

Research Article

Iron Status in Relation to Oral Contraceptive Use in Women of Reproductive Age

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Abstract

This study investigated the iron status in women of reproductive age in relation to oral contraceptive (OC) use. 178 women (18-34 years) who had never been pregnant and did not take iron supplements were sampled as part of a cross-sectional study to determine nutrient status in different life stages (DRKS00004789). Iron status was assessed by haemoglobin, ferritin, soluble transferrin receptor (sTfR) and sTfR-ferritin index. Frequency of anaemia (haemoglobin < 12 g/dL) was 2.8% and that of depleted iron stores without anaemia (ferritin < 20 µg/L) was 13.5%. In multiple linear regression models, OC use was associated with higher ferritin concentration (B=0.144, p=0.037), especially the fourth progestin generation (B=0.177, p=0.015). Further determinants of lower ferritin concentration were higher intensity of menstruation (B=-0.201, p=0.001), lower time since last period (B=-0.004, p=0.024), blood donation (B=-0.360, p=0.003) and vegetarian diet (B=-0.206, p=0.042). Although the prevalence of anaemia was low, women of reproductive age should ensure an adequate intake of highly available iron because depleted iron stores increase the risk of anaemia.

Keywords: Iron; Ferritin; Haemoglobin; Soluble Transferrin Receptor; Oral Contraceptive; Women of Reproductive Age

Abbreviations

AE: non-iron-deficiency anaemia; BMI: body mass index; ID: iron deficient non-anaemia; IDA: iron-deficiency anaemia; IDE: iron-deficient erythropoiesis; OC: oral contraceptives; SD: standard deviation; sTfR: soluble transferrin receptor; sTfR-F index: soluble transferrin receptor ferritin index; VitaMinFemin: Vitamin and mineral status among German women

Introduction

Worldwide, the risk of anaemia (haemoglobin < 12 g/dL) is estimated at 29% (496.3 million) in non-pregnant women aged 18 to 49 years [1]. Anaemia is caused by inadequate iron balance, which initially results in reduced iron stores (depressed ferritin levels) [2]. An iron deficiency without anaemia may already result in impaired neurocognitive functions [3]. Progressive iron deficiency leads to restricted erythropoiesis (increased soluble transferrin receptor [sTfR]). Further imbalance may lead in a deficiency of haemoglobin (anaemia) [2].

Iron status is particularly important among women of reproductive age who want to become pregnant because of the increased iron requirements during pregnancy [4] and the maternal and fetal health [5]. However, women of reproductive age are considered at-risk for iron deficiency [6] as menstruation-related blood losses strongly impact iron status [7]. The use of oral contraceptives (OCs) is associated with shorter bleeding times [8] and less blood loss [9] and may therefore positively affect iron status, as has been observed in a few studies [8,10] but the data situation is inconsistent [11].

Iron status also depends on the availability of iron from food. Both, the chemical form [12] and the presence of inhibiting and enhancing

factors influence the iron absorption [13]. Moreover, iron absorption from food depends on iron status [13] and body mass index (BMI) [14]. Blood donations [15] and endurance sports participation [16] also affect the iron status. As a result, the assessment of iron status via iron-specific laboratory test (e.g., haemoglobin, ferritin, soluble transferrin receptor and soluble transferrin-ferritin index) is more appropriate than the evaluation of iron intake.

The present study describes the iron status in women of reproductive age in relation to OC use and in consideration of other factors that might influence the iron status.

Material and Methods**Subjects and study design**

Subjects were recruited as part of the nationwide, cross-sectional, multicentre VitaMinFemin study (Vitamin and mineral status among German women), which determined the status of selected nutrients in women at different life stages (n=2367). The cross-sectional study was conducted in cooperation with 125 study sites (general practitioners and gynaecologists) between April 2013 and March 2015. Study design and implementation were conducted in accordance with the principles of Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved by the ethics commission of the Medical Chamber of Lower Saxony (26.03.2013) and every involved ethic commission from the different study sites. The study was registered in the German Clinical Trial Register with the identification number DRKS00004789 [17].

Iron status was measured in a subgroup of 192 women of reproductive age who had never been pregnant and did not take iron supplements. Of these, one subject was excluded from the analysis

due to missing data on the type of OC used. Another 13 subjects with intrauterine devices or vaginal ring use were excluded, as the study aimed to compare the influence of OC use to non-use. Therefore, the study population included 178 women.

Iron indices

Iron status was assessed by haemoglobin, serum ferritin, sTfR and sTfR-ferritin (sTfR-F) index. The haemoglobin determination was conducted using sodium lauryl sulphate, a photometric method. Ferritin reflects iron stores [18] and was measured in serum by electrochemiluminescence immunoassay (cobas®, Roche Diagnostics, Mannheim, Germany). Ferritin is an acute phase protein and can be increased by inflammation [19], therefore sTfR – an indicator of tissue iron deficiency [18] – was also assayed in serum by immunonephelometry (BN II/BN ProSpec® System, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). sTfR was used to calculate the sTfR-F index (sTfR/log10 ferritin), which reflects total body iron [20].

Iron status was categorized by the cut-offs for anaemia (haemoglobin < 12 g/dL) and depleted or absent iron stores (ferritin < 15 µg/L) [21]. Moreover, as physical performance is already decreased at ferritin levels < 20 µg/L [22], this cut-off was used for reduced iron stores. Iron erythropoiesis is restricted at increased sTfR-F index [20]. As the measurement of sTfR is highly method dependent, the laboratory-dependent value for sTfR-F index was used (> 1.54) (N Latex sTfR, 2011, Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany). A severe risk of iron overload exists at a ferritin level > 150 µg/L [21].

Therefore, the following classifications were used: iron-deficient non-anaemia (ID) (ferritin < 15 µg/L, haemoglobin ≥ 12 mg/L, sTfR-F index ≤ 1.54), iron-deficient erythropoiesis (IDE) (ferritin < 15 µg/L, haemoglobin ≥ 12 g/dL, sTfR-F index > 1.54), iron-deficiency anaemia (IDA) (ferritin < 15 µg/L, haemoglobin < 12 g/dL, sTfR-F index > 1.54) and non-iron-deficiency anaemia (AE) (ferritin > 15 µg/L, haemoglobin < 12 g/dL, sTfR-F index ≤ 1.54).

Factors influencing iron status

Different data were collected using a questionnaire (self-report) to consider factors that might influence the iron status: (1) OC use [8,10] (non-OC users vs. OC users), (2) OCs were classified by ethinyl estradiol concentration and progestin generation (second: levonorgestrel and norgestimate; third: desogestrel; new progestine with antiandrogen activity were classified as fourth: drospirenone, nomegestrol acetate, dienogest [23], chlormadinone acetate and cyproterone acetate [24]), (3) blood donation [15], (4) dietary pattern [13] (omnivore vs. non-omnivore [vegetarian and vegan]), (5) menstruation [25] (average duration in days, intensity [amenorrhoea, less-intense, more-intense, strong-intense] and time since last menstruation period), (6) smoking habits [26] and (7) BMI (calculated by weight and height) [14]. The group of blood donation included subjects which donated blood in the last twelve month before their involvement in the study. BMI was divided according to the WHO classification [27]. Women were classified as having amenorrhoea if the last menstruation has been more than 60 days ago or they had long cycles.

Table 1: Description of study population.

		Total study population (n = 178)	Non OC-users (n = 58)	OC-users (n = 120)	P value
Age (years)	Mean ± SD	23.0 ± 3.5	23.7 ± 3.9	22.7 ± 3.3	0.177 ^a
BMI (kg/m²)	Mean ± SD	22.6 ± 3.8	22.8 ± 4.5	22.6 ± 3.4	0.727 ^a
Intensity of menstruation^c		20 (11.4)	4 (7.0)	16 (13.4)	0.024 ^b
Amenorrhoea		32 (18.2)	5 (8.8)	27 (22.7)	
Less-intense	N (%)	109 (61.9)	40 (70.2)	69 (58.0)	
Normal-intense		15 (8.4)	8 (14.0)	7 (5.9)	
More-intense					
Last menstruation (days)^d	Mean ± SD	15.7 ± 9.1	14.9 ± 8.6	16.2 ± 9.5	0.160 ^a
Duration of menstruation (days)^d	Mean ± SD	4.9 ± 1.2	5.1 ± 1.2	4.8 ± 1.1	0.558 ^a
Donate blood^e	N (%)	13 (7.3)	2 (3.4)	11 (9.2)	0.165 ^b
Food pattern^f					
Omnivore		156 (87.6)	47 (81.0)	109 (91.6)	0.041 ^b
Non-omnivore ^f	N (%)	21 (11.8)	11 (19.0)	10 (8.4)	
Smoking	N (%)	44 (24.7)	12 (20.7)	32 (26.7)	0.386 ^b

Abbreviations: BMI, body mass index; OC, oral contraceptive

^a Mann Whitney U test

^b Chi square test

^c n = 176

^d n = 156 due to subjects with amenorrhoea (n = 20) and missing data (n = 2)

^e n = 177

^f three vegans

Statistical analysis

The statistical analyses were performed in the Statistical Package for the Social Sciences (SPSS) software version 22.0 (SPSS, Inc., Chicago, Illinois, USA). The results are presented as the mean ± standard deviation (± SD), frequency and percentage. The Kolmogorov-Smirnov test was used to analyse the normal distribution. Significant differences between subgroups were tested by Kruskal Wallis tests (> two groups) or nonparametric Mann Whitney U tests (two groups) for abnormally distributed data and by univariate ANOVA (> two groups) or t-tests for independent subsamples (two groups) for normally distributed data. To evaluate differences between categorical variables, Chi square tests were performed. Due to the skewed distribution of ferritin, a square root transformation was used for the multiple linear regression analysis. The models can be regarded as robust, as the forward and backward selection showed equal variable combinations. The significance level was p < 0.05.

Results

Subject characteristics

The characteristics of the study population are shown in Table 1. Here, 67.4% (n=120) of the study population used OCs. Frequencies of amenorrhoea and low-intensity menstruation were significantly higher in OC users than in non-users (p=0.024). Omnivorous dietary pattern were more common in OC users (p=0.041). Among OC users, five subjects used progestin-only pills. Regarding the progestin generation, 24.2% (n=29) took second-generation OCs, 18.3% (n=22) third-generation OCs and 57.5% (n=69) fourth-generation OCs.

Table 2: Haemoglobin, ferritin, sTfR-concentration and sTfR-F index in total study group and depending on determinants of iron status.

	N		Haemoglobin (g/dL)	P value	Ferritin (µg/L)	P value	sTfR (mg/L)	P value	sTfR-F index	P value
Total	178	Mean ± SD	13.7 ± 0.9	/	51.5 ± 35.9	/	1.18 ± 0.30	/	0.78 ± 0.41	/
OC use										
Non-users	58	Mean ± SD	13.7 ± 0.9	0.417 ^a	41.8 ± 23.7	0.029 ^b	1.20 ± 0.32	0.548 ^b	0.82 ± 0.42	0.156 ^b
Users	120	Mean ± SD	13.6 ± 0.9		56.2 ± 39.7		1.16 ± 0.29		0.76 ± 0.41	
Ethinylestradiol concentration in OC users^e										
0.02 mg	35	Mean ± SD	13.7 ± 0.7	0.417 ^a	59.8 ± 53.3	0.662 ^b	1.17 ± 0.39	0.923 ^b	0.78 ± 0.55	0.724 ^b
≥ 0.03 mg	80	Mean ± SD	13.6 ± 0.6		54.0 ± 32.2		0.75 ± 0.34		0.76 ± 0.41	
Progestin generation in OC users										
Second	29	Mean ± SD	13.8 ± 0.7		58.8 ± 58.3		1.13 ± 0.43		0.79 ± 0.61	
Third	22	Mean ± SD	13.6 ± 0.9	0.605 ^b	47.9 ± 32.0	0.186 ^c	1.21 ± 0.26	0.103 ^c	0.84 ± 0.43	0.280 ^c
Fourth	69	Mean ± SD	13.6 ± 0.9		57.7 ± 31.9		1.16 ± 0.22		0.72 ± 0.21	
Intensity of menstruation^f										
Amenorrhea	20	Mean ± SD	13.8 ± 0.9		61.7 ± 32.8		1.17 ± 0.18		0.70 ± 0.16	
Less-intense	32	Mean ± SD	13.9 ± 1.0	0.359 ^d	63.6 ± 44.9	0.005 ^c	1.11 ± 0.19	0.066 ^c	0.67 ± 0.20	0.020 ^c
Normal-intense	109	Mean ± SD	13.6 ± 0.8		48.4 ± 33.6		1.17 ± 0.31		0.78 ± 0.39	
More-intense	15	Mean ± SD	13.8 ± 1.1		34.0 ± 24.9		1.41 ± 0.48		1.16 ± 0.78	
Donate blood^g										
Non-donors	164	Mean ± SD	13.7 ± 0.9	0.402 ^a	53.5 ± 36.2	0.003 ^b	1.16 ± 0.26	0.063 ^b	0.75 ± 0.32	0.004 ^b
Donors	13	Mean ± SD	13.5 ± 1.0		28.8 ± 21.6		1.41 ± 0.57		1.23 ± 0.90	
Food pattern^h										
Omnivore	156	Mean ± SD	13.7 ± 0.9	0.862 ^d	53.6 ± 37.1	0.048 ^c	1.17 ± 0.31	0.206 ^c	0.78 ± 0.43	0.034 ^c
Non-omnivore	21	Mean ± SD	13.6 ± 0.7		37.5 ± 21.4		1.21 ± 0.21		0.83 ± 0.23	
Smoking										
Non-smoker	134	Mean ± SD	13.6 ± 0.9	0.292 ^a	49.1 ± 32.4	0.315 ^b	1.21 ± 0.32	0.019 ^b	0.82 ± 0.45	0.046 ^b
Smoker	44	Mean ± SD	13.8 ± 0.8		58.7 ± 44.6		1.09 ± 0.22		0.68 ± 0.22	

Abbreviations: OC, oral contraceptive; sTfR, soluble transferrin receptor; sTfR-F index, soluble transferrin receptor ferritin index

^a t-Test for independent subsamples

^b Mann Whitney U test

^c Kruskal Wallis test

^d Univariate ANOVA

^e n = 115 due to five used progestin-only pills

^f n = 176

^g n = 177

The majority of the total study population were normal weight (BMI 18.5 - 24.9 kg/m², 71.9%, n=128) and only 5.6% (n=10) had obesity (BMI ≥ 30 kg/m²), which distribution did not vary between OC users and non-users (p=0.158).

Iron status

In the total study population, the average haemoglobin concentration was 13.7±0.9 g/dL, ferritin concentration 51.5±35.9 µg/L, sTfR concentration 1.18±0.30 mg/L and sTfR-F index 0.78±0.41 (Table 2). With regard to OCs, only ferritin concentration was affected by the use of OCs, resulting in significantly higher concentrations in users than in non-users (p=0.029) (Table 2). Ferritin levels did not vary across ethanol estradiol concentrations among OC users. Interestingly, ferritin values were only significantly higher among users of fourth-generation compared to non-users (p=0.004). Among OC users, iron status did not differ by estradiol concentration or progestin generation.

Most of the iron markers did not depend on possible influencing factors in the total study population (intensity of menstruation, blood donation, dietary pattern, and tobacco use (Table 2) and BMI classification [data not shown]). However, significantly lower ferritin concentrations and higher sTfR-F index values were seen among women with intense menstruation (ferritin: p = 0.005, sTfR-F index: p=0.020), blood donors (ferritin: p=0.003, sTfR-F index: p=0.004) and non-omnivores (ferritin: p=0.048, sTfR-F index: p=0.034). The sTfR concentration and sTfR-F index value were significantly lower in smokers than in non-smokers (sTfR: p=0.019, sTfR-F index: p=0.046).

Prevalence of cut-off values for iron status

Of the study population, 82.6% had normal iron status. Iron deficiency without anaemia was present in 11.8% (n=21) of women and erythropoiesis was restricted in 1.7% (n=3) (IDE). Anaemia was present in 2.8% (n=5) of women, but only 1.7% (n=3) also had iron deficiency (IDA). The other 1.1% (n=2) showed anaemia without iron

Table 3: Determinants of square root ferritin concentration by multiple linear regressions^{a,b}.

	Regression coefficient	Standard-error	Standardized regression coefficient	P value
Constant	3.177	0.183		0.000
Non OC-users/OC users	0.144	0.069	0.154	0.037
Non OC-users/ second generation OC	0.073	0.053	0.157	0.169
Non OC-users/ third generation OC	0.120	0.087	0.142	0.172
Non OC-users/ fourth generation OC	0.177	0.072	0.220	0.015
Intensity of period (amenorrhoea/less-/normal-/more-intense)	-0.201	0.059	-0.361	0.001
Last period	-0.004	0.002	-0.232	0.024
Blood donation (no/yes)	-0.360	0.118	-0.218	0.003
Food pattern (omnivore/vegetarian)	-0.206	0.100	-0.144	0.042

OC, oral contraceptive.

^a multiple linear regressions considering the terms OC use, intensity of period, last period, blood donation and food pattern

^b n = 175, without vegans (n = 3)

deficiency (AE). Another 1.1% (n = 2) of women had a severe risk of iron overload.

The frequency of reduced iron stores (ferritin < 20 µg/L) was higher in women with more-intense menstruation than in women with less-intense menstruation (p=0.004) and in blood donors (p=0.007) (Figure 1). However, the prevalence did not vary across OC use (p=0.252) or dietary patterns (p=0.490, data not shown).

Determinants of ferritin concentration

In the multiple linear regression analysis, OC use was positively associated with ferritin concentration (p=0.037) (Table 3). However, a relationship was only seen for OCs of fourth-generation (p=0.015). The menstruation intensity (p=0.001), time since last menstruation (p=0.024) and blood donation (p=0.003) were negatively associated with ferritin levels (Table 3). With regard to dietary pattern, ferritin concentration was only associated with mixed and vegetarian diets (excluding vegan diets), with lower ferritin levels among vegetarians compared to omnivores (p=0.042). Therefore, vegans were not included in the model.

Ethinylestradiol concentration, average duration of menstruation, tobacco use and BMI did not contribute to the model and were therefore excluded from the multiple linear regressions.

Discussion

Our findings show that iron status was inadequate in 16.3% of study subjects. Anaemia was prevalent in 2.8% (haemoglobin < 12 g/dL), and 13.5% had depleted iron stores without anaemia (ferritin < 20 µg/L). The prevalence of anaemia was not critical. However, with depleted iron stores, neurocognitive function can be reduced and the risk of anaemia increases [3].

Previous studies showed similar rates of iron deficiency (10 - 20%) and anaemia (2 - 6%); however, different cut-offs for iron deficiency were used (ferritin < 12 µg/L, < 15 µg/L or < 16 µg/L) [8,28,29]. We thus find a lower prevalence of depleted iron status than these trials.

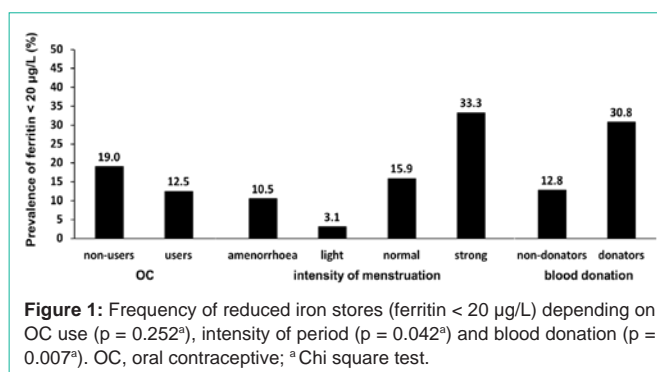


Figure 1: Frequency of reduced iron stores (ferritin < 20 µg/L) depending on OC use (p = 0.252^a), intensity of period (p = 0.042^a) and blood donation (p = 0.007^a). OC, oral contraceptive; ^a Chi square test.

OC use was only associated with higher ferritin concentrations and, therefore, with higher iron stores, whereas the prevalence of depleted iron stores (ferritin < 20 µg/L) was independent of OC use. Haile et al. (2016). also showed that risks of iron deficiency (sTfR > 43.9 nmol/L), iron deficiency anaemia (sTfR > 43.9 nmol/L and haemoglobin < 12 g/dL) and anaemia non-iron deficient (haemoglobin < 12 g/dL) are 49%, 80% and 58% lower, respectively, in OC users than in non-users [10]. However, the prevalence of inadequate iron stores was higher in the study population of Haile et al. (2016) than in our population. Iron status may vary with the type of OC. Although oestrogen concentration had no effect in our study, interestingly, the progestin generation was associated with ferritin concentration. However, differences in ferritin concentration between non-users and OC users were only seen for the fourth-generation. In contrast to the other progestin generations, fourth-generation has antiandrogen activity [24], which results in a lower intermenstrual bleeding rate, lower intensity of menstrual bleeding and lower frequency of dysmenorrhoea [30]. This finding probably explains the non-existent influence of OC use on ferritin concentration, sTfR concentration and sTfR-F index found by Casabellata et al. (2007). Their study population used only third-generation OCs [11]. Therefore, OC use may result in smaller menstruation-related blood losses [8]. Iron losses are higher in women with menorrhagia than in women with normal menstruation (5.2 mg vs. 0.87 mg iron/cycle) [31], and 27% and 60% of women with strong menstrual bleeding show anaemia and iron deficiency, respectively [25]. Although details on menstruation were subjectively described in our study, the lower the intensity of menstruation and the longer the time since the last menstruation, the higher the observed ferritin concentration. Menstruation-related blood loss is an independent contributor to ferritin levels, as blood loss strongly depends on individual factors [31] and also intra-individual [32].

Regarding diet, omnivores had significantly higher ferritin concentrations and lower sTfR-F index values than vegetarian, as shown by Leonard et al. [33]. These results conflict with the iron intake, which is surprisingly highest among vegans followed by vegetarians and lowest among omnivores [34]. However, iron absorption from a vegetarian diet is lower than from an omnivorous diet [35]. Furthermore, vegetarians consume higher amounts of iron inhibitors, such as phytic acid in soy protein [36] and dietary fiber [37].

Blood donation was associated with depleted iron stores and higher sTfR-F index values, which can result in an impaired

erythropoiesis with increasing iron imbalance. Moreover, depleted iron stores increase the risk of iron deficiency erythropoiesis, as shown by Cable et al. (2012) – 62% of the blood donors had iron deficiency erythropoiesis ($sTfR \geq 2.07$) [15].

In obese women, iron absorption is restricted by obesity-related inflammation compared to normal-weight women with similar iron intake [14]. However, the ferritin concentration is higher in obese women [38]. We find no influence of BMI on ferritin status.

This study has some limitations. First, the prevalence of reduced iron stores may be higher than observed as serum ferritin concentration increase with infection and inflammation [19]. Therefore, we used the $sTfR$ concentration, which is not affected by inflammation. Second, information about the intensity and duration of menstruation were subjective descriptions. Furthermore, although we have considered the type of diet, dietary records would be more appropriate. Moreover, $sTfR$ concentration is influenced by physical activity [16]. However, this factor was not considered in this study.

Conclusion

In conclusion, of the various factors considered (menstruation, blood donation, food pattern), use of fourth-generation OCs is positively correlated with ferritin concentration, which reflects higher iron status. Overall, anaemia was prevalent in 2.8% and reduced iron stores in 13.5% in women of reproductive age who did not take iron supplements. Menstruation-related blood losses, in particular, negatively affect iron status [7] and during pregnancy iron requirements are particularly necessary [5]. For those reasons women of reproductive age should ensure adequate intakes of highly available iron.

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