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Underweight as a Risk Factor for Iron Depletion and Iron-Deficient Erythropoiesis among Young Women in Rural Areas of East Java, Indonesia

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ABSTRACT

Introduction: Underweight and iron deficiency are serious problems in Indonesia. A good understanding of the association of these problems is required. **Methods:** A cross-sectional study was conducted in Probolinggo Regency, East Java Province, Indonesia on 115 non-pregnant, apparently healthy women, aged 21.7±3.7 years who were recruited after physical examination and pregnancy test at the Public Health Centre. Body weight and height were measured to calculate body mass index. Levels of haemoglobin, serum ferritin and soluble transferrin receptor (sTfR) were determined to assess parameters of iron status (WHO, 2007). Independent *t*-test was used to compare the mean difference of underweight group (n=27) and non-underweight group (n=88). Binary logistic regression was used to determine the association between underweight and iron status, and odds ratio. **Results:** The results indicate that 23.5% of women were underweight, and 33% anaemic. Anaemia among underweight women was 48.1%, while in the non-underweight (normal and overweight) women, it was 28.4%. Rates of iron depletion (37%) and iron-deficient erythropoiesis (IDE) (48.1%) among underweight women were higher than among the non-underweight (9.1% and 17% respectively). After adjusting for nutrient intake, underweight women were seven times more likely to have depleted iron store (OR: 7.05; 95% CI: 1.17-42.41; p=0.03), and approximately four times more likely to be IDE (OR: 3.93; 95% CI: 1.46 - 10.54; p=0.007) compared to those who were not underweight. **Conclusion:** Iron deficiency is more prevalent among underweight young women. Underweight is a risk factor for IDE and iron depletion rather than for anaemia. In addition, the risk for iron depleted iron store is higher than the risk for IDE among underweight young women. Therefore, iron supplementation to prevent iron deficiency among non pregnant women should be simultaneously followed with high energy density supplementary feeding for underweight women in this group.

Key words: Anaemia, iron deficiency, iron-deficient erythropoiesis, iron depletion

INTRODUCTION

Iron deficiency is the most widespread nutrient deficiency in the world, and women of reproductive age are highly susceptible to this problem (World Health Organization, 2015; Black *et al.*,

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2008). Several studies in some countries report that iron deficiency is more likely to occur among overweight and obese individuals,both in adolescents and adults (Aigner, Feldman & Datz, 2014; Eftekhar, Mozaffari-Khosravi & Shidfar, 2009; Nead *et al.,* 2004; Pinhas-Hamiel *et al.*, 2003). The high prevalence of iron deficiency among overweight and obese adolescents might be due to their excessive dietary and calories intake particularly their high fat diet (Sonnweber *et al.*, 2012), excess adiposity and inflamation (Tussing-Humphreys *et al.*, 2009). In contrast, a study in China demonstrates that anaemia is more prevalent among underweight women rather than among overweight and obese women (Qin *et al.*, 2013); this is also found in Bangladesh (Kamruzzaman *et al.,* 2015) and India (Gupta *et al.,* 2012). Another study revealed that both body mass index (BMI) below the 5thpercentile and BMI above the 85th percentile are risk factors for iron deficiency anaemia among adolescents in Southwest Iran (Keikhaei, Askari & Aminzadeh, 2012). Based on these studies, it appears that either underweight or overweight are associated with anaemia or iron deficiency.

Many countries face a double burden of malnutrition that includes both under-nutrition and overweight, especially in low-and middle-income countries (Shrimpton & Rokx, 2012; World Health Organization, 2015). Underweight, which is reflected by low body mass index as well as iron deficiency, is very prevalent in many developing countries, including Indonesia. Based on national data survey, there is increasing evidence of the prevalence of both underweight and overweight among adolescents aged 16-18 years old as well as among adult women (> 18 years old) over the last three years. The prevalence of underweight among 16-18 years old increased from 8.9% in 2010 to 9.4% in 2013, but decreased among adults from 12.6% in 2010 to 8.1% in 2013. On the other hand, there was greater prevalence of overweight among 16-18 years old from 1.4% in 2010 to 7.3% in 2013, and a very high prevalence of overweight (BMI> 25) among adult women which increased from 14.8% in 2010 to 32.9%. Moreover, prevalence of anaemia among women in the reproductive age was 22.7% (Indonesian Health Ministry, 2010; Indonesian Health Ministry, 2013).

In developing countries, underweight is still a serious problem, and may also relate to micronutrient deficiency, including iron deficiency. Moreover, iron deficiency, particularly among women in the reproductive age, also has a role to play in poor pregnancy outcomes, such as low birth weight, preterm delivery and intra-uterine growth retardation (Allen, 2001; Rasmussen, 2001; Allen, 2005, Scholl, 2011). Several studies have demonstrated that iron status before conception is a strong predictor of maternal iron status and anaemia in later pregnancy and pregnancy outcomes (Casanueva *et al.*, 2003; Ronnenberg *et al.,* 2004), and also has a negative impact on iron status of their children (Abu-Ouf & Jan, 2015). Attention should also be paid to women in reproductive age with low BMI (underweight) as well as high BMI (obesity) who are vulnerable to poor pregnancy outcomes (Neggers & Goldenberg, 2003; Leddy, Power & Schulkin, 2008). In an effort to address thus problems, the present study objectives were to (i) obtain a further understanding on the association between weight status, as measured by BMI and iron deficiency, and (ii) understand whether weight status is a risk factor for iron deficiency which is reflected by a low level of haemoglobin and serum ferritin, and the elevation of serum transferrin receptor concentration.

METHODS

A cross-sectional study was conducted in Probolinggo Regency, East Java Province, Indonesia, because according to the

Indonesian Health Ministry (2013) figures, the prevalence rates of both underweight and overweight in East Java is higher than national data. Probolinggo Regency was selected because a previous study showed that the prevalence of underweight as well as anaemia among women in reproductive age in this regency is 27.3% and 48.5%, respectively (Sumarmi *et al.*, 2010). The most recent study showed that the proportion of anaemia among newly married women in Probolinggo is 15% (Putri & Sumarmi, 2013).

The sample size was determined by using multistage-random sampling. In the first stage, we selected nine sub-districts based on percentage of underweight women in reproductive age reported in a previous study (Sumarmi *et al.,* 2010). In the second stage of sampling, 420 pre-marital women (PMW) who were listed in nine sub-districts Office of Religious Affairs were selected based on the inclusion criteria of age 16-35 years, not pregnant, and apparently healthy. Of the 420 PMWs in the list, 45 had moved out from the study area. A physical examination and a pregnancy test were carried out on the remaining 375 women. The physical examination was carried out by a medical doctor in Public Health Centre, while the pregnancy test was done by midwives using the human chorionic gonadotrophin (hCG) kit from OneMed® to test their urine. Eighty-six women were found to be pregnant, while 12 did not meet the age criteria (< 16 years old = 7, and> 35 years old = 5), resulting in 277 women for the sample. Based on sample size calculation using software Sample Size version 2.0 (Lwanga & Lemeshow, 1991), with confidence level (1-α) 95%, anticipated population proportion (P) was 15%, and absolute precision (d) 0.05, a sample size of 115 women was needed for our study.

Anthropometric measurements, including the measurement of weight, height, and mid-upper arms circumference (MUAC), were carried out by trained field workers under the supervision of the researcher. Body weight was measured using Seca® type 803 digital body scales, with increment of 100 grams. Height was measured using microtoise with 0.1 cm increment. BMI was calculated as a ratio of weight in kilograms and square of height in meter (kg/m^2). Age-specific BMI reference values for adolescents were used to evaluate the nutritional status of 15-20-year-old participants (De Onis *et al.*, 2007). Underweight was determined by a BMI value of less than the fifth percentile. For participants older than 20 years underweight was defined as BMI less than 18.5 (Gibson, 2005).

Venous blood samples (8 ml) were collected and analysed to assess the level of serum ferritin and soluble transferrin receptor (sTfR). Haemoglobin level was determined from samples of approximately 10μL of capillary whole blood drawn using HemoCue Hb 201 micro-cuvettes. The samples were then analysed using a portable haemoglobin meter kit HemoCue® AB, from Anglehome, Sweden. Ferritin was determined from samples of serum (0.5 cc) obtained from approximately 3-4 ml venous blood, and analysed using Micro Particle Enzyme Immunoassay (MEIA) using Axym MEIA. Soluble transferrin receptor was also assessed from samples of serum (0.5 cc) obtained from approximately of 3-4 ml venous blood. The samples were then analysed using Particle-Enhanced Immuno-turbidimetric Assay (PEIA) method, with commercial reagents Tina-quant® Soluble Transferrin Receptor on a Modular P analyser (*Roche* Diagnostics) and read by Cobas Integra 400/600/800 analyser machine. Procedure and principle test have been described in detail by Souminen *et al.* (1999). Levels of haemoglobin, ferritin and sTfR were used as parameters of iron status. Anaemia was determined using single cut-off point of haemoglobin less than 12 g/dL; iron depletion was stated if serum ferritin concentration was less than 15mg/L; serum ferritin concentration of less than 30 μg/L was considered as marginal iron store; and serum ferritin concentration 30 μg/L to 60 μg/L was considered as normal repleted. If serum ferritin concentration was greater than 60μg/L, it was considered as excess iron store. A serum ferritin concentration of greater than 250 μg/L was considered as sub-clinical infection or inflammation, and was excluded from the analysis (World Health Organisation, 2007). Iron deficiency erythropoiesis (IDE) was stated when concentration of sTfR was more than 4.4 mg/L. Because normal threshold of transferrin receptor in blood serum or plasma has not been defined, the World Health Organization (2007) suggests using the sTfR threshold recommended by the manufacturer of the assay. Therefore, we defined cut-off points for IDE using the Roche Diagnostic standard. Dietary data were obtained from a single-day 24-h dietary recall, and nutrients intake was analysed by using Nutrisurvey software.

Data processing and analysis were executed using the SPSS for windows statistical software package version 13.0 (SPSS Inc., Chicago, IL, USA). Normal distribution test of data set was done using one-sample Kolmogorov Smirnov test. Based on one-sample Kolmogorov Smirnov test, most of our data set were found to be normally distributed ($p > 0.05$), including haemoglobin concentration and serum ferritin, but concentration of sTfR appeared to be a typical right-skewed distribution ($p < 0.05$). Much of the dietary data were normally distributed as well, except for data intake of Poly Unsaturated Fatty Acid (PUFA), vitamin A and vitamin C which had a right-skewed distribution. Normally distributed data were expressed by means and standard deviation (SD), while the right-skewed distributed data were log transformed in all calculations. For data presentation, these variables were transformed back to the original scale and presented as mean (±SD). Bivariate logistic regression (confidence interval of 95%, and $\alpha = 0.05$) was used to determine the association between weight status (underweight or overweight) and iron deficiency, or the risk factor of iron deficiency. Multivariate logistic regression was executed to analyse weight status and dietary intake simultaneously to ensure that weight status has strong association with iron status, even though it had been adjusted by several dietary intakes. Stepwise multivariate logistic regression was carried out to determine the candidates of confounding variables. The eligible candidates of confounding variables were selected from bivariate analysis between underweight status and each variable of dietary intake, which had a p value < 0.25.

Ethics approval

The study protocol was approved by the ethical committee of the Faculty of Medicine, Gajah Mada University, Yogyakarta, Register No. KE/FK/202/EC. All participants were informed about the aim of the study by the researcher. On hearing or reading the explanation for this study, the participants agreed to participate in the study and signed the informed consent form.

RESULTS

Overall, the sample population consisted of young women with an average age of 21.7 ± 3.7 years (Table 1). More than 40% of the women had a low level of schooling. Percentage of underweight (23.8%) was greater compared to those who were overweight (9.6%) and obese (2.6%). The average levels of haemoglobin, serum ferritin and soluble transferrin receptor among participants were 12.4 ± 1.1g/dL, 50.6 ± 35.7 μg/L and 3.7 ± 1.4 mg/L, respectively. Of the 115 participants (33% were anaemic (Hb < 12 g/dL), 15.7% had depleted iron stores (serum ferritin < 15 μg/L), and 16.5% had marginal iron

Table 1. Characteristics of study subjects

Variables	*n*	*Mean±SD (range)*
Age (years)	115	21.7 ± 3.7 (16 - 31)
Weight (kg)	115	47.3 ± 8.6 (29.0 – 72.2)
Height (cm)	115	150.4 ± 5.6 (136.5 – 164.5)
BMI	115	20.9 ± 3.7 (15.4 – 34.5)
Iron status		
Hemoglobin (g/dL)	115	12.4 ± 1.14 (9.1 – 15.7)
Feritin (μg/L)	115	50.6 ± 35.7 (3.2 – 188.3)
sTfR (mg/L)	115	3.7 ± 1.4* (1.9 – 13.6)
Nutrients intake		
Energy (kcal)	115	1575 ± 428 (720 – 2612)
Protein (g)	115	60 ± 20 (23 – 131)
Total fat (g)	115	66.1 ± 24.6 (17.1 – 138.6)
PUFA (g)	115	12.2 ± 1.9* (2.2 – 39.0)
Vitamin A(μg)	115	616.4 ± 3.1* (0 – 6116)
Vitamin C (mg)	115	15.9 ± 3.2* (0 – 193.2)
Folic acid (μg)	115	116.5 ± 59.1 (23 – 318)
Iron (mg)	115	7.7 ± 3.2 (2.2 – 16.7)
Zn (mg)	115	7.4 ± 2.6 (2.3 – 14.7)
		%
Nutritional status base on BMI		
Underweight	27	23.5
Normal weight	74	64.3
Overweight	11	9.6
Obese	3	2.6
Hb (g/dL)		
< 9.5	1	0.9
9.5 ≤ Hb < 12	37	32.1
≥ 12	77	67.0
Serum ferritin (μg/L)		
< 15	18	15.7
15 ≤ ferritin < 30	19	16.5
30≤ferritin < 60	37	32.2
≥ 60	41	35.6
sTfR (mg/L)		
≥ 4.4	28	24.3
1.9 ≤ sTfR < 4.4	87	75.7
< 1.9	0	0
Age (years)		
< 20	40	34.8
20 ≤ age < 25	55	47.8
> 25	20	17.4
Education level		
Middle or less	47	40.9
High school	48	41.7
College or above	20	17.4
Income level (Rupiahs)		
< 1000 000	63	45.8
1000 000 – 2500 000	48	41.7
> 2500 000	4	3.5

* Mean and SD were derived from log-transformed data due to data being not normally distributed.

store. About 32% of women fell into the normal repleted store category while 35.7% were categorised as having excess iron stores (serum ferritin ≥ 60 μg/L). Based on maximum value of serum ferritin concentration (range: 3.2 – 188.3 μg/L), this data indicate that inflammation does not co-exist in our study sample. Based on sTfR concentration, 24.3% had iron-deficient erythropoiesis. Table 1 describes the characteristics of the sample.

For further analysis, data are presented as dichotomous data based on weight status. In category I, the dichotomous data consist of underweight and non-underweight (normal weight, overweight and obese), while category II consists of overweight (overweight and obese) and non-overweight (normal and underweight). Anaemia among the underweight group was 48.1% compared to the non-underweight group, which was 28.4%. Iron depletion (37%) and IDE (48.1%) rates among underweight were higher than those who were not underweight (9.1% and 17% respectively). On the other hand, in categoy II, anaemia among the overweight group was 14.3% compared to 35.6% in the non-overweight group. Meanwhile, iron depletion and IDE among overweight women were 11.5% and 26.9%, respectively.

Binary logistic regression analysis was applied to determine underweight as a risk factor for low iron status. The bivariate analysis demonstrated that underweight was not associated with anaemia (OR 2.34; 95% CI: 0.965-5.674; p=0.060), but was significantly associated with iron depletion state and iron-deficient erythropoiesis. Women who were underweight were six times more likely to have depleted iron stores (OR: 5.882; 95% CI: 2.024 -17.097), and approximately four times to be IDE (OR: 4.519; 95% CI: 1.770-11.538; p=0.002) compared to the non-underweight. Logistic regression results demonstrated that overweight was not associated with all the parameters of iron status considered in this study (Table 2).

Dietary intakes of energy, protein, total fat and PUFA intake as well as micronutrients, including several vitamins and minerals are presented in Table 1. Significant associations between energy intake level and iron depletion, and IDE were observed. However, the levels of protein intake, total fat and polyunsaturated fatty acid (PUFA) were not associated with iron depletion and IDE (Table 3). Dietary data were also analysed simultaneously with weight status category I (underweight and non-underweight), as confounding variables. The multiple logistic regression analysis indicated that after adjustment for dietary intake (energy, total fat, folic acid, vitamin C, iron and zinc), underweight showed significant association with iron depletion state (Table 4). The risk of iron depletion store was 7-fold among underweight women, compared to non-underweight (OR=7.05; 95% CI= 1.17 – 42.43; p= 0.03). After adjusting for dietary intake (energy, total fat, vitamin A, vitamin C, iron and zinc), underweight continued to have a strong association with IDE. Women who were underweight were approximately four-fold more likely to be IDE compared to those who were non-underweight (OR= 3.929; 95% CI: 1.464 – 10.540; p = 0.007).

DISCUSSION

Women of reproductive age are one of the vulnerable groups for iron deficiency. Under-nutrition among women aged 15-45 years, represented by low body mass index (BMI less than 18.5 kg/m^2 or less than fifth percentile), is prevalent in many regions. In South-central Asia, more than 20% of women in the reproductive age have a BMI of less than 18.5 kg/m^2, indicating underweight (Black *et al.*, 2008). Our results demonstrate a higher rate of underweight among young women, at 23.5%. This rate is higher than the prevalence of underweight

Table 2. Association between nutritional status and iron status

Nutritional status	*Anemic status*			*Iron depletion status*			*Iron-deficient erythropoiesis status*		
	Anaemic (n=38)	*Normal (n=77)*	*Statistical test*	*Iron depletion (n=18)*	*Normal iron repletion (n=97)*	*Statistical test*	*Iron-deficient erythripoiesis (n=28)*	*Normal erythropoesis (n=87)*	*Statistical test*
Category I									
Underweight (n=27)	13 (48.1%)	14 (51.9%)	OR=2.34	10 (37.0%)	17 (63.0%)	OR=5.88	13 (48.1%)	14 (51.9%)	OR=4.52
Non-underweight (n=88)	25 (28.4%)	63 (71.6%)	CI= 0.97-5.67 p=0.06	8 (9.1%)	80 (90.9%)	CI = 2.02-17.10 p=0.001**	15 (17.0%)	73 (83.0%)	CI=1.77-11.54 p=0.002**
Category II									
Overweight (n=14)	2 (14.3%)	12 (85.7%)	OR=0.30	6 (11.5%)	46 (88.5%)	OR=0.38	14 (26.9%)	38 (73.1%)	OR=0.83
Non-overweight (n=101)	36 (35.6%)	65 (64.4%)	CI= 0.06-1.42 p=0.129	6 (9.5%)	57 (90.5%)	CI= 0.05-3.10 p=0.367	14 (22.2%)	49 (77.8%)	CI= 0.21-3.21 p=0.786

* significant in $\alpha=0.05$, **significant in $\alpha=0.01$, Univariate analysis using logistic regression $\alpha = 0.5$, Confidence Interval (CI) = 95%

Tabel 3. Association between dietary intake level and iron status

Nutrients intake	*Anemic status*			*Iron depletion status*			*Iron-Deficient Erythropoiesis status*		
	Anaemic (n=38)	*Normal (n=77)*	*Statistical test*	*Iron depletion (n=12)*	*Normal iron repletion (n=103)*	*Statistical test*	*Iron-deficient erythripoiesis (n=28)*	*Normal erythropoesis (n=87)*	*Statistical test*
Energy (kcal)									
< 1800	28 (35%)	52 (65%)	OR=1.225	12 (15%)	68 (85%)	OR=0.850	24 (30%)	56 (70%)	OR=2.625
≥ 18000	10 (28.6%)	25 (71.4%)	CI= 0.670-2.2390	(0%)	35 (100%)	CI = 0.775-0.932	4 (11.4%)	31 (88.6%)	CI= 0.984-7.002
			p=0.50			p=0.015**			p=0.033*
Protein (g)									
< 56	21 (40.4%)	31 (59.6%)	OR=1.497	6 (11.5%)	46 (88.5%)	OR=1.212	14 (26.9%)	38 (73.1%)	OR=1.212
≥ 56	17 (27%)	46 (73%)	CI= 0.887-2.526	6 (9.5%)	57 (90.5%)	CI= 0.887-2.526	14 (22.2%)	49 (77.8%)	CI= 0.637-2.305
			p=0.128			p=0.725			p=0.559
Total fat (g)									
< 75	24 (33.8%)	47 (66.2%)	OR=1.062	9 (12.7%)	62 (87.3%)	OR=1.859	19 (26.8%)	52 (73.2%)	OR=1.308
≥ 75	14 (31.8%)	30 (68.2%)	CI= 0.618-1.825	3 (6.8%)	41 (93.2%)	CI= 0.532-6.498	9 (20.5%)	35 (79.5%)	CI= 0.651-2.630
			p=0.826			p=0.318			p=0.444
PUFA (g)									
< 13.1	21 (41.2%)	30 (58.8%)	OR=1.550	5 (9.8%)	46 (90.2%)	OR=0.896	14 (27.5%)	37 (72.5%)	OR=1.255
≥ 13.1	17 (26.5%)	47 (73.4%)	CI= 0.919-2.615	7 (10.9%)	57 (89.1%)	CI= 0.302-2.658	14 (21.9%)	50 (78.1%)	CI= 0.660-2.387
			p=0.098			p=0.843			p=0.489

* significant in α=0.05
** significant in α=0.01

Tabel 4. Association between underweight and iron status, adjusted for nutrients intake

Bivariate analysis (unadjusted)				*Multivariate analysis (adjusted)*			
Variables	*p*	*OR*	*95% CI for OR*	*Variables*	*p*	*OR*	*95% CI for OR*
Risk for anaemia				Risk for anaemia			
Underweight	0.60	2.34	0.97 – 5.67	Underweight	0.29	2.03	0.55 –7.53
Energy	0.12	0.99	0.99 – 1.00	Energy	0.78	1.00	0.99 – 1.00
Protein	0.28	0.99	0.97 – 1.01				
Total fat	0.38	0.99	0.98 – 1.01				
PUFA	0.27	0.97	0.92 – 1.02				
Vitamin A	0.44	1.00	1.00 – 100				
Vitamin C	0.32	1.01	0.99 – 1.02				
Folic acid	0.69	0.99	0.99 – 1.01				
Iron	0.49	0.96	0.85 – 1.08				
Zinc	0.40	0.94	0.99 – 1.09				
Risk for iron depletion				Risk for iron depletion			
Underweight	0.001**	5.88	2.02 – 17.09	Underweight	0.03*	7,05	1.17 –42.41
Energy	0.015*	0.99	0.96 – 1.00	Energy	0.68	1.00	0.99 – 1.00
Protein	0.37	0.99	0.96 – 1.02	Total fat	0.90	1.00	0.96 – 1.05
Total fat	0.07	0.98	0.96 – 1.00	Folic acid	0.89	0.99	0.98 – 1.01
PUFA	0.32	0.97	0.90 – 1.03	Vitamin C	0.47	0.99	0.96 – 1.02
Vitamin A	0.44	1.00	1.00 – 1.00	Iron	0.76	0.95	0.68 – 1.32
Vitamin C	0.17	0.98	0.95 – 1.01	Zinc	0.96	1.01	0.67 – 1.52
Folic acid	0.10	0.99	0.98 – 1.00				
Iron	0.03*	0.82	0.68 – 0.99				
Zinc	0.06	0.82	0.67 – 1.01				
Risk for iron-deficient erythropoiesis				Risk for iron-deficient erythropoiesis			
Underweight	0.002**	4.52	1.77 – 11.54	Underweight	0.007**	3.93	1.46 – 10.54
Energy	0.014*	0.99	0.99 – 1.00	Vitamin A	0.17	0.99	0.99 – 1.00
Protein	0.53	0.99	0.97 – 1.02				
Total fat	0.13	0.98	0.97 – 1.00				
PUFA	0.36	0.97	0.92 – 1.03				
Vitamin A	0.03*	0.99	0.99 – 1.00				
Vitamin C	0.06	0.98	0.95 – 1.00				
Folic acid	0.47	0.99	0.99 – 1.01				
Iron	0.14	0.90	0.78 – 1.04				
Zinc	0.08	0.86	0.72 – 1.02				

* significant in α=0.05, **significant in α=0.01, Univariate analysis using logistic regression α = 0.5, Confidence Interval (CI) = 95%

among adolescents aged 16-18 years and adults (> 18 years old) in national survey data (Indonesian Health Ministry, 2013), and also higher compared to several findings in other countries (Keikhaei, Askari & Aminzadeh, 2012; Anticona & Sebastian, 2014). The percentage of both overweight and obesity in our study was 12.2%, consisting of 9.6% overweight and 2.6% obese women. This rate is similar to other findings (Hanafi, Abdallah & Zaky, 2013). In several countries, prevalence of overweight and obesity is higher than underweight (Eftekhari, Mozaffari-Khosravi & Shidfar, 2009; Nead *et al.*, 2004; Pinhas-Hamiel *et al.*, 2003; Qin *et al.*, 2013). It appears that the higher prevalence of underweight is apparent in low and middle- income countries compared to the more developed ones (Black *et al.*, 2008). The characteristics of underweight women in our sample were similar to the characteristics of underweight women in Black's paper (2008), particularly in relation to socio-economic level. Our sample was also categorised as falling into the low socio-economic status, reflected by low education and low-income level (Table 1). This situation raises a serious problem, because nutritional status prior to pregnancy plays an important role for a healthy pregnancy, where BMI before pregnancy is a strong predictor for birth weight (Neggers & Goldenberg, 2003). Low maternal BMI is also associated with intra-uterine growth restriction (Black *et al.*, 2008).

Anaemia and iron deficiency remain the most common nutritional deficiency worldwide (WHO, 2015; Black *et al.*, 2008). Our study found anaemia to be more prevalent among underweight women, compared to overweight or obese women, at 48.1% and 28.4% in underweight and non-underweight (normal & overweight), respectively. This finding is consistent with the study of Qin *et al.* (2013) in China. The prevalence of anaemia among women in our study is not far from the rate of 33% and 31.1% respectively observed in Qin's study. However, the anaemia rate in our study is lower than that in the study of Kamruzzaman *et al*. (2013) in Bangladesh who reported anaemia among underweight non-pregnant ever-married women in Bangladesh to be more than 50%. It is expected because our subjects were newly married, non-pregnant women when recruited. Compared to Kamruzzaman's study, the association between underweight and anaemia in our study is not statistically significant (p=0.060); however, Kamruzzaman's findings clearly showed underweight to be significantly associated with anaemia ($\chi 2$ = 94.302; p<0.001).

The range of serum ferritn concentration in our study did not exceed the maximal value of normal range. According to WHO (2007), the normal value of serum ferritin concentration is 15 – 250 μg/L (Worwood, 2007; Northrop-Clewes, 2007). As ferritin is an acute phase reactant that is influenced by inflammation state or infection, if serum ferritin is used as an indicator of iron status, the use of a marker for inflammation state such as C-reactive protein (CRP) or other cytokines should be considered. Otherwise, the maximum value of serum ferritin concentration must not be more than 250μg/L to ensure that the coexistence of inflammation with iron deficiency is not a coincidence (Northrop-Clewes, 2007). The descriptive data of serum ferritin shows the maximum value to be 188.3 μg/L (Table 1), therefore it can be assumed that inflammation does not co-exist with anaemia in our study participants. It means that physical examination during recruitment was quite effective in eliminating the possibility of infection or inflammation, although it was carried out without assessment of inflammatory markers such as CRP or other cytokines

The average sTfR concentration among the women in our study was 3.7 mg/L, a normal state as the normal range of sTfR concentration is 1.9 – 4.4 mg/L. The percentage of women with sTfR concentration ≥ 4.4 mg/L was 24.3%, and they were considered as in an iron-deficient erythropoiesis (IDE) state. Both serum ferritin and serum transferrin receptor are known to be indicators of the sequence of change in the body as iron decreases from normal-repleted level to that of depleted iron stores and iron deficiency anaemia. Low serum ferritin (ferritin concentration of less than 15μg/L) indicates a depleted state of iron stores in the body (WHO, 2007), and the elevation of serum transferrin receptor reflects a functional iron deficiency or iron-deficient erythropoiesis state (Infusino *et al.,*2012; Jain*et al.,* 2010; Suominen *et al.,* 1998). The overall rate of iron deficiency among the women in our study was higher than those reported in other countries (Eftekhari *et al.,* 2009; Nead *et al.,* 2004; Pinhas-Hamiel *et al.,* 2003). Our results reveal that iron deficiency, indicated by low serum ferritin and elevation of serum transferrin receptor, is more likely to occur in underweight women than in normal and overweight women.

As we reflect on the percentage of anaemia (33%), and percentage of iron depletion (15.7%), it could be inferred that the occurrence of iron-deficient anaemia (IDA) was 24.3%, while 8.7% could be considered as having non-iron deficient anaemia. Of the IDA cases, 15.7% had anaemia accompanied by iron depletion store, while 8.6% had marginal iron store (serum ferritn concentration < 30 μg/L). An interesting observation was that the percentage of anaemia and IDE among underweight women were exactly similar (48.1%) (Table 2). This indicates that anaemia among underweight women is caused by iron deficiency.

The mean value of energy intake was 1575 kcal, meeting only 74% of energy requirements among girls aged 16-18 years or 70% of energy adequacy of adult women (Indonesian Institute of Science, 2012). The average protein consumption (66 gram) met the daily recommendation for adolescent girls or adult women based on the Indonesian Reference Intake. Dietary data revealed that only protein intake reached the standard reference intake (56 g/d) (Indonesian Institute of Science, 2012). The limitation of our study is that our dietary data was derived from a single-day 24-h dietary recall. Due to this limitation, we could not assume that the data reflected nutrient adequacy, hence the data is expressed as dietary intake level. However, our findings reveal that the average energy intake is similar to the national data of the National Health Survey (Indonesian Health Ministry, 2010).

Several studies indicate that the key to the occurrence of a high rate of iron deficiency among overweight and obese adolescents is a high fat diet and excess adiposity (Tussing-Humphreys *et al.,* 2009; Sonnweber *et al.,* 2012), but in contrast a study by Jamieson *et al.* (2013) shows that higher long-chain polyunsaturated fatty acid status is associated with reduced risk of iron depletion. Our findings do not support previous findings from several developed countries. Total fat intake and polyunsaturated fatty acid were not found to be associated with iron depletion or IDE. It might be that most women (61.7%) were deficient in total fat intake (fat intake < 75 g/d), and 44.3% were deficient in polyunsaturated fatty acid (PUFA intake < 13.1 g/d). Only energy intake of less than 1800 kcal appears as a risk factor for iron depletion and IDE (Table 3).

Based on multivariate logistic regression, the dietary data do not appear to be confounding factors for the association of underweight with iron status. It implies that underweight is a strong risk factor for iron status, particularly for iron depletion as well as for IDE. However, the bivariate analysis shows that energy intake is a strong candidate for iron depletion and IDE, but in multiple logistic regression, all dietary data do not appear as confounding variables (Table 4).

The mechanism of anaemia and iron

deficiency among undernourished women is not only explained by differences in intake of several nutrients but also by the biochemical features of several indicators. A previous study by Magdougall *et al*. (1982) provides detailed explanation about the mechanism of anaemia in protein-energy malnutrition among children under 5 years old (Macdougall *et al.,* 1982). In an energy-protein malnutrition state, the increased activity of glycolytic enzymes such as hexokinase, pyruvate kinase and glucose 6-phosphate dehydrogenase (G6PD) alters membrane permeability, leading to the breakdown of red cells. These findings provide detailed explanation why underweight women in our study are more likely to have iron deficiency reflected by low haemoglobin level, low serum ferritin concentration and the elevation of serum transferrin receptor concentration.

CONCLUSION

There is greater prevalence of iron deficiency among underweight young women than among normal and overweight ones. The risk for depleted iron stores and IDE are approximately seven times and four times higher compared to those who are not underweight. In addition, the risk for iron depleted iron store is higher than the risk for IDE among underweight young women. To improve the effectiveness of health programs, preventing and combating iron deficiency anaemia among non-pregnant women should be simultaneously implemented with programs addressing underweight women. Iron supplementation should be followed with high energy density supplementary feeding to improve weight status.

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Conflicts of interest

The author(s) declare no potential conflict of interest with respect to the authorship and/or publication of article.

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