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## **Intestinal absorption of iron alone and in combination with authentic or natural vitamin C and carotene**

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With 3 tables

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Intestinal absorption of iron is an important process that controls the beneficial action of food iron or therapy. This process is affected by endogenous factors such as transferrin saturation (8), state of iron stores (9) rate of erythropoiesis (13), and by exogenous factors such as valency of iron given (4) and the type of nutrient present with iron in the gastrointestinal tract.

Several studies were performed to investigate the effect of combination of iron with different nutrients including vitamins (2, 16) and proteins (5, 15). However, little could be traced in the literature dealing with the effect of these nutrients as present in vegetables or fruits and in the form they are usually eaten.

The present study aim at investigating the effect of two vitamins, namely vitamin C and carotene derived from authentic sources on intestinal iron absorption. Besides, juices of two vegetables, namely parsley and pepper and one fruit, namely orange, proved to be rich in these two vitamins, were tested when given with oral doses of iron.

### **Material and Methods**

The present study was carried out on normal white albino rats of body weight ranging from 152 to 225 gm. These animals comprised both sexes, and to ensure the absence of nutritional disorders, their blood hemoglobin concentration and plasma total proteins were estimated (table 1). The animals were categorized into 6 groups each of 5.

Table 1. Body weight, blood hemoglobin and plasma proteins of rats used in the experiment.

Group	Body weight gm. %	Blood Hb gm. %	Plasma proteins gm. %
1. Iron alone	166-193 175.4 ± 4.7	13.98-17.57 15.55 ± 0.59	6.08-7.17 6.37 ± 0.19
2. + ascorbic acid	152-202 176 ± 9.19	14.85-16.74 15.77 ± 0.33	6.33-7.66 7.0 ± 0.27
3. + Carotene	160-209 183.4 ± 8.3	13.77-17.01 15.61 ± 0.82	6.33-7.66 7.0 ± 0.28
4. + orange	155-225 184 ± 13.0	14.99-17.55 16.20 ± 0.45	6.08-7.17 6.40 ± 0.64
5. + parsley	175-220 197 ± 7.18	15.66-17.28 16.63 ± 0.29	6.08-7.00 6.35 ± 0.18
6. + pepper	155-184 174.8 ± 5.15	14.45-17.55 15.50 ± 0.53	6.08-7.66 7.05 ± 0.28

The rate of iron absorption was tested alone and in combination with either ascorbic acid or carotene derived from authentic or natural sources as orange, parsley or pepper juices.

Rate of intestinal iron absorption was evaluated by giving each rat an oral dose of iron equivalent to 10 mg./rat in the form of ferrous sulphate. The elevation in the level of plasma iron was followed during intervals of 1, 2 and 3 hours after the orally administered dose. To test the effect of added vitamins, 50 mg of vitamin C or 0.2 mg. carotene were given. The carotene dose was

Table 2. Fasting plasma iron and its values 1, 2 and 3 hours after the given dose of iron either alone or with different compounds.

Compound	Plasma iron $\mu\text{g.}/100\text{ ml}$				Ab- sorption index
	fast	1 hour	2 hours	3 hours	
1. Alone	195-237 219.6 ± 16	495-575 516 ± 19.6	375-750 540 ± 32.2	375-495 446 ± 9.2	2.40
2. Ascorbic acid	195-225 209 ± 6.6 0.2	400-575 510 ± 32.2 0.4	400-500 480 ± 20.0 0.1	325-500 420 ± 34.8 0.3	2.45
3. Carotene	125-225 185 ± 18.7 0.1	325-500 400 ± 29.6 0.005	375-575 450 ± 33.5 0.05	325-525 405 ± 33.9 0.2	2.40
4. Orange	160-255 207 ± 20.2 0.3	250-450 363 ± 33.0 0.005	375-501 478 ± 36.9 0.2	330-405 349 ± 1.5 0.005	2.30
5. Parsley	150-250 193 ± 23.4 0.2	330-360 336 ± 13.5 0.005	364-425 400 ± 12.8 0.005	280-360 331 ± 18.3 0.005	2.00
6. Pepper	175-240 232 ± 13.2 0.3	375-575 455 ± 38.2 0.1	375-550 437 ± 41.5 0.05	375-425 415 ± 10.0 0.025	1.90

given dissolved in corn oil. When vitamin C or carotene were supplied from vegetables or fruits (orange, parsley and pepper), 5 ml. of each juice was given twice during the day before dosing and another 5 ml. with the iron dose.

Hemoglobin concentration was estimated as described by Wong (18).

Plasma total proteins was done according to the procedure of Wootton (19). Iron was estimated by the method of Ramsay (12). Vitamin C and carotene were determined by the method of association of vitamin chemists (1951).

## Results

Body weight, blood hemoglobin and plasma proteins of rats used in this experiment are tabulated in table 1.

The level of fasting plasma iron and its values after the orally given dose (10 mg. elemental iron/rat) are given in table 2.

The absorption index calculated as the ratio of maximum plasma iron level reached after the dose to the fasting level is shown in table 2.

The vitamin content (C and carotene) of the three juices and is tabulated in table 3.

## Discussion

Most reports in the literature agree that iron must be in a reduced and soluble form to be easily absorbed from the intestine (3). This actually necessitates the existence of a reducing agent with iron either in food or given separately. Ascorbic acid is one of the most familiar reducing agents recommended in this respect (10).

Some compounds have been reported to enhance iron absorption either through their reducing action or formation of chelates with the metal (6). Of these compounds are amino acids, as histidine, cysteine and lysine have been reported to enhance iron uptake from ligated segments of the intestine (5). Iron supplementation with valine and histidine brought about enhancement of absorption in healthy and iron deficient children (7). Certain other compounds are carbohydrates, polyalcohols and organic acids enhance iron absorption across the intestine by formation of low molecular weight compounds with iron (17).

On the other hand, certain nutrients or compounds present in foods have been reported to hinder intestinal iron absorption as phytic acid (14).

Food is considered as a combination of nutrients including most of these components whether enhancing or retarding intestinal iron absorption.

Table 3. Vitamin C and carotene content of the three juices used in this experiment.

English name	Latin name	Vitamin C mg/100 ml	$\beta$ -carotene mg/100 ml
Orange	Citrus sinesis	48	2.6
Pepper	Capsicum annuum	147	1.5
Parsley	Petroselinum crispum	176	25.0

For this reason, it is felt valuable to investigate the effect of combination of certain juices from fruits or vegetables known to be rich in vitamin C together with other compounds on intestinal iron absorption.

Our data showed that oral administration of iron to rats caused a marked increase in plasma iron level, which reached its maximum after two hours, then declined. Such a rise in the level of plasma iron is expected since the elemental iron was given in the form of ferrous sulphate which was dissolved in water immediately before dosing. This minimizes the chance of oxidation of the element and in turn makes it readily available for absorption. When ascorbic acid was added to the iron dose, no significant change was noticed between absorption index of both iron alone or with added ascorbic acid, but the maximum was reached one hour after dosing in case of addition of vitamin C. Such effect of ascorbic acid may indicate that the compound act as a preservative for iron, maintaining it in the ferrous form. However, it is noticed that at the two and three hours' intervals, the level of plasma iron in the group of rats receiving iron plus vitamin C was lower than corresponding values in the group receiving iron alone. This, together with the appearance of the maximum at the first hour in case of addition of ascorbic acid, may indicate enhanced utilization of iron after being absorbed and confirm previous finding reported by *Israels* (10) that ascorbic acid caused marked improvement in iron utilization.

The addition of carotene dissolved in corn oil to the iron dose resulted in the following:

- a) The absorption curve in case of supplementation with carotene was lower than when iron was given alone.
- b) The difference between maximum level of plasma iron and the fasting level was higher in the group receiving iron alone.
- c) The difference between the fasting level of plasma iron in the two groups was nonsignificant while the level at the one and two hours interval was significantly lower, in those given carotene with the iron dose.

These previously mentioned findings may indicate a hindering effect of carotene to intestinal iron absorption. The absorption indices in both groups either receiving iron alone or in combination with beta carotene are more or less equal. We could not find any information in the available literature dealing with the effect of beta carotene on iron absorption. Our data may speculate certain hindering action of beta carotene, but it is not a clear cut conclusion.

The three juices selected in this study which are orange, parsley and pepper contain more or less equal amounts of carbohydrates as gm.%, but the amino acid contents are different.

Our data presented in table 3 show that parsley is distinctly rich in  $\beta$ -carotene, while pepper and orange are markedly lower in their carotene content relative to parsley. Carotene content of orange was found to be 1.5 times that of pepper. Regarding ascorbic acid content, parsley and pepper are relatively rich in vitamin C than orange. Their content was about 3 times that of orange.

The absorption index calculated for iron and orange was more or less equal to that of iron alone, while for parsley and pepper it was lower. Although parsley is very rich in ascorbic acid content (table 3), yet it is also rich in beta carotene. Its content of  $\beta$ -carotene is 10 times that of orange. The hindering action of parsley juice may be due to its high content of  $\beta$ -carotene as previously indicated. Pepper juice, which is rich in ascorbic acid, also hindered iron absorption. The absorption index amounted to 1.9. Pepper contains about double the value of carotene as orange, which may be the cause of such hindrance. Parsley juice, which contains about 16 times carotene, as pepper exerted more or less similar effect as the latter. It is suggested that at certain concentration of carotene, the hindering action to iron absorption is realized and that excess carotene after this level becomes of no effect, i.e., there is no progressive hindering action with increasing concentration of carotene.

In conclusion, it may be recommended that patients suffering from iron deficiency or receiving iron-hematinics should avoid nutrients rich in carotene as pepper and orange, at least during or near iron therapy.

#### Summary

The effect of vitamin C and carotene derived from authentic or natural sources on intestinal iron absorption was studied. Vitamin C caused slight enhancement to iron absorption, while carotene hindered it. The three juices tested, namely orange, parsley and pepper, which were found to be rich in these two vitamins, hindered intestinal iron absorption to different extents. It was recommended that patients suffering from iron deficiency are not supplied with nutrients rich in carotene particularly during iron therapy.

#### Zusammenfassung

Es wurde die Wirkungsweise von echtem bzw. natürlichem Vitamin C und Carotin auf die Eisenresorption im Darm untersucht. Vitamin C bewirkte eine leichte Steigerung der Eisenresorption, während Carotin diese behinderte. Orangen-, Petersilien- und Pfeffersaft, sämtlich reich an beiden Vitaminen, behinderten in verschiedenem Maße die Eisenresorption im Darm. Es wird daher empfohlen, Patienten mit Eisenmangel nicht mit carotinreicher Nahrung zu versorgen, besonders nicht während der Eisentherapie.

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