

Oral iron absorption test should not be performed with iron drops containing ferric iron

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ABSTRACT

INTRODUCTION: In an oral iron absorption test (OIAT), the rise in plasma iron concentration after oral ingestion of iron is a measure of intestinal iron absorption. We describe results of the OIAT using two different formulations of oral iron drops.

METHODS: The study included all patients who had an OIAT performed at the Department of Internal Medicine, Farsø, Aalborg University Hospital, Denmark, from 1 January 2013 to 17 June 2014 (n = 24) using ferrous iron drops "Glycifer" and from 18 June to 3 November 2014 (n = 17) using ferric iron drops "Medic". A venous blood sample was drawn before and then 90, 180 and 240 min. after the intake of 9 ml iron drops of the "Glycifer" brand (270 mg ferrous iron) or the intake of 11 ml iron drops of the "Medic" brand (264 mg ferric iron).

RESULTS: The patient characteristics (ferrous versus ferric iron drops) were similar in terms of gender, age, haemoglobin, ferritin and previous gastric bypass surgery. The fasting baseline plasma iron concentration was median 5 µmol/l in both groups (p = 0.4). The maximum plasma iron concentration increase from baseline after oral intake of the iron drops was median 2 µmol/l (range: 0-8 µmol/l) in the group given ferric iron drops and 48 µmol/l (range: 14-78 µmol/l) when ferrous iron drops were used (p < 0.001).

CONCLUSION: OIAT performed with ferrous or ferric iron drops showed very different results with a lack of plasma iron concentration increase after ingestion of ferric iron drops.

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The oral iron absorption test (OIAT) is a method used to evaluate intestinal iron absorption in humans. In this test, the plasma iron concentration increases after oral ingestion of iron is used as a measure of intestinal iron absorption [1, 2]. The test can be performed in patients with microcytic anaemia and low plasma iron concentration who do not respond to oral iron treatment [3]. Such non-response may be seen in patients with iron deficiency anaemia caused by impaired intestinal iron absorption and in patients with anaemia due to chronic disease where the inflammation-induced hepatic production of hepcidin decreases the release of iron from

the enterocytes to the circulation [4]. Lack of an increase in plasma iron concentration after oral ingestion of iron may indicate impaired intestinal iron absorption and a need for intravenous iron treatment.

Iron (Fe) is an essential trace mineral that is naturally present in many foods, added to some foods and available as a dietary supplement. Iron exists in a wide range of oxidation states, but mainly as Fe²⁺ (ferrous) and Fe³⁺ (ferric) [5]. After oral ingestion of iron, the intestinal absorption of iron occurs in the duodenum and the proximal jejunum (**Figure 1**) [6]. Fe²⁺ is actively transported into the enterocytes by the divalent metal transporter 1 (DMT1), and the expression of DMT1 is regulated by the intracellular level of iron. Fe³⁺ must be reduced to Fe²⁺ by the duodenal cytochrome B before translocation can occur (**Figure 1**) [6].

In our Department of Clinical Biochemistry, the OIAT was previously performed with iron drops of the "Glycifer" brand which contains ferrous iron. However, this formulation was withdrawn from the Danish market in the beginning of 2014, and the OIAT was then switched to iron drops of the "Medic" brand containing ferric iron. The OIAT was discontinued in the beginning of November 2014 as it was recognised that all OIAT performed after the change in oral iron drops showed no increase in plasma iron concentration.

In the present observational study, we describe our results relating to OIAT performed before and after the change from ferrous to ferric iron drops.

METHODS

Study population

All patients included in the study had an OIAT performed at the Department of Internal Medicine, Farsø, which forms part of Aalborg University Hospital in Denmark. At the Department, the change in test procedure from "Glycifer" iron drops to "Medic" iron drops was implemented in June 2014. We consecutively included all patients who had an OIAT performed with "Glycifer" iron drops in the period from 1 January 2013 to 17 June 2014 (n = 24) and all patients who had an OIAT performed with "Medic" iron drops in the period from 18 June to 3 November 2014 (n = 17). One patient was not included in the study because it was uncertain with which formu-

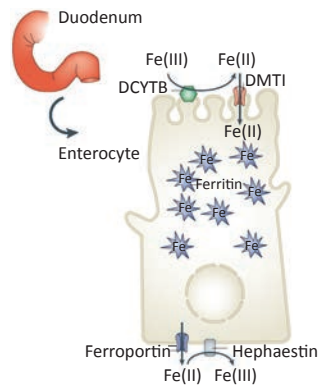
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FIGURE 1

Illustration of the transport of iron (Fe) into the enterocyte by the divalent metal transport 1 in the apical membrane. Fe(III) must be reduced to Fe(II) by the duodenal cytochrome B before translocation.



DCYTB = duodenal cytochrome B; DMT1 = divalent metal transport 1. Source: reprinted by permission from Macmillan Publishers Ltd [6].

lation of iron drops the OIAT had been performed. Two healthy controls had an OIAT performed with “Medic” iron drops.

Procedure for oral iron absorption test

All OIAT were performed fasting in the morning. The first venous blood sample was drawn immediately before iron was given orally (between 7.43 and 9.12 a.m.). Iron was given to the patient orally as either 9 ml iron drops of the “Glycifer” brand (Takeda Pharma, Denmark; ferrous glycine sulphate, abricot and lemon flavour, sorbitol, sodium saccharin, ethanol, and concentrated sulfuric acid) containing 270 mg of ferrous iron (30 mg/ml) or 11 ml iron drops of the “Medic” brand (Meda, Denmark; ferric glycine, purified water, sorbitol, glycerol) containing 264 mg of ferric iron (24 mg/ml). The patients were offered a glass of water to clean their mouth and to ensure that all iron was swallowed. Additionally, they were asked not to eat or drink before the next venous blood sample had been drawn 90 min. after the intake of iron. The subsequent blood samples were drawn after 180 and 240 min. Any patient complaint about abdominal discomfort was registered during the OIAT. Information was obtained from the medical record on most recent haemoglobin, ferritin and mean erythrocyte cell volume (MCV) measurement as well as any performed duodenal biopsy.

Biochemical analyses

Biochemical analyses were performed at the Department of Clinical Biochemistry, Aalborg University Hospital, in the section located in Farsø or in the section in Aalborg. Plasma iron concentration was measured using a colorimetric method on a Cobas 6000 c501 in the Farsø section before 1 June 2014 and on a Cobas 8000 c702 in the Aalborg section after 1 June 2014 (Roche,

Schwitzerland). According to the manufacturer, the limit of detection was 0.9 $\mu\text{mol/l}$, and the coefficient of variation for repeatability (within-day variation) and intermediate precision (within-day and between-day variation) was < 2%. Internal and external controls were satisfactory in both sections, and no bias in the results of external controls between the two sections was observed. Plasma ferritin concentration was measured using a particle-enhanced immunoturbidimetric method on a Cobas 8000 c702 (Roche, Switzerland). Plasma haemoglobin concentration and MCV were measured on a Pentra 120 DX (Horiba Medical, France).

Statistical analyses

Results were expressed as range, median and interquartile range (IQR); and comparison by groups (iron drops of the “Glycifer” brand versus iron drops of the “Medic” brand) was performed using the Mann-Whitney U test. The maximum increase in plasma iron concentration (C_{max}) from baseline during the OIAT was calculated for each patient. Statistical analyses were performed using STATA 11 (Stata Corp., College Station, Texas, USA), and a 5% level of significance was chosen.

Trial registration: not relevant.

RESULTS

A total of 41 patients and two healthy controls had an OIAT performed and were included in the study. The majority of patients were women (Table 1). Patient gender and information on haemoglobin, ferritin and MCV as well as previous gastric bypass surgery were not significantly different between the group of patients in which the OIAT was performed with ferrous iron drops (Glycifer) and the group in which the OIAT was performed with ferric iron drops (Medic) (Table 1). A total of 22 patients had a duodenal biopsy performed (Table 1) which was normal in all cases. All patients given iron drops of the “Glycifer” brand complained about abdominal discomfort during the test, whereas neither the patients nor the healthy controls given iron drops of the “Medic” brand had such complaints.

Notable differences were observed in the results of the OIAT performed with ferrous iron drops (Figure 2A) and ferric iron drops (Figure 2B). Intake of ferrous iron drops caused an increase in the plasma iron concentration in all patients, whereas no increase in plasma iron concentration was observed either for the patients or for the two healthy controls after ingestion of ferric iron drops. The fasting baseline plasma iron concentration was similar in the two groups (Table 2); but in line with Figure 2, C_{max} was considerably higher when the OIAT was performed with ferrous iron drops.

In the group of patients in which the OIAT was per-

formed with ferrous iron drops (Glycifer), ten patients had iron deficiency after gastric bypass surgery (Table 1). The fasting baseline plasma iron concentration in this group was median 8 $\mu\text{mol/l}$ (IQR: 3-13 $\mu\text{mol/l}$) and not significantly different from the 14 patients with no gastric bypass surgery: median 4 $\mu\text{mol/l}$ (IQR: 3-8 $\mu\text{mol/l}$), $p = 0.4$. However, C_{max} was significantly higher in the group with no gastric bypass surgery (median 58 versus 30 $\mu\text{mol/l}$ in the gastric bypass surgery group, $p = 0.01$). In the group of patients in which the OIAT was performed with ferric iron drops (Medic), only three patients had iron deficiency after gastric bypass surgery (Table 1), and there was no increase in plasma iron concentration during the OIAT in these patients ($C_{\text{max}} = 0$). Similarly, C_{max} was low in the 14 patients with no gastric bypass surgery (range: 1-8 $\mu\text{mol/l}$) where ferric iron drops were used.

DISCUSSION

This study reported the results of OIAT performed with two different types of iron drops containing either ferrous iron (Glycifer) or ferric iron (Medic). In comparable patient groups, the results of the OIAT were markedly different with no increase in plasma iron concentration after oral ingestion of iron drops containing ferric iron.

Ferrous and ferric oral iron formulations

Oral iron formulations vary widely in terms of iron content, oxidation state of the iron (ferrous versus ferric) and iron chelates. The most likely reason for the disparity between our results of the OIAT performed with two types of iron drops is the difference in the chemical state of the iron. The importance of the valency of iron for iron absorption has been known for years. In a review in 1975 [7], it was concluded that all available results from haemoglobin regeneration tests, oral iron absorption tests and techniques based on administration of radioactive iron such as whole-body counting and erythrocyte incorporation had demonstrated that humans absorb ferrous iron 4-10 times better than ferric iron from therapeutic oral iron doses (50-250 mg). Today, it is still generally believed that oral ferrous iron formulations have advantages over ferric formulations [8], and ferrous iron formulations are recommended by The International Nutritional Anemia Consultive Group (INACG), the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) for treatment and prevention of iron deficiency [9].

In our observational study, the dosage of iron given orally during the test was approximately the same for iron drops "Glycifer" and "Medic" (270 mg versus 264 mg), and the dosage was calculated according to the manufacturer's information on the iron content of the

formulation. In both types of iron drops, iron was coordinated to glycine as an iron amino acid chelate, and the slightly different content of excipients is unlikely to explain our results. Thus, our results suggest that in the OIAT performed with iron drops of the "Glycifer" brand, ferrous iron was well-absorbed, whereas the absorption of ferric iron from iron drops of the "Medic" brand was much lower. This is in line with the clinical findings that only the patients who were given iron drops of the "Glycifer" brand complained about abdominal discomfort. We observed that C_{max} was lower in patients who had gastric bypass surgery performed, which is in line with the findings in a previous report [10]. After such surgery, iron absorption is reduced due to the fact that the duodenum and the proximal jejunum are bypassed and to the fact that the ingested iron comes into contact with the gastric acid to a lesser extent [11]. In all patients and controls, the OIAT that we describe were performed fasting. In a study comparing radioactive labelled



TABLE 1

Characteristics of the patients who had an oral iron absorption test performed with ferrous iron drops (Glycifer) or ferric iron drops (Medic).

	All patients	Glycifer	Medic	p-value ^a
<i>Patients</i>				
n	41	24	17	
<i>Female gender</i>				
n (%)	31 (76)	17 (71)	14 (82)	0.4
<i>Age, yrs</i>				
Range	24-80	24-80	39-71	
Median	51	46	56	0.1
IQR	43-65	42-62	45-65	
<i>Haemoglobin, mmol/l^b</i>				
Range	2.9-8.7	2.9-8.7	5.0-8.4	
Median	6.5	5.9	6.6	0.1
IQR	5.6-6.9	5.2-6.7	6.4-6.9	
<i>Ferritin, $\mu\text{g/l}^c$</i>				
Range	5-134	5-91	5-134	
Median	8	6	8	0.7
IQR	5-13	5-13	5-13	
<i>MCV, fl^d</i>				
Range	57-103	57-93	58-103	
Median	76	75	76	0.3
IQR	69-83	68-82	73-84	
<i>Gastric bypass surgery</i>				
n (%)	13 (32)	10 (42)	3 (18)	0.1
<i>Duodenal biopsy</i>				
n (%)	22 (54)	10 (42)	12 (71)	0.1

fl = 10^{-15} l; IQR = interquartile range (25th-75th percentile); MCV = erythrocyte mean cell volume.

a) Result of the comparison between groups (ferrous versus ferric iron drops) using the chi-squared test for categorical variables and the Mann-Whitney U test for continuous variables.

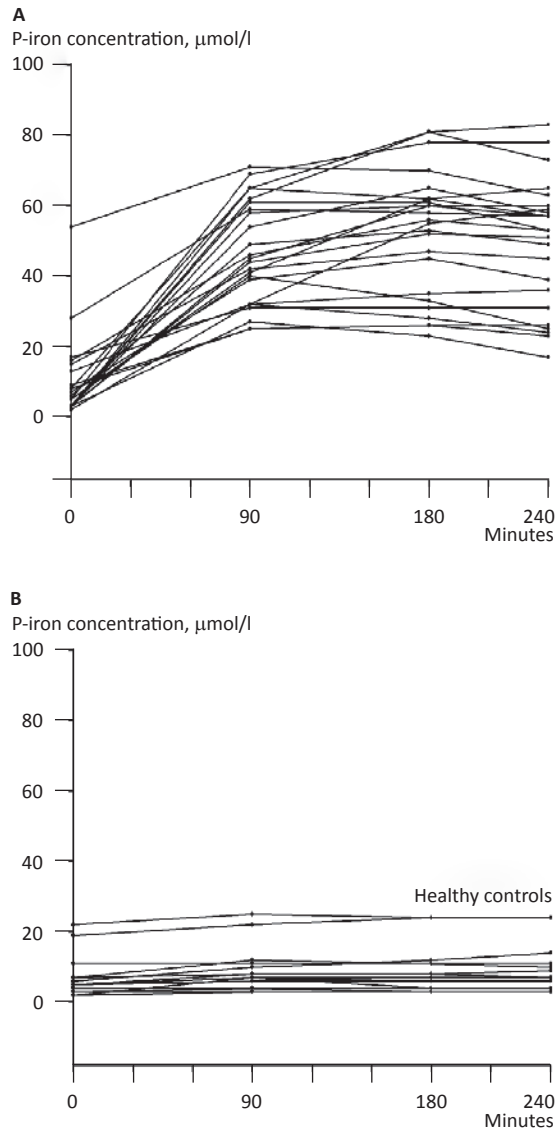
b) Reference range: 7.3-9.5 mmol/l in women, 8.3-10.5 mmol/l in males.

c) Reference range: 15-120 $\mu\text{g/l}$ in women < 50 yrs, 15-290 $\mu\text{g/l}$ in women > 50 yrs, 22-355 $\mu\text{g/l}$ in males.

d) Reference range: 80-100 fl.

FIGURE 2

Results of the oral iron absorption test in 24 patients who had the test performed with iron drops of the "Glycifer" brand (ferrous iron) (A) and in 17 patients and two healthy controls who had the test performed with iron drops of the "Medic" brand (ferric iron) (B). A venous blood sample was drawn immediately before oral intake of iron ($t = 0$) and 90, 180 and 240 min. after oral intake of iron. The two curves with the highest fasting baseline plasma iron concentration ($t = 0$) in B are the results of the two healthy controls.



ferrous and ferric iron formulations, intestinal iron absorption in the fasting state was much higher for ferrous iron [12]. After a meal, the absorption of ferrous iron was not affected, whereas the absorption of ferric iron increased. Thus, the fasting state during the OIAT we present may have further aggravated a poor absorption of ferric iron. In our study, the last blood sample was drawn after 240 min.; however, we cannot exclude that the results would have been different if the observation

TABLE 2

Fasting baseline plasma iron concentration and maximum increase in plasma iron concentration during the oral iron absorption test in patients who had the test performed with ferrous iron drops (Glycifer) or ferric iron drops (Medic).

	Glycifer	Medic	p-value ^a
<i>Patients</i>			
n	24	17	
<i>Fasting baseline plasma iron ($\mu\text{mol/l}$)^b</i>			
Range	2-54	2-15	
Median	5	5	0.4
IQR	3-11	3-6	
<i>C_{max} ($\mu\text{mol/l}$)</i>			
Range	14-78	0-8	
Median	48	2	< 0.001
IQR	31-61	1-3	

C_{max} = maximum increase in plasma iron concentration; IQR = interquartile range (25th-75th percentile).

a) Result of the comparison between groups (ferrous versus ferric iron drops) using the Mann-Whitney U test.

b) Reference range: 9-34 $\mu\text{mol/l}$.

period had been longer. Besides the change to a new type of iron drops, the OIAT test procedure employed in our study was similar as were the biochemical analyses. Plasma iron concentration was measured in the Farsø section of the Department of Clinical Biochemistry before 1 June 2014 and in the Aalborg section after 1 June 2014; but in both places, it was measured on a Cobas system using identical measurement principles and no measurement bias between the two sections was observed.

CONCLUSION

The principal implication of our observations is that an OIAT should not be performed with iron drops containing ferric iron. In Denmark, an 8 mg iron supplement is officially recommended to children aged 6-12 months, unless the child receives 400 ml infant formula, follow-up formula or industrially produced gruel daily [13]. In pregnant women, 40-50 mg of iron supplement is recommended as from gestational week ten [14]. The possible role of ferrous versus ferric iron formulations in treatment and prevention has not been examined in the present study.

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