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☐ **Original Article** ☐

Effect of Vitamin C Supplementation on Iron Status of Adolescent Girls with Low Hemoglobin Levels

Joung-Won Lee†, Chun-Keun Park*

Department of Home Economics Education, Chungnam National University, Taejon, Korea
Suwon Girls' High School, Suwon, Korea*

ABSTRACT

This study investigated the effects of vitamin C supplementation on the nutritional iron status of 31 adolescent girls, aged 12 - 15 years, with low hemoglobin levels. They were randomly divided into four groups, and for two groups daily 150mg or 900mg of L-ascorbic acid(AsA) was given in three equal doses at three meals during 9 weeks. To another group daily 60mg iron as ferrous sulfate was given in the same way as AsA. The control group was given sugar placebo. Body iron status was monitored through the determination of Hb, Hct, MCHC, and serum ferritin concentrations. Dietary AsA and iron intakes were measured from food consumption surveys performed by 3-day 24-hour recalls. The amount of absorbed iron was estimated from the model of Monsen et al. The average amounts of food iron for four groups were 12.3 - 15.0mg and 11.1 - 18.9mg at initial and at final peroid of the supplementation trial, respectively. The tentatively estimated amount of absorbed iron was significantly increased in the 900mg AsA and iron supplementing groups, but not in the 150mg AsA and placebo groups. Both Hb and MCHC were improved to above normal levels in all groups except the placebo group. Hct was elevated only in the AsA 900mg group whose Hct was relatively lower than the other groups. Serum ferritin concentrations of the four groups, which were as low as 8.50 - 14.39ng/mL on average at the initial periods, augmented significantly to 20.18ng/mL and 26.63ng/mL in the 900mg AsA and iron groups, respectively. Serum ferritin was not elevated in either the AsA 300mg group or the placebo group. The above data indicated that the daily supplementation of 150mg AsA to the meals containing 12 - 15mg iron per day promoted Hb levels of adolescent girls with low Hb, and the 900mg AsA supplementing improved not only Hb level but also body iron store. A supplementation of 60mg iron per day appeared to be slightly more effective in improving the iron status in comparison to the 900mg AsA supplement. (*Korean J Community Nutrition* 2(5) : 687~694, 1997)

KEY WORDS : vitamin C supplementation · adolescent girls · hemoglobin · MCHC · serum ferritin.

Introduction

Iron deficiency remains as a common nutritional problem in Korea, particularly at the peeriods of growth, puberty and pregnancy(Tschai et al. 1981). For adolescent girls their rapid growth and menarche require a high intake of dietary iron. However their irregular meals, frequent skipping of breakfast, heavy snacks, preferring fast-foods, anoretic response to

†**Corresponding author** : Joung-Won Lee, 220 Kung-dong, Yusong-ku, Taejon, 305-764
TEL) 042) 821-6841, FAX) 042) 822-8283

emotional stress, and dieting lower their iron intakes (Ko et al. 1991 ; Sim & Kim 1993). Iron deficiency in a severe state produces anemia and is associated with a reduction of work capacity, impaired behavior and intellectual performance, delayed adaptation of body temperature to a cold environment, and decreased resistance to infection.

According to 1993 Korean national nutritional survey 18% of girls in the 13 – 16years age range were anemic as defined by blood hemoglobin concentrations below 11.5g/dL for girls as suggested by WHO (1968). Ha et al.(1990) reported through various hematologic analyses that 7.2% of adolescent girls in the 12 – 15 years age range in urban areas and 13.2% in rural areas were iron-deficient anemic and 26% in both areas were in an iron depleted state without anemia.

The main cause of iron deficiency is thought to be the poor assimilation of dietary iron in addition to its low intake. The most efficient way of eliminating iron deficiency is the intake of nondairy animal foods, such as meats, fish and poultry, containing heme iron which is well absorbed in the intestine. The larger part of dietary iron, however, is poorly absorbable nonheme form. All of the iron in cereals, vegetables, eggs, and milk as well as approximately three-fifths of the iron in meats, fish, and poultry are nonheme iron(Monsen et al. 1978). In contrast to heme iron, the absorption rate of nonheme iron is highly influenced by other food components ingested concomitantly. Particularly vitamin C has been shown as the key enhancing factor, whereas phytic acid and tannins have been indicated as inhibiting factors (Callender et al. 1970 ; Cook et al. 1972 ; Hallberg et al. 1987). Hallberg and Rossander(1982) have predicted that absorption rate of nonheme iron may vary up to tenfold depending upon the content of enhancers and inhibitors.

Several recent studies have reported that the dietary iron intakes of Korean adolescent girls was insufficient, 8.5 – 15.0mg per day and more than two-thirds of the total iron was from cereals and vegetables(Ahn & Kim 1988 ; Kim et al. 1997 ; Lee & Yoo 1997). Thus the poor bioavailability of dietary iron as well as the low iron intake have been the problems of iron nutriture to be solved.

As one of the strategies to improve iron status, vitamin C supplementation can be effective because of its apparent effect on the absorption of food iron and because of the low cost. Moreover, taking high doses of vitamin C supplements have recently become popular for health and beauty. The daily amounts of vitamin C taken as the supplements varied from 90 – 2250mg with the average of 556.5mg (Kim 1994). A study using the extrinsic tag method with two radio-iron isotopes has reported that the amounts of vitamin C showing a clear-cut effect to enhance nonheme iron absorption was 50mg or more per meal(Hallberg et al. 1986). It seems that large amounts of vitamin C supplementation above the range of the usual dietary intake will be needed to obtain some promoting effect on iron nutritional status. Therefore an intervention trial was carried out in adolescent girls with quite low hemoglobin levels in order to investigate the hematologic response to the vitamin C supplementation at three daily meals of 50mg or 300mg per meal.

Subjects and Methods

1. Subjects and vitamin C supplementation

Thirty-five female adolescent students of a middle school in a rural area participated in this study. They ranged in age from 12 – 15 years and had experienced menstruation for more than 6 months. All of them were apparently healthy and individuals on any form of vitamin or mineral supplementation were excluded from the study. The subjects with relatively poor iron status were selected on the basis of preliminary hemoglobin concentration(Hb) and hematocrit(Hct) screening.

The subjects were randomly divided into four groups – 9, 9, 9, and 8 subjects for Group I, II, III, and IV, respectively. Group I(C-150) received 150mg vitamin C as L-ascorbic acid(AsA, coated, USP) daily, given in three doses of 50mg each as a tablet one hour after each meal, for a period of 9 weeks. Group II(C-900) received 900mg AsA daily in the same

way as in C-150. To group III(Fe-60) a daily 60mg of iron as ferrous sulfate was given in the same way as in C-150 for the comparison between iron and AsA supplementing effects on body iron status. Group IV(Placebo) was given sugar placebo. Two subjects of Group II and two of Group IV failed to complete the study and were excluded from the data analyses. During the trial period subjects continued with their usual diets and daily activities.

2. Dietary survey and the estimation of bioavailable iron

Dietary intakes of iron, AsA, and crude fiber by the subjects were determined at the initial and final periods of the AsA supplementing trial. Dietary surveys were performed through 24-hour recalls for 3 consecutive days and their nutrients contents were computed on the basis of a food composition table.

The bioavailability of dietary iron was tentatively estimated using the model developed by Monsen et al.(1978). This mathematical model was based on the amounts of vitamin C and MPF(meat, poultry, and fish) per meal and was also based on expected absorption rates depending on the body iron stores of individuals. The quantity of body iron store of the subjects was presumed as 125mg on the basis of their serum ferritin concentration(Cook et al. 1974 ; Finch & Huebers 1986). Thus the absorption rates of heme and nonheme irons were selected as the middle value between 0 and 250mg body iron store in the model of Monsen et al.

3. Hematologic measurements

Fasting venous blood was obtained from each subject in the morning before and after 9 weeks of the supplementing trial. An aliquot of blood was collected in a heparinized tube and was analyzed for Hb and Hct using cyanomethemoglobin(Drabkin & Austin 1932) and microcentrifuge methods, respectively. From these two values mean corpuscular hemoglobin concentration(MCHC) was calculated. Serum ferritin was measured by a solid phase enzyme immunoassay using Ferrizyme Kits(Abbott) with autoanalyzer(Quantum II) (Revenant & Beaudonnet 1982).

Serum total vitamin C concentration was analyzed by 2,4-dinitrophenylhydrazine method(Roe & Kuether 1943).

4. Statistical analysis

All values of the data were expressed as Mean±SD. The significance of the mean differences between the initial and the final supplementing periods of the same group was tested by paired t-test, and among groups, by Duncan's multiple range test.

Results and Discussion

1. Dietary intakes of vitamin C and iron

Baseline characteristics of the subjects are shown in Table 1. Their average body weights and heights were similar to Korean standards for age(The Korean Nutrition Society 1995) and their average BMIs belonged to the normal range. The average age of group C-900 was slightly higher than those of the other three groups and BMI of Fe-60 was a little bit larger than those of other three groups. But these slight differences would not influence the results of this supplementing study.

As presented in Table 2, average daily intakes of

Table 1. Age and body size of the subjects by groups

Measurements	C-150(n=9)	C-900(n=7)	Fe-60(n=9)	Placebo(n=6)
Age(yr)	14.0±0.0[a]	15.1±0.4[b]	14.7±0.7[ab]	14.3±0.5[a]
Initial weight(kg)	48.4±7.1	51.9±5.3	53.9±5.1	48.2±5.8
Final weight(kg)	48.1±7.3	51.4±5.2	53.5±4.2	48.3±6.5
Height(cm)	156.4±7.4	156.5±5.1	156.6±3.6	156.7±6.7
Initial BMI	19.8±2.4[a]	21.2±2.5[ab]	22.1±2.0[b]	19.6±1.8[a]

Mean±SD

[abc]Values in the same row without common superscript are significantly different at $p<0.05$ as determined by Duncan's multiple range test

BMI : Body mass index=weight(kg)/height(m)^2

C-150 or C-900 : 150mg or 900mg L-ascorbic acid supplementation daily

Fe-60 : 60mg iron supplementation daily

Table 2. Daily intakes of dieatry ascorbic acid and iron by the subjects during the supplementing period

Nutrients	C-150		C-900		Fe-60		Placebo	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Ascorbic acid(mg)	57± 22	60± 24	68± 20	50± 26	66± 23	61± 28	84± 17	59± 31
Iron(mg)	12.5± 4.4	12.3± 5.1	12.3± 3.0	13.3± 6.9	13.1± 5.1	11.1± 7.0	15.0± 4.4	18.9± 10.8
Available iron†	1.0± 0.7	1.3± 0.6[a]	1.3± 0.8	2.0± 0.9[a]*	0.8± 0.4	3.5± 0.7[b]**	1.1± 0.6	1.3± 0.8[a]

Mean±SD

*$p<0.05$, **$p<0.01$

[ab]Values in the same row of the final without common superscript are significantly different at $p<0.05$ by Duncan's multiple range test

†The amounts of available iron were tentatively calculated from the model suggested by Monsen et al.(1978). The body iron store of the subjects were assumed as 125mg on the basis of their serum ferritin determination. The amounts of available iron at the final were estimated including the intakes of supplemented ascorbic acid or iron

dietary vitamin C(AsA) and iron, by groups and by supplementing periods, were varying, but were not significantly different among groups and between supplementing periods. The amounts of dietary AsA intakes were 57-84mg at initial and 50-61mg at final period, all of which were more than 50mg of recommended dietary allowance for Koreans(RDA, the Korean Nutrition Society 1995). But if the loss of AsA occurring during cooking is considered, the amount may not be sufficient. The dietary intakes of iron, 12.3-15.0mg at the initial and 11.1-18.9mg at the final, were inadequate compared with 18mg of RDA, except that at the final period of placebo group.

The amounts of total absorbed iron were tentatively estimated from each meal using the model of Monsen et al.(1978). The model, which has been suggested for western meals and people, may not be reasonable to be applied for Korean diets and subjects. Nevertheless, this attempt seems to be meaningful to predict the iron absorbability roughly when the other direct methods are not available to apply. At the initial period of the trial, the total absorbed iron amounted to 0.8-1.3mg giving the absorption rates of 6.1-10.6%. However, the absorption rates of iron at final period were increased in AsA supplemented groups - C-150 and C-900, while no increase occurred in Placebo group. In Fe-60 group the estimated amount of absorbed iron was greatly increased from 0.8mg to 3.5mg due to the large iron supplementation though the absorption rate was decreased.

Table 3. Changes of hemoglobin level(Hb), hematocrits (Hct) and mean corpuscular hemoglobin concentrations(MCHC) by ascorbic acid or iron supplementation

Parameters	Groups	Supplementing periods	
		Initial	Final
Hb(g/dL)	C-150	10.1±2.5	16.1± 1.6**
	C-900	10.4±3.4	16.5± 2.3**
	Fe-60	10.5±3.3	15.0± 3.0**
	Placebo	11.8±3.0	13.4± 4.5
Hct(%)	C-150	39.1±1.9[ab]	40.1± 1.5[a]
	C-900	37.1±1.7[a]	40.5± 1.6[ab]*
	Fe-60	39.6±2.9[ab]	42.6± 2.0[b]
	Placebo	40.7±2.0[b]	41.7± 1.5[ab]
MCHC(%)	C-150	25.7±5.9	40.2± 4.1[a]**
	C-900	27.8±8.8	40.9± 6.4[a]*
	Fe-60	26.5±8.4	35.3± 7.7[ab]*
	Placebo	29.1±7.8	32.2±10.9[b]

Mean±SD

[ab]Values in the same column without common superscript are signigicantly different at $p<0.05$ as determined by Duncan's multiple range test

*$p<0.05$, **$p<0.01$

2. Changes of Hb, Hct and MCHC

Hematological indices and serum ferritin were measured for monitoring the changes of iron status by the supplementation of AsA or iron. Pre-supplementation values of Hb, Hct, and MCHC on average were not significantly different among four groups, except that Hct value in C-900 was slightly lower than that of the Placebo group(Table 3). Pre-supplementation levels of Hb and MCHC were poor on the whole. Mean values of both Hb and MCHC were lower than the ICNND standards(1963) for the same age with the subjects and those of other reports(Ha et al. 1990 ; Tschai 1981). According to

the WHO standards(1968) for the same age, 27 for Hb and 18 for MCHC of the 31 subjects belonged to the anemic catagories. Average levels of Hct, however, were acceptable for all groups.

As shown in Table 3, the supplementation of AsA or iron during 9 weeks elevated both Hb and MCHC markedly from impaired to above normal levels($p<0.05$ or $p<0.01$). Such significant elevations of Hb and MCHC were not shown in placebo group although there were some insignificant increments. In case of Hct, only C-900 group of which the initial value of Hct was lower than the other groups, showed a significant increase compared with Placebo($p<0.01$).

The degree of improving effects on Hb and MCHC were similar among the three supplemented groups. There were no differences between 150mg and 900mg of AsA supplementation, and no differences between 150 or 900mg AsA and 60mg iron supplemented to the diets containing 12–15mg iron. If both AsA and iron were supplemented together to the same subjects, the improvement of iron status could be expected to be much better. These results were consistent with other reports. Ajayi and Nnaji(1990) reported marked elevations of Hb, Hct, and red blood cell count through the daily supplementation with 50–100mg AsA for 8 weeks in young female adults. Seshadri et al.(1985) also reported that anemic preschool children who received daily 200mg AsA supplementation for 60 days showed a significant improvement in Hb and red cell morphology. Kang et al.(1983) and Freire(1989) carried out iron supplementing trials, 20mg and 78mg per day respectively and showed significant improvements in Hb levels.

The improvements of iron status by AsA supplementation observed in Hb and MCHC levels is mainly ascribed to the promotion of food iron absorption(Hallberg et al. 1986 ; Layrisse et al. 1974 ; Smith 1986). It is well established that AsA enhances nonheme iron absorption owing to its ability to reduce Fe^{3+} to Fe^{2+}, possibly followed by the formation of Fe^{2+}–ascorbate chelates(Roeser 1983). In addition, AsA has been reported to influence the mobilization of storage iron, the uptake and release of iron by transferrin, and the exchange of iron between cytosol and ferritin(Roeser 1983). The function of AsA on the maintenance of folic acid in the reduced form may be another point of AsA contribution to the improving hematologic status (Seshadris 1984).

3. Change of serum ferritin

Serum ferritin was determined as an indicator of body iron stores. Serum ferritin is well known to be a useful tool for assessing nutritional iron status since it reflects from the early stages of iron deficiency. Serum ferritin levels of all the subjects ranged 0–45.4ng/mL with the mean value of 10.9±9.8ng/mL. Eighteen of 30 subjects had lower serum ferritin levels than 12ng/mL suggested as a cutoff value of iron deficient anemia(Herbert 1987). This cutoff value also indicated that the storage compartment of iron was depleted(Skikne et al. 1990).

As seen in Table 4, average serum ferritin levels at initial periods were different among the four groups, but a statistical significance did not exist. During the AsA of iron supplementation serum ferritin levels of C-900 and Fe-60 were greatly increased($p<0.05$ and $p<0.01$, respectively), whereas C-150 and Placebo did not show any changes. No increase of body iron store in group C-150 coincided with Malone et al.'s study(1986), which reported the lack of serum ferritin change by the supplementation of 300mg AsA daily for 8 weeks. Above results may be explained as that additional iron absorbed more by 150mg AsA was first used to replenish Hb and was not enough to increase storage iron pools during the 9 weeks.

The mean serum ferritin values of C-900 and Fe-

Table 4. Change of serum ferritin level by ascorbic acid or iron supplementation

Groups	Serum ferritin (ng/mL)		
	Initial	Final	Δ Change
C-150	12.30±13.63	12.75±5.45[a]	+ 0.45
C-900	8.50±7.28	20.18±6.14[ab]*	+11.68
Fe-60	9.46±8.93	26.63±13.15[b]**	+17.17
Placebo	14.39±10.12	14.99±9.71[a]	+ 0.61

Mean±SD

[ab]Values in the same column without common superscript are significantly different at $p<0.05$ as determined by Duncan's multiple range test

*$p<0.05$, **$p<0.01$

60 groups were somewhat lower than C-150 and Placebo. Moreover group C-900 showed slightly lower value of Hct than the Placebo. These may be suspected as influencing factors upon iron absorption rate and thus the change of serum ferritin because the less body iron storage is, the more food iron is absorbed(Monsen et al. 1978). However this possibility does not seem to be enough to dilute the effects of 900mg AsA or 60mg iron since those effects of elevating serum ferritin were great. The increased amounts of body iron store could be assumed as 72mg in group C-900 and 110mg in Fe-60, when using the general guideline that a serum ferritin concentration of 1ng/mL was equivalent to approximately 120μg of storage iron per kg of body weight (Finch & Huebers 1986). Cook et al.(1984) could not find an increase of body iron storage through the supplementation of daily 2g AsA, 1g each with lunch and supper, for 16 weeks. This result may be explained by the iron status of the subjects. They had normal iron status, 46ng/mL of serum ferritin, and their ordinary diets contained substantial amounts of animal tissues such as meat, a known enhancer for nonheme iron absorption and thus the AsA supplement did not affect the iron status. Hunt et al. (1990) also reported that AsA supplementation of daily 1500mg with diets containing 13.5mg of iron for 5.5 weeks improved Hb levels, but did not elevate the serum ferritin. This negative result on body iron storage may be attributed to the fact that the supplementing period was not long enough to replenish the body iron storage occurring after the improvement of Hb, compared with 9 weeks of this study, particularly for the subjects who were iron-depleted so that their serum ferritin concentrations were below 8.5ng/mL.

4. Serum total vitamin C concentration

The average serum total vitamin C concentrations of the four groups during the experiment belonged to the normal range of 0.5 – 1.5mg/dL and the initial serum vitamin C levels of the Fe-60 group was a little bit lower than that of the placebo group(Table 5). The 150 or 900mg of AsA supplementation augmented the serum vitamin C levels, while such increasing effects were not shown in both Fe-60 and placebo groups.

Table 5. Change of serum totoal vitamin C concentration during the experiment

Groups	Serum total vitamin C (mg/dL)	
	Initial	Final
C-150	0.96 ± 0.13^{ab}	$1.31 \pm 0.31^{b*}$
C-900	0.95 ± 0.19^{ab}	$1.38 \pm 0.23^{b*}$
Fe-60	0.78 ± 0.22^{a}	0.84 ± 0.31^{a}
Placebo	1.08 ± 0.27^{b}	1.23 ± 0.33^{b}

Mean ± SD

[ab]Values in the same column without common superscript are significantly different at $p<0.05$ as determined by Duncan's multiple range test

*$p<0.05$

Summary and Conclusion

The effects of vitamin C suplementation on the nutritional iron status of adolescent girls with low hemoglobin levels were examined. Thirty-one girls aged 12 – 15years were divided into four groups, and for two groups daily amounts of 150mg or 900mg of L-ascorbic acid(AsA) was given in three equal doses at three meals during 9 weeks. To another group daily 60mg of iron as ferrous sulfate was given in the same way as AsA. The control group was given sugar placebos.

The average amounts of dietary iron for the four groups were 12.3 – 15.0mg and 11.1 – 18.9mg at the initial and at the final peroid of the supplementation trial, respectively. The absorbed iron, predicted from the calculating model of Monsen et al., was significantly increased in 900mg AsA and iron groups at the final period, but not in the 150mg AsA and placebo groups. Both Hb and MCHC were improved to above normal levels in all groups except the placebo group. Hct was elevated only in the AsA 900mg group whose Hct was relatively lower than the other groups. Serum ferritin concentrations of the four groups, which were low as 8.50-14.39ng/mL on average at the initial periods, augmented significantly to 20.18ng/mL and 26.63ng/mL in the 900mg AsA and iron groups, respectively. Serum ferritin was not elevated in either the AsA 150mg or

the placebo group.

In conclusion, the daily supplements of 150mg AsA to the meals containing 12 - 15mg iron per day during 9 weeks promoted Hb levels of adolescent girls with low Hb, and the 900mg AsA supplementation improved not only Hb level but also body iron store. The supplementation of 60mg iron per day appeared to be slightly more effective in improving the iron status in comparison to the 900mg AsA supplementation.

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