie van bloedarmoede (met 22%, 95% BI: 9-35%), ijzergebrek (met 12%, 95% BI: 2-22%), en een verhoging van de prevalentie van meisjes met een ijzerstatus die als minimaal werd beschouwd (met 28%, 95% BI: 15-41%). Bij 16 weken nadat ijzersuppletie was gestaakt, was deze winst in ijzerstatus weer grotendeels

verloren gegaan.

De dwarsdoorsnedenonderzoeken om de relaties tussen riboflavinestatus en ijzerstatus te onderzoeken, leidden tot tegenstrijdige resultaten. De studie in Oost-Jakarta (*hoofdstuk 3*) betrof adolescente meisjes in de leeftijd van 15-18 jaar (*n=107*). Deze studie toonde aan dat een verlaging van riboflavinestatus, overeenkomend met een verhoging met 1 eenheid van de 'erythrocyte glutathione reductase activity' (EGRAC), geassocieerd was met een verlaging van hemoglobineconcentratie van 33.5 g/L (95% BI: 27.8-39.2 g/L), en met een verlaging in de plasmaconcentratie van ferritine met 92% (95% BI: 89-94%). Statistische correctie voor worminfecties en sociaal-economische status leidde tot vergelijkbare schattingen. Echter, in studies in Tangerang en Centraal Jakarta (*hoofdstuk 4*) onder adolescente meisjes die hun eerste menstruatie hadden gehad en in de leeftijd van 11-17 jaar (*n=749*), kon geen relatie worden aangetoond tussen riboflavinestatus en concentraties van hemoglobine en ferritine in plasma.

Een duidelijke relatie tussen vitamine A-status en hemoglobineconcentratie kon alleen worden aangetoond als de resultaten van alle dwarsdoorsnedenonderzoeken werden samengevoegd. Evenzo werd de relatie tussen vitamine A-status en plasmaconcentratie van ferritine alleen gevonden als de gegevens van alle dwarsdoorsnedenonderzoeken werden samengevoegd en in Centraal Jakarta (*n=307*), waar tevens ook de hoogste prevalenties werden waargenomen voor bloedarmoede (57%), en voor gebrek van ijzer (57%) en vitamine A (21%).

In een tweede interventiestudie (hoofdstuk 5) werd de werkzaamheid onderzocht van dagelijkse suppletie gedurende 8 weken met riboflavine of vitamine A, die alleen of in combinatie werd gegeven aan adolescente meisjes (*n=258*) met bloedarmoede. Alle meisjes in deze studie kregen ook dagelijkse ijzersupplementen. Suppletie met riboflavine of vitamine A resulteerde in geen of slechts marginale effecten op de concentraties van hemoglobine en ferritine in plasma. Uit een verkennende subgroep-analyse bleek dat vitamine A-suppletie leidde tot een verhoging van de plasmaconcentratie van ferritine (met 19%, 95% BI: 1-39%). Bovendien bleek bij de analyse van de effecten op hemoglobine-concentraties en van plasmaconcentraties van ferritine en transferrine-receptor, dat meisjes die bij begin van de studie de laagste ijzerstatus hadden, méér baat hadden van suppletie met riboflavine en ijzer dan meisjes die slechts ijzer ontvingen. De prevalentie van ijzergebrek werd beduidend minder door de ijzersuppletie (van 58% naar 4%);

desondanks had aan het eind van de studie nog 30% van de meisjes verhoogde plasmaconcentraties van transferrine-receptor, hetgeen duidde op resterend ijzergebrek. Ondanks de marginale effecten op ijzerstatus, leidde suppletie met riboflavine en vitamine A tot een beduidende reductie van de prevalentie van gebrek van riboflavine respectievelijk vitamine A.

In de interventiestudies (*hoofdstukken 2, 5*) bleef bloedarmoede bestaan in 21-29% van de meisjes die ijzersupplementen ontvingen.

De percentages meisjes met een inname van riboflavine, vitamine A of ijzer die lager waren dan de voor Indonesië geldende aanbevolen dagelijkse hoeveelheden was respectievelijk 45-88%, 75%, en 95-99% (*hoofdstukken 3,5*).

De hoge prevalentie van bloedarmoede in onze studiepopulaties (*hoofstukken 2, 5*) duidt erop dat dit een groot probleem voor de volksgezondheid blijft vormen. De prevalentie van gebrek van riboflavine, vitamine A en ijzer varieert respectievelijk tussen 21-96%, 7-25%, en 20-58%. Tussen 50% en 82% van de meisjes had onvoldoende lichaamsvoorraden van ijzer om bloedarmoede te voorkomen in daaropvolgende zwangerschappen; de prevalentie van meisjes met onvoldoende lichaamsvoorraden aan vitamine A varieerde daarentegen tussen 40% en 73%.

Samenvattend is uit dit werk gebleken dat bloedarmoede, en gebrek van riboflavine, vitamine A en ijzer vaak voorkomen onder Indonesische adolescente schoolmeisjes, en dat de inname van deze micronutriënten laag is. De belangrijkste oorzaak van bloedarmoede is ijzergebrek, terwijl slechts een klein deel te wijten is aan gebrek aan andere micronutriënten. Adolescente meisjes die trachten een adequate ijzerstatus te bereiken voor zwangerschap moeten worden aangemoedigd om wekelijks ijzersupplementen te nemen tijdens hun schooljaren.

Suppletie met riboflavine of vitamine A naast ijzer leidt tot geen of slechts marginale verbetering van ijzerstatus in vergelijking met suppletie met slechts ijzer.

De hoge prevalentie van gebrek van vitamine A en riboflavine zijn zorgelijk; echter, omdat er van de functionele consequenties van deze gebreken in adolescente meisjes weinig bekend is, vormen onze bevindingen onvoldoende reden om gezondheidsprogramma's voor vitamine A en riboflavine te rechtvaardigen.

Verdere studies in adolescente meisjes zijn nodig om de functionele consequenties van lage status voor riboflavine en vitamine A te onderzoeken, en om de werkzaamheid te bepalen van suppletie met micronutriënten op de uitslag van zwangerschap.

Ringkasan

Di Indonesia, anemia, terutama pada wanita hamil, masih merupakan masalah kesehatan masyarakat. Kekurangan zat besi merupakan penyebab utama, namun seperti di negara-negara berkembang lainnya, kekurangan zat gizi mikro umum dijumpai juga dan hal ini mungkin merupakan penyebab terjadinya anemia. Remaja puteri, terutama yang telah mengalami haid, dibandingkan dengan yang belum haid, lebih rentan terhadap anemia, sehubungan dengan kehilangan darah yang dialami sewaktu haid. Data menunjukkan bahwa asupan makanan para remaja putri tidak dapat menyediakan cukup zat gizi untuk memenuhi kebutuhan mereka. Kekurangan zat gizi mikro pada masa remaja dapat berdampak negatif pada proses pertumbuhan dan kematangan organ-organ reproduksi. Sehubungan dengan hal tersebut, adalah penting bagi remaja puteri untuk mencapai status zat gizi mikro yang optimal sebagai dasar utama kesehatan gizi bagi seluruh masa reproduksi yang akan dilaluinya kelak. Kegagalan mencapai status yang optimal akan berdampak pada status zat gizi mikro saat ini, dan pada gilirannya dapat berdampak pada status gizi generasi penerus. Program penanggulangan anemia telah lama berjalan, namun remaja puteri tidak dijadikan target. Lagi pula, hanya sedikit data status gizi remaja puteri yang tersedia.

Disertasi ini bertujuan untuk mempelajari peran riboflavin dan vitamin A sebagai faktor yang turut menentukan status zat besi diantara remaja puteri di Indonesia.

Berbagai survei dan studi eksperimental pada remaja puteri berumur 11-18 tahun di sekolah menengah pertama, telah dilakukan untuk mempelajari hubungan antara status riboflavin, vitamin A dan zat besi dengan anemia. Penelitian dilakukan di daerah kumuh di kota, Jakarta, maupun di pedesaan di Jawa Barat, baik pada saat musim hujan maupun kemarau, pada tahun 1995, 1998-2000 dan 2002.

Uji eksperimental acak tersamar ganda dengan kontrol (bab 2) dilakukan untuk mengetahui hasil pemberian suplementasi zat besi untuk 16 minggu (n = 202). Data menunjukkan bahwa kadar hemoglobin dan ferritin serum meningkat, dan prevalensi anemia dan kekurangan zat besi menurun berturut-turut 22% (95% CI: 9-35%) dan 12% (95% CI: 2-22%). Prevalensi remaja puteri yang mencapai target minimal status zat besi meningkat 28% (95% CI: 15-41%). Namun peningkatan ini pupus setelah dihentikannya pemberian zat besi selama 16 minggu berikutnya.

Survei menunjukkan bahwa hubungan antara riboflavin dan status zat besi tidak selamanya terungkap dengan jelas. Penelitian pada remaja puteri umur 15-18 tahun (n=107) yang telah mengalami haid di Jakarta Timur (bab 3) memperlihatkan ada hubungan antara riboflavin dan status zat besi, dengan dan tanpa memperhitungkan faktor adanya infestasi cacing dan keadaan sosial ekonomi: penurunan status riboflavin yang ditandai dengan meningkatnya 1 unit aktivitas enzim glutation reduktase, setara dengan menurunnya kadar hemoglobin sebesar 33.5 g/L (95% CI: 27.8-39.2 g/L), dan menurunnya 92% (95% CI: 89-94%) kadar feritin plasma. Namun, pengamatan baik di Tangerang maupun di Jakarta Pusat (bab 4), yang melibatkan remaja puteri yang telah mengalami haid dengan umur 11-17 tahun (n=749) tidak menunjukkan adanya hubungan seperti itu.

Hubungan antara status vitamin A dengan kadar hemoglobin baru telihat bila hasil dari berbagai survei digabung. Sedangkan hubungan antara status vitamin A dengan kadar feritin plasma dapat diamati bila berbagai survei digabung ataupun di Jakarta Pusat (n=307), dimana diamati prevalensi anemia (57%), kekurangan zat besi (57%) dan vitamin A (21%) tertinggi dibandingkan dengan lokasi lainnya.

Uji eksperimental acak tersamar ganda dengan kontrol berikutnya (bab 5) bertujuan untuk mengetahui efek dari pemberian riboflavin atau vitamin A, baik sendiri maupun bersama-sama setiap hari selama 8 minggu pada remaja puteri di sekolah (n = 258). Zat besi diberikan kepada semua peserta setiap hari. Pemberian riboflavin atau vitamin A tidak atau sangat kecil pengaruhnya atas kadar hemoglobin atau feritin plasma. Hasil analisa pada sebahagian kelompok menunjukkan adanya peningkatan kadar feritin plasma sebesar 19% (95% CI: 1-39%). Selain itu, terlihat pula bahwa penambahan pemberian riboflavin meningkatkan kadar hemoglobin, feritin dan reseptor transferin plasma lebih tinggi pada remaja puteri dengan status zat besi yang lebih rendah dibandingkan remaja puteri dengan kadar zat besi cukup. Walaupun pemberian zat besi sangat menurunkan prevalensi kekurangan zat besi (58% menjadi 4%), kadar reseptor transferin plasma pada 30% remaja puteri tetap tinggi; hal ini menunjukkan masih adanya kekurangan zat besi tubuh. Walaupun penambahan pemberian riboflavin atau vitamin A hampir tidak membantu peningkatan keadaan zat besi tubuh, namun mampu menurunkan prevalensi keadaan kekurangan riboflavin dan vitamin A tubuh.

Uji eksperimental acak tersamar ganda dengan kontrol yang telah dilakukan (bab 2,5), menunjukkan bahwa walaupun remaja puteri mendapat zat besi, anemia menetap pada 21-29% diantara mereka.

Jumlah remaja puteri dengan asupan akan riboflavin, vitamin A dan zat besi dibawah kecukupan zat gizi yang dianjurkan adalah berturut-turut sebesar 45-88%, 75% dan 95-99% (bab 3,5).

Prevalensi anemia (9-57%) pada remaja puteri yang tergabung dalam penelitian ini, menunjukkan bahwa anemia masih merupakan masalah kesehatan masyarakat pada kelompok ini. Prevalensi kekurangan riboflavin, vitamin A dan zat besi berturut-turut berkisar antara 21-96, 7-25% dan 20-58%. Prevalensi remaja puteri dengan cadangan zat besi dibawah target minimal untuk mencegah kemungkinan terjadinya anemia pada kehamilan mendatang berkisar antara 40 dan 73%.

Dapat disimpulkan bahwa prevalensi anemia, kekurangan riboflavin, vitamin A dan zat besi, status zat besi yang rendah, rendahnya cadangan vitamin A tubuh serta rendahnya asupan zat gizi mikro masih tinggi diantara remaja puteri. Penyebab terbesar anemia adalah kekurangan zat besi, dan hanya sebagian kecil berhubungan dengan kekurangan zat gizi selain zat besi. Remaja puteri yang hendak mencapai kecukupan status zat besi tubuh sebelum mengalami kehamilan, dianjurkan untuk tetap mengkonsumsi tambahan zat besi selama mereka berada dalam lingkungan sekolah.

Pemberian riboflavin atau vitamin A sebagai tambahan pemberian zat besi dibandingkan dengan pemberian zat besi saja, tidak atau sedikit sekali menyumbang pada peningkatan status zat besi.

Tingginya prevalensi kekurangan riboflavin dan vitamin A memprihatinkan, namun pengetahuan tentang dampak kekurangan tersebut pada remaja puteri masih sangat sedikit, sehingga hasil penelitian ini belum mempunyai dasar yang utuh untuk menganjurkan pemberian tambahan riboflavin maupun vitamin A.

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Februhartanty J, Dillon D, Khusun H. Will Iron supplementation during menstruation improve iron status than weekly supplementation? *Asia Pacific J Clin Nutr* 2002;11(1): 36-41.

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The Author

Drupadi was born on May 28th, 1951 in Jakarta, Indonesia. She spent her childhood in a small town, surrounded by beautiful plantations in North Sumatra. Her father, a physician, used to take her along on his visits to health clinics belonging to the plantations, where she first saw malnutrition among the poor plantation workers, especially pregnant women.

After completing her secondary education at SMA Teladan Jakarta in 1969, she pursued medicine at the Faculty of Medicine, University of Indonesia, and graduated in 1975. Immediately upon graduation she volunteered to work at the Pediatric Department in an army hospital, in central Jakarta, where she witnessed many malnutrition problems among her patients. Since then, her interest in maternal and child health has continued to grow unabated.

In 1976, during her sojourn in Ithaca accompanying her husband, she took a number of courses in nutrition at Cornell University and became convinced that nutrition is a very important determinant of health and well-being. She found a good opportunity to supplement vitamins and minerals to the plantation workers while accompanying her husband doing his field research for his doctoral dissertation in North Sumatra in 1978.

In 1980, she joined the Department of Nutrition, Faculty of Medicine, University of Indonesia. She became head of the SEAMEO-TROPMED Regional Center for Community Nutrition laboratory after completion of her Master's in Applied Human Nutrition at the same institution in 1990. The GTZ Nutrition project in this institution helped her to realize her dream to turn the laboratory specializing in micronutrients. She was then appointed as the Deputy Director of the same institute. She attended the European Nutrition Leadership Training in Luxembourg in 2000 as part of her task to found a training of nutrition leadership for the South East Asia region. Now, the South East Asia Nutrition Leadership Training Program has been running for three consecutive years.

She had the opportunity to attend some training programs with nutrition related topics at Cornell University and Boston University in USA; MRC Dunn Nutrition Laboratory Cambridge, LSHTM University of London, King's College London, and University of Southampton, in England.

After making sure that she would not be neglecting her three growing sons, she then started her PhD program at Wageningen University in June 1999. Being convinced of the importance of maternal and child health, she decided to focus on adolescent girls as a group of women preparing for the maternal stage and chose anemia among adolescent schoolgirls as the topic of this thesis.

Despite the long separation from her beloved family in Indonesia, she survived the campus life, happily biking around Wageningen. To her, the process of writing the thesis has proven to be very exciting.

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