

## Effect of vitamin C on the absorption of non Heme Iron in apparent healthy volunteers

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### Abstract

The study was acute and conducted on two groups of individuals. Group A (treatment) consisted of 30 healthy volunteers who were administered Iron and VitC for two hours and then followed another two hours without VitC and Iron. Group B (control) 30 healthy subjects served as control they were administered Iron without VitC and then followed another two hours without Iron. VitC Ingestion lead to nonsignificantly increased ( $p>0.05$ ) in plasma Iron concentration in male and female after two hour, but it has significantly increased effect ( $p<0.05$ ) in plasma Iron concentration in total (male and female) after two hours from ingestion. But the change in the concentration of the plasma iron in three groups (male, female, total) were no significantly effect ( $p>0.05$ ) after the last two hours without VitC and Iron.

### الخلاصة

الدراسة الحالية كانت من نوع قصيرة الامد, أجريت على مجموعتين من الأشخاص الاصحاء , المجموعة الاولى ( A ) مجموعة المعاملة وتتكون من 30 شخص, تناولوا الحديد و فيتامين C لمدة ساعتين. المجموعة الثانية ( B ) وتتكون من 30 شخص يمثلون مجموعة المقارنة , تناولوا الحديد فقط . ان تناول الفيتامين وكمية (180mg/2h) مع الحديد ادى الى زيادة غير معنوية ( $P>0.05$ ) في تركيز الحديد في بلازما الدم في مجموعة الاناث والذكور بعد ساعتين, لكن كانت الزيادة معنوية ( $p<0.05$ ) في تركيز الحديد في بلازما الدم في مجموعة الكل(الاناث والذكور) بعد ساعتين من تناول الحديد والفيتامين بالمقارنة مع المجموعة ( B).

### Introduction:

VitC is the only dietary constituent other than animal tissue that has been shown repeatedly to augment the absorption of non heme Iron in humans<sup>(1-5)</sup> the stimulating influence of ascorbic acid has been shown when it is given with inorganic Iron alone and is even more pronounced which it is taken with food<sup>6,7</sup>. The facilitating effect of VitC is dose related in one student in which increasing amounts of ascorbic acid ranging from 25 to 1000mg where added to a liquid formula meal containing 4.1mg non heme iron, absorption increased progressively from 0.8% to 7.1%<sup>3</sup>.

# **Effect of vitamin C on the absorption of non Heme**

In contrast with striking effect of the ascorbic acid on iron absorption, the improvement in iron status when the diet is supplemented with the VitC has been minimal. In one study the addition of 2000mg VitC/d to the diet for  $\leq 2y$  did not alter iron stores significantly as measured by serum ferritin concentration<sup>8</sup>.

Repeat iron absorption studies after 16 wk of supplementation showed that the lack of effect on iron status was not explained by the adaptation of the gastrointestinal tract to a high intake of VitC in another studies, 100mg ascorbic acid given 3 times daily with meals to menstruating women for 9 mo. Had no significant effect on iron status<sup>9</sup>. Similarly 25 healthy women aged 20-45y with low iron stories as defined by a serum ferritin concentration  $\leq 20\mu\text{g/l}$  were given 500mg ascorbic acid 3 time daily with meals for 10wk. there was no significant alternation in biochemical indexes of iron status in these women, even in those consuming a diet low in bioavailable iron<sup>10</sup>.

About 70 percent of the total iron is circulating in erythrocyte hemoglobin and up to 25 percent of the body iron is stored reticuloendothelial system (liver, spleen and bone marrow) and only about 50-70  $\mu\text{mol}$  (3-4mg) or about 0.1 percent of the total body iron is circulating in the plasma. This fraction which is bound to the protein transferrin<sup>11</sup>.

## ***Subject and methods***

### **Subject**

A total of 60 subject 20-22 years of age were selected for the study. The body max with normal range all the subjects were interviewed extensively before they were enrolled in the study to establish their willingness. All the subjects stated that they were in good health, were taking no iron medication and had no history at recent infections or disorders known to influence iron absorption. The participants were mainly selected from undergraduate students, third stage, Pharmacy College, university of Basra.

The volunteers were randomly allocated into two groups (30 subject as control, 30 subject as treatment). Group A the study group received VitC 180mg and iron (ferrous gluconate) 300mg for two hours and then followed another two hours without VitC and Iron . Group B the control group received iron (ferrous gluconate) 300mg for two hours and then followed another two hours without iron.

### **Collection of the sample**

It is drew from the vein of the subjects (5ml) by the syringe, stand for 10 min. then centrifuge(3000 RPM) for 5min. drew 1ml serum and stored in vial in refrigerator until to use

### **Preparation of VitC tablet**

VitC was prepared previously by Kruger GmbH and Co. KG D-51469 Bergesch Gladbach in germany [www.kruger.de](http://www.kruger.de).

### **Preparation of Iron tablet**

It was prepared previously by co. in Egypt

Every tablet (300mg total weight) contains 300mg non-heme iron (ferrous gluconate).

## Method

The principle of reaction is: the iron dissociated from transferrin-iron complex in the serum by solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex<sup>12</sup>.

### Reagents

Reagent1	guanidine, HCL	4,5mmol/L	
	Acetate buffer solution	pH=5	
Reagent2	Ascorbic acid		
Reagent3	Ferrozine	40mmol/L	
Reagent4	Standard	1mg/L	17.9 $\mu$ mol/L

### Preparation and stability

Reagent A: add one measure (250mg) of reagent2 at 50ml of reagent1.

Reagent B: mix one volume of reagent3 with 25 volumes of reagent A

### Stability

2 weeks at 2-8 C<sup>0</sup>

3 days at 20-25C<sup>0</sup>

### Procedure

	Reagent blank	standard	Sample blank	sample
D.W	200 $\mu$ l			
StandardR <sub>4</sub>		200 $\mu$ l		
sample			200 $\mu$ l	200 $\mu$ l
Reagent A			1ml	
Reagent B	1ml			

Mix and let stand for 10 min. at 20-25C<sup>0</sup> then read the absorbance the color of reaction is stable for at least 30min. wave length is 562nm.

### Calculation

$$\text{Iron} = (\text{sample} - \text{sample blank} / \text{standard}) * n$$

$$n = 1 \text{ mg/l}$$

$$n = 17.9 \mu\text{mol/l}$$

### Statistical analysis

All values were expressed as the mean  $\pm$  S.D, significance differences between treatment and control was determined using ANOV: two factors. Difference were considered significant at lowest  $p < 0.05$  level.

### Result and Discussion

# Effect of vitamin C on the absorption of non Heme

VitC administration for level of iron in blood plasma was non-significant ( $p>0.05$ ) in male and female after 2 hour as in table (1, 2) and figure (1, 2), whereas, it was significant ( $p<0.05$ ) in total after 2 hour as compare with control as in table (1, 2) and figure (3), but was nonsignificantly ( $p>0.05$ ) in three groups(male, female, total) after the last 2 hours without VitC and Iron as compare with control as in table(1,2) and figure (1,2,3).

The recent study was conducted on healthy volunteers; therefore, the physiological factors only are concerned like:

1-Sex and age differences (two sex were in the study and they have nearly equal ages

2-cyclical variation (circadian rhythm, the blood was drew from all the subjects in the morning. monthly variation in women (women during menstrual period are refused from the study.

3- Random variation, all the subjects in that morning were without physical or mental stress.

4-effects pregnancy and oral contraceptives, all the pregnancy women's or she was ingestion contraceptive are refused from the study. The results were significant in total group while non-significant in male and female groups may because number of volunteers in total group was larger number (60 subjects), or because high S.D. (standard deviation). The rising effect of VitC on level of iron in plasma may result to its effect on attaching of Iron with the protein apoferritin to form ferritin in the upper small intestine, which, as elsewhere in the body, is a storage compound. Or because of VitC effects on marrow erythropoietic activity, therefore, increase form of erythrocytes lead to increase consumption of iron and because that obtain rise absorption of Iron in the intestine. In the otherwise may be because effect of VitC on oxygen tension in the intestinal cell, or because reducing properties of VitC which lead to reduce ferric ( $Fe^{+3}$ ) to ferrous ( $Fe^{+2}$ ) form in the intestine and by which Iron can only cross cell membranes; therefore, increase absorption and concentration in the plasma. Return the values approximately to the level of the zero time in the treatment group after last two hours without VitC as compare with control group enhance the rising effect of the VitC on the level of the Iron in plasma.

## Conclusion

The absorption of Iron by small intestine and its level in healthy subjects plasma is more increase when ingestion VitC (180mg) with Iron (300mg) than iron only after two hours.

## Reference

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**Table (1) effect of VitC on level of iron in blood plasma (control).**

	age	No.	0 hour	2 hour	4hour
Male	21-23	15	1.509±0.4	1.307 <sup>d</sup> ±0.4	1.48 <sup>d</sup> ±0.4
Female	21-23	15	2.213±0.5	1.323 <sup>d</sup> ±0.4	1.9 <sup>d</sup> ±0.5
Total	21-23	30	1.772±0.5	1.313 <sup>d</sup> ±0.4	1.6 <sup>d</sup> ±0.5

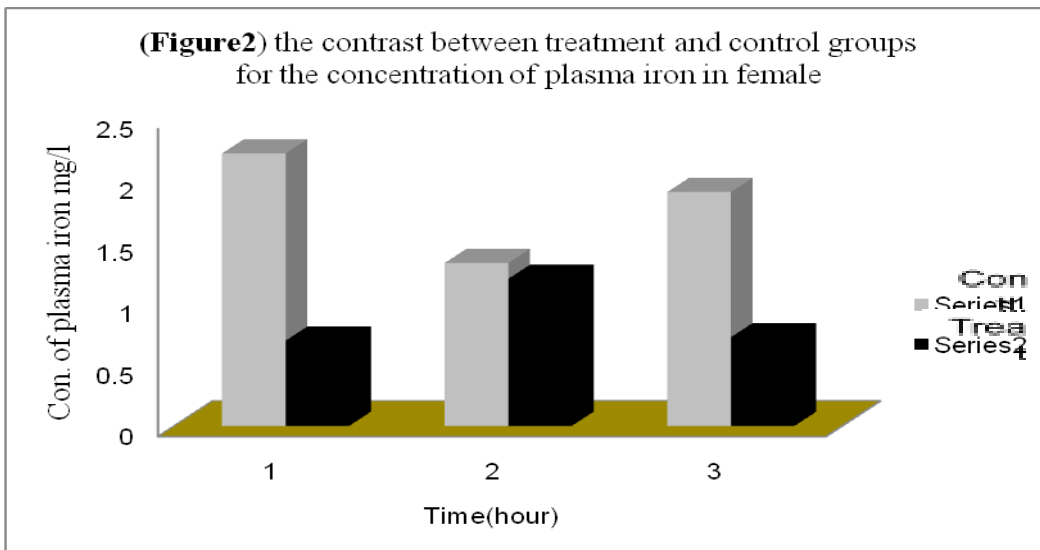
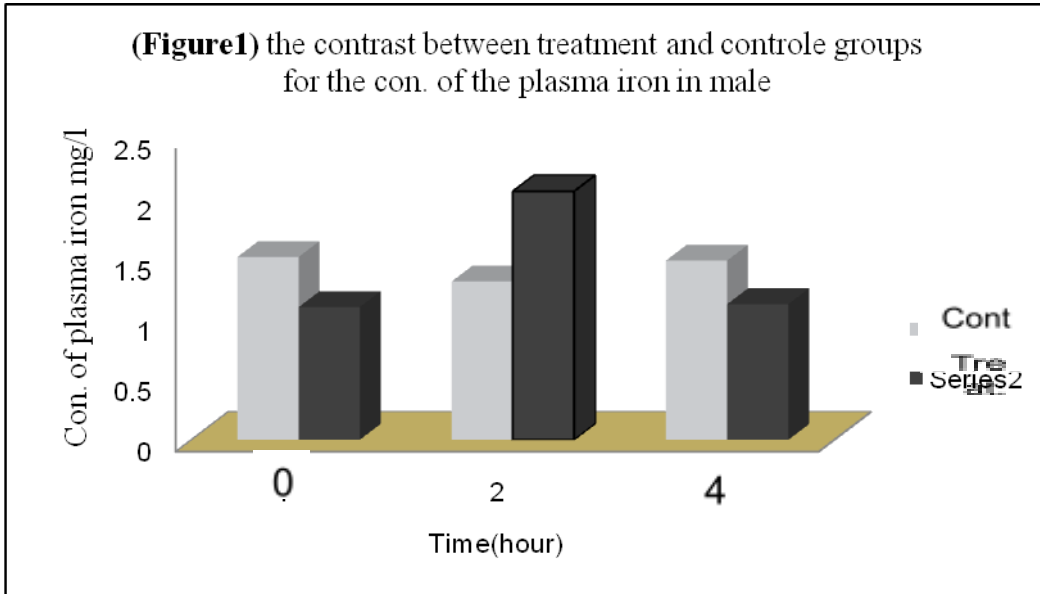
**Table (2) effect of VitC on level of iron in blood plasma (treatment).**

	age	No.	0 hour	2 hour	4hour
Male	21-23	15	1.095±0.7	2.052 <sup>d</sup> ±0.5	1.12 <sup>d</sup> ±0.6
Female	21-23	15	0.692±0.09	1.194 <sup>d</sup> ±0.4	0.72 <sup>d</sup> ±0.6
Total	21-23	30	0.894±0.4	1.578 <sup>a</sup> ±0.7	0.93 <sup>d</sup> ±0.4

→ a significant increase

→ d no-significant increase

# Effect of vitamin C on the absorption of non Heme



**(Figure3)** the contrast between treatment and control groups for the concentration of plasma iron in total

