Introduction

The physiologic and metabolic changes that occur during pregnancy increase requirements of energy and nutrients. Adequate amounts of nutrients during pregnancy are essential for maternal, fetal and child health [1]. Among the essential micronutrients for pregnant women, iron and folate are recognized to play an extremely important role in the development...
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Iron is needed to support the increased maternal red blood cell mass, and is involved in the growth of the fetus and the development of the fetal brain. Furthermore, a higher amount of iron is needed during the late stage of pregnancy to store iron within the body of the fetus. Iron deficiency can lead to anemia of pregnant women, premature birth or low birth weight of the infant [4].

Folate, a soluble B vitamin which plays an important role in DNA synthesis and amino acid metabolism, is involved in cell division and normal development of the neural tube. The requirement of folate is also increased with the growth of the fetus, expansion of the uterus, development of placenta and increased blood supply. Folate deficiency during pregnancy can cause megaloblastic anemia of pregnant women, miscarriage, adverse birth outcomes such as low birth-weight and neural tube defects (NTDs) [5].

However, dietary intakes of iron and folate in pregnant women have been reported to be very low compared with the Recommended Nutrient Intakes (RNIs) [6-15]. It is recommended to take dietary supplements to meet the increased requirements during pregnancy [4]. Cho et al. [15] showed that mothers in the third trimester consumed an average of 19 mg from food and 89.1 mg daily from dietary supplements. Although the iron intake of pregnant mothers can be increased by supplement use, excessive consumption of iron needs to be carefully monitored as well.

The U.S. Centers for Disease Control (CDC) recommends pregnant mothers to start oral, low-dose (30 mg/day) supplements of iron at the first prenatal visit [16]. On the other hand, Health Canada recommends pregnant mothers to take a supplement that has 16 to 20 mg of iron in each daily dose, and also recommends to take a multi-vitamin supplement containing 400 µg of folic acid [17]. The 2010 Dietary Reference Intakes for Koreans specifies that the RNIs for pregnant women are 24 mg of iron and 600 µg DFE of folate, respectively, which are much higher than the RNIs for non-pregnant women. However, there are no specific recommendations in terms of the appropriate amount of iron or folic acid from supplements for pregnant women even though 400 µg of folic acid supplements are recommended for women of childbearing age to prevent NTDs [5].

We investigated the intakes of iron and folate from dietary supplements as well as from food sources in pregnant women and evaluated hematologic indices according to the type of the supplement i.e. single nutrient supplements (folic acid or iron) and multi-vitamins containing folic acid and iron.

Materials and Methods

Subject

Among the pregnant women after 20 weeks of gestation who were receiving prenatal care at a women’s hospital located in Seoul, 165 subjects participated in the study after completing the consent forms. Survey and blood tests were conducted from March to November, 2009. This study was approved by the Institutional Review Board (IRB) of Cheil General Hospital & Women’s Healthcare Center at Kwandong University College of Medicine and was carried out according to the policies of the IRB.

Dietary intake survey

Trained interviewers collected the information of general characteristics and dietary supplement use using a questionnaire. The questionnaire included age, level of education, and family income of the subjects as well as product name, amount, and frequency of consumption of dietary supplements. Intakes of iron and folic acid from dietary supplements were estimated using the nutrition label displayed in the bottle of the dietary supplements.

At 29 weeks of gestation, dietary intake was assessed by a food frequency questionnaire (FFQ) developed by the Korean CDC [18]. Intakes of iron and folate from food were estimated using a nutrient conversion program (Nutritional Assessment Management using FFQ, version 1.0) provided along with FFQ by Korean CDC. This program used nutrient database in the 7th revision of the Recommended Dietary Allowances for Koreans [19].

Collection of blood samples and laboratory assays

Blood was obtained in the EDTA tube from the subjects during the 36th gestational week. Hematologic indices, namely, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and red blood cell (RBC) counts were measured by Sysmex XE-2100 hematology analyzer. To diagnose iron-deficiency anemia of pregnant women, the following cut points according to Goonewardene et al. [4] were used: Hb < 11 g/dL, Hct < 33%, MCV < 80 fl, MCH < 30 pg, MCHC < 30 g/dL, RBC < 4.1 × 10⁸/μL. Folate de-
Deficiency was diagnosed by MCV > 100 fl.

Data analysis

The data were analyzed by using SAS 9.2 and all measurements were presented as mean ± standard deviation, frequencies or percentages. The subjects were classified into the four groups according to the type of dietary supplements: the subjects who did not use any dietary supplements (non-supplement group, N), the subjects who used iron or folic acid as a single nutrient supplement (S), the subjects who used multi-vitamins containing folic acid and iron (M) and the subjects who used iron or folic acid as a single nutrient supplement in addition to multi-vitamins containing folic acid and iron (S+M). The differences in nutrient intake and hematologic indices between the four groups were tested using One way ANOVA and Tukey Test. χ²-test was used to verify the differences in the distribution of the subjects who consumed less than the Estimated Average requirement (EAR) or more than the Tolerable Upper Intake Level (UL) according to the type of supplements. Statistical significance was set at the significance level of < 0.05.

Results

The general characteristics of the subjects are presented in Table 1. The average age of the subjects was 32.5 years. The subjects reported a relatively high level of education; 19.4% and 53.5% had completed college and university, respectively and 15.2% had completed graduate school. And 35.6% had their average monthly household incomes of 300,000-450,000 won.

Among the subjects, 25.5% did not take any supplements (N), 12.7% used single nutrient supplements for iron or folic acid (S), 38.2% used multi-vitamins containing folic acid and iron (M), and 23.6% used both single nutrient and multi-vitamins containing folic acid and iron (S+M). While 73.3% took a supplement containing iron, 66.7% took a supplement containing folic acid.

Table 2 shows mean daily intakes of iron and folate from food and dietary supplements. The mean intake of iron from food was 11.1 mg, which is 46.3% of the RNI for pregnant women. The iron intakes in the M or S+M group were significantly lower than that in the S group. The average daily intake from supplements was 66.8 mg. The average intake of iron of 19 women consumed iron in S group was 37.5 mg, whereas the mean intakes in the M and S+M group were 56.2 mg and 98.1 mg, respectively. The intake in the S+M group was highest, followed by the M and S groups. These differences were statistically significant. The total daily intakes of iron from food and supplements were 11.9 mg in the N group, 49.1 mg in the S group, and 66.2 mg in the M group, and 108.1 mg in the S+M group, which showed significant differences between the groups. The average iron intakes of the S, M, S+M groups were higher than the RNI of iron, even though the intake of the N group was lower.

The average daily intake of folate from food was 231.2 µg, which only accounted for 38.5% of the RNI of pregnant women. Similar to iron, the intake of S+M group was significantly

| Table 1. General characteristics of the subjects* |
|-----------------------------------------------|
| **Variables** | **N (%)** |
| Age (n = 165) | 32.5 ± 3.7 |
| Education (n = 165) | |
| High school | 20 (12.1) |
| College | 32 (19.4) |
| University | 88 (53.5) |
| ≥Graduate school | 25 (15.2) |
| Income level, 10,000 won/mon (n = 163) | |
| <150 | 3 (1.8) |
| 150-300 | 54 (33.1) |
| 300-450 | 58 (35.6) |
| ≥450 | 48 (29.5) |
| Type of supplement (n = 165) | |
| None (N) | 42 (25.5) |
| Single nutrient supplements (S) | 21 (12.7) |
| Iron alone | 13 (7.9) |
| Folic acid alone | 2 (1.2) |
| Iron + folic acid | 6 (3.6) |
| Multi-vitamins containing iron and folic acid (M) | 63 (38.2) |
| Single nutrient + multi-vitamins (S+M) | 39 (23.6) |
| Taking a supplement containing iron (n = 165) | |
| No | 44 (26.7) |
| Yes | 121 (73.3) |
| Taking a supplement containing folic acid (n = 165) | |
| No | 55 (33.3) |
| Yes | 110 (66.7) |

*Values are presented as mean ± SD or N (%).
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Table 2. Intakes of iron and folate from food and supplements*

| Supplement Type | N§ (n = 42) | S" (n = 21) | M* (n = 63) | S+M (n = 39) | Total (n = 165) |
|-----------------|-------------|-------------|-------------|-------------|-----------------|
| Iron            | 11.9 ± 4.5 [a,b] | 13.3 ± 4.9 [b] | 10.6 ± 3.6 [a] | 10.1 ± 3.3 [a] | 11.1 ± 4.1 |
| From food**     | 11.9 ± 4.5 [a] | 49.1 ± 14.1 [b] | 66.2 ± 17.7 [c] | 108.1 ± 27.9 [d] | 60.1 ± 38.7 |
| Folate          | 253.2 ± 119.8 [a,b] | 271.8 ± 111.7 [b] | 221.4 ± 90.4 [a,b] | 202.9 ± 68.8 [a] | 231.2 ± 99.2 |
| From supplements| 612.5 ± 379.6 [b] | 773.0 ± 190.9 [a] | 946.2 ± 424.2 [b] | 822.7 ± 320.7 (110) | 773.0 ± 190.9 |

*Values are presented as mean ± SD; †None; ‡Single nutrient; §Multivitamins & minerals; *Significantly different among the groups by ANOVA at p < 0.05; †Values with different superscript letters [a,b,c] are significantly different by Tukey test at p < 0.05.

Table 3. Distribution of the subjects who consumed iron and folate from food and supplements less than EAR or more than UL*

| Supplement Type | N§ (n = 42) | S" (n = 21) | M* (n = 63) | S+M (n = 39) | Total (n = 165) |
|-----------------|-------------|-------------|-------------|-------------|-----------------|
| Iron            | < EAR (18.3 mg or 18.6 mg)¶ | 36 (85.7) | 2 (9.5) | 3 (4.8) | 0 (0.0) | 41 (24.9) |
| > UL (45 mg)¶   | 0 | 0 | 0 | 0 | 0 |
| Folate          | < EAR (520 μgDFE)¶ | 40 (95.2) | 13 (61.9) | 0 (0.0) | 0 (0.0) | 53 (32.1) |
| > UL (1000 μg)¶ | 0 | 1 (4.8) | 1 (1.6) | 10 (25.6) | 12 (7.3) |

EAR: estimated average requirement, UL: tolerable upper intake level, DFE: dietary folate equivalent.

*Values are presented as N (%); †None; ‡Single nutrient; §Multivitamins & minerals; ¶EAR is different depending on the age of the subjects; 18.3 mg in 19-29 year-old women and 18.6 mg in 30-49 year-old women; †Significantly different among the groups by χ²-test at p < 0.001.

lower than the intake of S group. The average intake of 8 women consumed folate in S group was 612.5 μg. The average intakes of the M and S+M groups were 773.0 μg and 946.2 μg, respectively. The intake of the S+M group was significantly higher than that of the S and M groups alone. The total intake of folate from food and supplements was calculated as the Dietary Folate Equivalent (DFE) in order to account for the absorption efficacy. The intake was highest in the S+M group with 1,611 μgDFE. The intake was lower in the order of M, S, and N group. Significant differences were observed between the groups. While the average intake of the N group was 253.2 μgDFE, which was lower than the RNI, the S, M, and S+M groups that consumed the supplements had more than the RNI.

Table 3 shows the distribution of pregnant women who consumed iron and folate less than EAR or more than UL. The proportion of pregnant women who consumed less than EAR was 85.7% in the N group. However, only 9.5% in the S group, 4.8% in the M group, and no one in the S+M group. On the other hand, although the proportion of subjects who consumed more than UL of iron was nonexistent in the N group, the proportion was very high among the supplement users (S group - 81.0%, M group - 88.9%, S+M group - 97.4%). Significant differences existed in the distribution of subjects classified based on the UL according to the type of supplements.

The proportion of pregnant women who consumed less than EAR of folic acid was 95.2% in the N group, 61.9% in the S group and 0% in the M group and S+M group presenting significant differences according to the supplement type. In case of the S group, most subjects consumed the single iron supplement and only 8 subjects consumed folic acid. This is related to the fact that 61.9% of subjects who did not consume folic acid supplements had less than EAR. The UL of folic acid was set as 1,000 μg consumed in supplement form [5]. Although no pregnant women consumed more than UL in the N group, significant differences were observed in the other groups (4.8%, 1.6% and 25.6% in the S, M, and S+M groups, respectively).

Table 4 presents the hematologic indices according to the type of supplements of pregnant women. The average hemoglobin concentration was 11.9 g/dL in the N and S group and 12.4 g/dL in the M and S+M groups, respectively. The hemoglobin concentration of the M group was significantly higher than the N group. Furthermore, the hematocrits were 35.0% and 35.4% in the N and S group respectively, which were lower than those in the M and S+M groups. The hematocrit of the M group was significantly higher than the N group. MCV, MCH, MCHC did not show any significant differences between the groups and the average values were all in the normal range except for MCH of the S group. However,
the average values of RBC counts were lower than the normal ranges in all groups.

Table 5 shows the distribution of pregnant women diagnosed as anemia according to hematologic indices based on the type of supplements. 7.9% of the subjects had hemoglobin concentration below 11 g/dL (16.1% in the N group, 17.6% in the S group, 1.8% in the M group and 5.9% in the S+M group). 10.1% of subjects had hematocit less than 33%. The possible folate deficiency exists in one subjects in the N group presenting with MCV above 100 fL. The proportions of pregnant mothers diagnosed as anemia according to the hematologic indicators in the N or S group was higher than in the M or S+M group. As the frequencies in the distribution of subjects diagnosed as anemia were too low for testing the significant differences, $\chi^2$-test was performed between the N group and the combined group of the S, M and S+M groups as well as between the combined groups of N and S groups and the combined groups of M and S+M groups. There were significant differences between the combined groups ($p < 0.01$).

Table 6 presents the correlation between hematologic indices and the intake of iron and folate. The concentrations of hemoglobin and hematocrit were significantly correlated

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### Table 4. Hematologic indices of the subjects*

| Cut point for anemia | N (n = 31) | S (n = 17) | M (n = 57) | S+M (n = 34) | Total (n = 139) |
|----------------------|-----------|-----------|-----------|-------------|----------------|
| Hb $^1$ >11 g/dL     | 11.9 ± 1.0 [a]$^1$ | 11.9 ± 1.0 [a,b]$^1$ | 12.4 ± 0.8 [b]$^1$ | 12.3 ± 0.7 [a,b]$^1$ | 12.2 ± 0.9 |
| Hct $^2$ >33%        | 35.0 ± 3.0 [a]$^2$ | 35.4 ± 2.4 [a,b]$^2$ | 36.5 ± 2.2 [b]$^2$ | 36.4 ± 2.1 [a,b]$^2$ | 36.0 ± 2.5 |
| MCV                  | 80-100 fL | 89.7 ± 5.3 | 88.8 ± 5.7 | 90.2 ± 8.3 | 91.7 ± 4.1 | 90.3 ± 6.6 |
| MCH                  | >30 pg    | 30.5 ± 2.0 | 29.9 ± 2.4 | 31.1 ± 1.5 | 31.0 ± 1.5 | 30.8 ± 1.8 |
| MCHC                 | >30 g/dL  | 34.0 ± 1.3 | 33.6 ± 1.3 | 34.1 ± 1.0 | 34.7 ± 1.0 | 34.2 ± 2.8 |
| RBC                  | >4.1 x 10$^6$/µL | 3.9 ± 0.3  | 4.0 ± 0.3  | 4.0 ± 0.3  | 4.0 ± 0.3  | 4.0 ± 0.3  |

Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RBC: red blood cells.

*Values are presented as mean ± SD; $^1$None; $^2$Single nutrient; $^3$Multivitamins & minerals; $^4$Significantly different among the groups by ANOVA at p < 0.05; $^5$Values with different superscript letters [a,b] are significantly different by Tukey test at p < 0.05.

### Table 5. Distribution of the subjects by anemic cutpoints*

|        | N (n = 31) | S (n = 17) | M (n = 57) | S+M (n = 34) | Total (n = 139) |
|--------|-----------|-----------|-----------|-------------|----------------|
| Hb < 11 g/dL | 5 (16.1) | 3 (17.6) | 1 (1.8) | 2 (5.9) | 11 (7.9) |
| Hct < 33%    | 8 (25.8) | 2 (11.8) | 2 (3.5) | 2 (5.9) | 14 (10.1) |
| MCV < 80 fL  | 1 (3.2) | 1 (5.9) | 1 (1.8) | 0 | 3 (2.2) |
| MCV > 100 fL | 1 (3.2) | 0 | 0 | 0 | 1 (0.7) |

Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume.

*Values are presented as N (%); $^1$None; $^2$Single nutrient; $^3$Multivitamins & minerals.

### Table 6. Correlation of intakes of iron and folate from food and supplements with hematological indices*

|        | Hb | Hct | MCV | MCH | MCHC | RBC |
|--------|----|-----|-----|-----|------|-----|
| Iron   |    |     |     |     |      |     |
| From food | -0.090 | -0.039 | -0.040 | -0.173$^4$ | -0.060 | 0.052 |
| From supplements | 0.191$^1$ | 0.201$^1$ | 0.067 | 0.094 | 0.112 |
| Total  | 0.197$^1$ | 0.209$^1$ | 0.068 | 0.086 | 0.092 | 0.124 |
| Folate |    |     |     |     |      |     |
| From food | -0.138 | -0.097 | 0.031 | -0.096 | -0.027 | -0.057 |
| From supplements | 0.193$^1$ | 0.233$^1$ | 0.050 | 0.055 | 0.011 | 0.149 |
| Total  | 0.184$^1$ | 0.223$^1$ | 0.055 | 0.043 | 0.008 | 0.143 |

Hb: hemoglobin, Hct: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RBC: red blood cells.

*Pearson correlation coefficient; Significantly correlated at $^*p < 0.05$ and $^**p < 0.01$. 

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with the intake of iron and folate from supplements as well as the total intakes. However, there was no correlation with the dietary intakes of iron and folate. Although dietary intake of iron showed a significant inverse correlation with MCH, it was difficult to explain the reason.

Discussion

We investigated the intakes of iron and folate from dietary supplements as well as from food sources in pregnant women according to the type of supplements. No differences were observed in the level of education or income between the 4 groups. However, the average age of the subjects in the S group was 30.5 years, which was significantly lower than the average ages, 32.9-33.4 years, in the other three groups (results were not shown).

Previous studies reported that the intakes of iron and folate in pregnant women were much lower than the RNIs [6-15]. Since it is difficult for pregnant women to consume a sufficient amount of these nutrients through diet alone, taking a dietary supplement is recommended [4,16,17]. Despite the relatively high level of education and income of our subjects, 25.7% did not take any supplements containing iron or folic acid (Table 1).

Joung et al. [12] investigated iron intake of pregnant women in Daegu by the 24-hour recall method and reported that nearly 85% of pregnant women consumed iron less than 75% of the RNIs and that pregnant women in the third trimester consumed 12.6 mg on average, which was similar to 11.1 mg in this study. Furthermore, they showed that 73.5% took iron supplements in the third trimester, which was comparable to the 73.1% in this study. Jang & Ahn [13] reported that the average intake of dietary iron was 17.8 mg in the third trimester, whereas the average intake of supplemental iron was 52.7 mg. Cho et al. [15] reported that 83.9% of pregnant mothers took iron supplements. Also, they showed that the pregnant mothers in the third trimester consumed 19 mg from food and 89.1 mg from supplements on average when estimating nutrient intake using the same food frequency questionnaire as used in this study. No significant differences were found when they compared pregnancy outcomes such as weight increase during pregnancy and weight and height of newborn baby according to the intake of iron supplements. The average intake of dietary folate was 231.2 µg, which only accounted for 38.5% of the RNI. However, dietary folate intake might be underestimated as the food folate database used in this study was not completely established [20].

Although the UL of iron is 54 mg, the average intake of iron consumed from supplements was 66.8 mg; 37.5 mg in the S group, 56.2 mg in the M group, and 98.1 mg in the S+M group (Table 2). Iron intake from dietary supplements ranged widely from 5 mg to 210 mg, and 90.2% of the subjects who used supplements consumed more than UL (Table 3). Therefore, it is needed to suggest recommendations on the appropriate range of iron intake from supplements. The average intake of folic acid from supplements was 822.7 µg, which was lower than the UL (1,000 µg). However, there was a pregnant mother who consumed as much as 2,900 µg from a supplement.

The average concentrations of hemoglobin and hematocrit in the M group were significantly higher than in the N group. The M group also had the lowest proportions of the subjects diagnosed as anemia, which were less than 4%. Even though the S+M group had significantly higher intake of iron and folate than the M group, the S+M group did not show any better results than the M group in hematologic indices. Thus, it may not be advisable to take single nutrient supplements in addition to multi-vitamins. Although Jang & Ahn [13] reported that nearly 30% of 33 pregnant women in the early stage of pregnancy were diagnosed as anemia when both hemoglobin and hematocrit were lower than the cut point, less than 10% of the subjects were anemic in this study.

Joung et al. [12] reported that the proportions of pregnant mothers who consumed iron less than 75% of RNI were reduced by the use of iron supplements. Cho et al. [15] also showed that iron supplements improved iron intake. The use of dietary supplements containing folic acid in pregnant women increased folate intake [6]. In this regard, although it is most favorable to consume a variety of foods to meet increased amount of nutrients during pregnancy, it may be advisable to take dietary supplements if it is difficult to have meals with adequate nutrients.

Choi [21] suggested that at least 30 mg of iron should be supplemented every day from the third month of pregnancy. The Korean Nutrition Society recommends women of child-bearing age to take 400 µg of folic acid supplements to prevent NTDs of newborns [5]. However, as it does not specify the recommended amount of iron and folic acid from supplements, specific guidelines had better be presented in the future on the basis of further research.
Pregnant women are generally recommended to take iron and folic acid supplements. Our results showed that the use of multi-vitamins containing iron and folic acid is more effective in improving hematologic indices when compared with the use of single nutrient supplements (Table 4, 5). This implies that balanced intake of essential nutrients, not a single nutrient such as iron or folic acid, is important for erythropoiesis. Furthermore, the use of single nutrient supplements such as iron or folic acid in addition to multi-vitamins can lead to excessive intake.

Dietary intakes of iron and folate in supplement users in our study were found to be lower than non-users, indicating the need for nutrition education on adequate intake of nutrients for pregnant women. As the contents of iron and folic acid ranged widely in dietary supplements available in Korea, pregnant women should cautiously select supplements. Also, dietary supplements could be better formulated to reduce the risk of excessive intakes and the nutrient contents of supplements should be carefully monitored.

Conclusion

We investigated the intakes of iron and folate from dietary supplements as well as from food sources and evaluated hematologic indices according to the type of the supplement in 165 pregnant women who were in their late pregnancy period. As the intakes of iron and folate consumed by pregnant women from food sources alone were much lower than the RNIs, it is necessary to provide pregnant women with nutrition education to have healthy dietary habits for improving intakes of essential nutrients. Furthermore, our results showed it is more advisable to select multi-vitamins than single nutrient supplements.

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