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Randomized Control Trials

Effect of procyanidin on dietary iron absorption in hereditary hemochromatosis and in dysmetabolic iron overload syndrome: A crossover double-blind randomized controlled trial☆

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SUMMARY

Background & aims: Type I hereditary hemochromatosis (HH) and dysmetabolic iron overload syndrome (DIOS) are the two most prevalent iron overload diseases. Although many food components, particularly polyphenols, reduce iron bioavailability, there is no clinically validated nutritional strategy to reduce food-iron absorption in patients with these diseases. We aimed to determine whether supplementation with 100 mg of procyanidins during a meal reduces dietary iron absorption in patients with HH or DIOS.
Methods: 20 HH and 20 DIOS patients were enrolled in a double-blind three-period crossover randomized study. Basal serum iron level was measured following an overnight fast. Each patient consumed a standardized test iron-rich meal containing 43 mg of iron with two capsules of placebo or procyanidin supplementation. Each period was separated by a 3-day wash-out period. The primary objective was a reduction of dietary iron absorption, assessed by a reduction of serum-iron area under the curve (AUC) corrected for baseline serum iron.
Results: All patients completed the study. The meal and the procyanidin supplements were well tolerated. In both HH and DIOS patients, the iron-rich meal induced a significant increase of serum iron compared with baseline at 120, 180, 240 min, from 8 to 9.1% ($p = 0.002$, 0.001 and 0.003, respectively) in DIOS and from 15.8 to 25.7% ($p < 0.001$) in HH. Iron absorption was 3.5-fold higher in HH than in DIOS ($p < 0.001$). Procyanidin supplementation did not significantly modify iron absorption in DIOS (AUC of added iron 332.87 ± 649.55 vs 312.61 ± 678.61 μmol.h/L, $p = 0.916$) or in HH (1168.62 ± 652.87 vs 1148.54 μmol.h/L ± 1290.05, $p = 0.917$).
Conclusions: An iron-rich test meal led to a marked increase in iron absorption in HH but a mild increase in DIOS. Procyanidin supplementation does not significantly reduce dietary iron absorption in either disease.
Clinical trial registry: clinicaltrials.gov (NCT03453918).

1. Introduction

Iron is involved in multiple functions mediated by iron-containing proteins [1]. Owing to its capacity to exchange electrons, non-transferrin-bound iron induces the Fenton reaction, producing reactive oxygen species involved in cellular and DNA damage [2]. Whole-body iron stores are tightly regulated. Dietary iron absorption in mature enterocytes balances uncontrolled iron

Abbreviations: AUC, Area under the curve; BMI, Body mass index; C_{last}, Last observed value; C_{max}, Maximum observed value; DIOS, Dysmetabolic iron overload syndrome; HH, Hereditary hemochromatosis.

☆ Data described in the manuscript, code book, and analytic code will be made available on request.

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losses (about 1–2 mg/day) [3]. Iron fluxes are regulated by hepcidin, a 25-amino-acid peptide synthesized by hepatocytes in response to high iron stores [4].

The prevalence of type I hereditary hemochromatosis (HH) is estimated to be 0.4% in the European population [5] and 0.44% in non-Hispanic whites in North America [6]. Type I HH is caused by hypohepcidinemia due to p.Cys282Tyr homozygous mutation on the *HFE*-gene, that lead to iron accumulation through an increase of dietary iron absorption [7]. Dysmetabolic iron overload syndrome (DIOS) [8] prevalence is 14.5% in patients with metabolic syndrome [9]. This syndrome combines various components of the metabolic syndrome with a mild increase in body and hepatic stores of iron. The pathophysiology remains unclear: although the hepcidin level is consistent with iron storage leading to decreased iron absorption [10], iron accumulation could occur during the phase of increased iron availability at the onset of weight gain, as supported by animal experimentation [11]. Therapeutic phlebotomy is the main treatment to prevent organ damage due to iron excess in HH [12], whereas in DIOS, phlebotomy is not recommended because it does not improve metabolic and hepatic features [13,14]. Improvement of diet and lifestyle remains the mainstay of therapeutic management of DIOS. Surprisingly, nutritional studies focusing on the modulation of dietary iron absorption in iron overload diseases are scarce. Low dietary iron intake was not associated to lower ferritin levels [15], and chelation therapy using deferoxamine is not recommended in HH.

Polyphenols are well-known iron chelators that are present in a wide variety of meals and beverages. *In-vitro* iron-chelating ability of grape seed proanthocyanidin extract was shown to be equivalent to 30 μM of deferoxamine to chelate iron in a 50 μM solution of ferrous sulfate [16] Several studies investigated the effect of nutritional polyphenols on iron absorption in healthy or iron-deficient humans using radiolabeled iron [17–19] and showed a dose-dependent inhibitory effect on non-heme iron absorption [20]. Tea consumption providing 90 mg of polyphenols was evaluated in HH [21]: radiolabeled test showed reduced iron absorption from 22.1 to 6.9% (70% of reduction) and regular consumption three times a day was shown to be associated with a significantly reduced frequency of phlebotomy. Furthermore, the potential anti-oxidant activity of polyphenols could be of interest in counteracting the pro-oxidant effect of high iron stores [22]. Finally, dietary supplementation with polyphenols to prevent iron absorption could be promising after the removal of iron by phlebotomy. Indeed, bloodletting may induce erythropoiesis, enhancing erythroferrone production, leading to a reduction of hepcidin and in turn enhancing iron absorption [23].

We conducted a crossover double-blind randomized trial versus placebo to investigate the effects of procyanidin supplementation (Oligopin®) on dietary iron absorption in the two most common iron overload diseases: HH and DIOS.

2. Materials and methods

2.1. Trial design

Three-period randomized double-blind crossover study to assess the effect of procyanidin supplementation versus placebo on iron absorption during a test iron-rich meal. In line with French regulations, ethical approval was obtained from the "Comité de Protection des Personnes Sud-Méditerranée I" on 20 February 2018 (reference 18-10). The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice recommendations. Written informed consent was obtained from each participant. The study was registered on clinicaltrials.gov (NCT03453918) and on the European Clinical Trials Database (EudraCT 2017-A01955-48). Patients were screened for participation during their normal medical follow-up in the Internal Medicine Department of Clermont-Ferrand University Hospital, France. The sequence of administration was randomized through a computer generator using block randomization with stratification by disease. Patients, experimenters, care-providers and statisticians were blinded to the sequence of administration.

2.2. Patients

The inclusion criterion for type I HH was the presence of a p.Cys.282Tyr homozygous mutation on the *HFE* gene. The inclusion criteria for DIOS were [1]: high hepatic iron content assessed by magnetic resonance imaging of the liver, at ≥50 μmoL/g dry weight, associated with a high ferritin level of ≥450 μg/L measured at least twice [2]; at least one of the following metabolic features: body mass index (BMI) ≥ 25 kg/m^2, abdominal obesity defined by waist circumference (≥80 cm for women, ≥ 94 cm for men), blood pressure ≥ 130/85 mmHg or anti-hypertensive therapy, high serum triglyceride level (≥1.7 mmoL/L), low level of high-density lipoprotein (≤1.29 mmoL/L for women, ≤1.03 for men), high level of fasting blood glucose (≥5.6 mmoL/L) or type 2 diabetes mellitus. The exclusion criteria were: age <18 years; concomitant or use in the last 2 months of drugs or dietary supplements that could affect iron absorption (e.g. ascorbic acid, iron chelators, iron supplements, herbal tea); intestinal malabsorption of any cause; anemia (hemoglobin ≤9 g/dL); lactating, pregnant or planning pregnancy; alcohol consumption ≥20 g/day for women, ≥30 g/day for men; inability to understand and comply with the instructions. Therapeutic phlebotomies before and during the study were not an exclusion criterion.

2.3. Intervention

The patients attended for three visits, at the same hour each time, after a minimum of 6 h fasting overnight. They were asked not to modify their nutritional habits during the 5 days before each visit. Clinical features were measured by the same physician during each visit: height, weight, BMI, blood pressure, and waist circumference. During the first visit (V0), under fasting conditions, serum iron concentrations were measured six times (0 min = T0, 30 min = T1, 60 min = T2, 120 min = T3, 180 min = T4, 240 min = T5) to record the basal intrinsic variation in fasting serum iron. At 0 min, we also measured ferritin, C-reactive protein, glucose, lactate dehydrogenase, haptoglobin, total bilirubin, alanine transaminase and aspartate transaminase. During the first visit, water intake was limited to 100 mL distributed regularly throughout the duration of the visit. At the following visits (V1 and V2), the patients were randomly assigned to receive polyphenols (Oligopin®) or placebo as capsules during a test meal containing 43 mg of iron (Table 1). Oligopin® is a French Maritime Pine Bark extract rich in procyanidins, available in a readily-administrated form. Procyanidins are catechin and epicatechin oligomers, which are major polyphenols found in black tea infusion, which consumption showed reduced iron absorption in HH [21]. Assumed the high antioxidant properties of procyanidin [24], Oligopin® could also be of interest to prevent oxidative stress due to the high iron-stores.

To ensure adequate iron intake to achieve a significant elevation of serum iron level, we elaborated an iron-rich meal based on the average nutritional composition of the most commonly consumed food in France [25]. The test meal comprised two courses: 50 g poultry liver and 40 g green salad as a starter, 120 g black pudding and 100 g pasta as the main course, and 30 g cheese with 100 g soft bread. Each ingredient was weighed in grams to ensure

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Table 1
Standardized meal.

Meal	Weight (g)	Total iron content (mg)	Non-heme iron (mg)	Heme-iron (mg)
Green salad	40	0.25	0.25	–
Poultry liver	50	5.3	3.18	2.12
Black pudding	120	27.36	16.4	10.96
Pasta	100	0.34	0.34	–
Cheese	30	0.08	0.08	–
Soft bread	100	10	10	–
Total	–	43.3	30.25	13.08

reproducibility; each participant was asked to eat the full meal in no more than 20 min. Immediately after the first course and before the second, two capsules of procyanidin (50 mg/capsule, Oligopin®, Dérivés Résiniques et Terpéniques, DRT laboratory) or placebo were administered with a glass of water (Table 2); polyphenols and placebo capsules were visually identical. Iron capsules content was not measured. Serum samples were collected at the same time: 0 (just before the meal), and 30, 60, 120, 180 and 240 min after the beginning of the meal. During V1 and V2, water intake was limited to 500 mL distributed regularly throughout the visit. The patients were asked to stay in a restful position with minimal physical activity during the entire visit. Between V1 and V2, the wash-out period was 3 days. Clinical tolerance was assessed at the end of each visit.

2.4. Outcome assessment

Blood samples were collected through a catheter inserted in the median cubital vein at the beginning of each visit (before T0). To reduce blood hemolysis, samples were analyzed in the usual laboratory flow. All biochemical parameters were measured using an automatic biochemical analyzer (Dimension-Vista, Siemens®). Hemoglobin was measured by a hematological analyzer (XN 9000, Sysmex®).

2.5. Statistical analysis

The primary outcome was the iron area under the curve (AUC) 4 h after the meal, corrected for the basal value in the fasting condition, and the secondary outcome was the immediate tolerance of procyanidin supplementation. Due to insufficient resource funding, oxylipin analyses were not performed during the first phase of the study; blood samples were frozen and stored to be tested in a second phase as ancillary study.

For a two-sided type I error at 5%, a statistical power >80%, and with an intra-individual correlation coefficient = 0.5 (owing to the crossover design and no carryover effect assumed), 20 patients for each disease (DIOS and HH) were needed to highlight a difference of at least 30% in the primary outcome, for a standard deviation (SD) of 43% and 10% drop out. The assumption of a 30% difference was based to be clinically relevant in iron-overloaded population.

Table 2
Capsule composition of polyphenols and placebo.

Component	Oligopin®	Placebo
Proanthocyanidin	50 mg	–
catechin	4–10%	
taxifoliol	0.5–4%	
taxifoliol glucoside	3–8%	
ferrulate glucoside	4–10%	
gallic acid	0.1–1%	
protocatechic acid	0.5–3%	
caffeic acid	0.5–3%	
p-coumaric acid	0.3–2%	
ferulic acid	1–5%	
Wheat maltodextrin	150 mg	218.9 mg
Magnesium stearate	1.1 mg	1.1 mg
Iron	Not available	Not available

Statistical analysis was performed using SAS® version 9.4 software (Cary, NC, USA); hypothesis testing was two-sided with significance interpreted as $p < 0.05$. The AUC, C_{max} (maximum observed value of serum iron concentration) and C_{last} (last observed value of serum iron concentration) were calculated using Phoenix 64® Winnolin V7 (Certara Princeton NJ, USA). The AUCs were calculated using the linear trapezoidal rule. For continuous parameters, mean ± SD or median and interquartile range were calculated, according to the statistical distribution. The Shapiro–Wilk test was used to investigate the assumption of normality for continuous data.

The primary endpoint was compared between the groups using analysis of variance for correlated data including sequence, visit, treatment and time fixed effects in addition to the patient effect (as a random effect). When appropriate, the pathology was added to the model as a fixed effect. Type III sum of squares and least squares (LS) means were used. We used the Bonferroni correction for multiple comparisons. The normality of residuals was studied using the Shapiro–Wilk test. Owing to negative AUC values no logarithmic transformation was proposed.

First, analysis of the values observed at T0 was carried out (without correction for missing values) to evaluate within- and between-subject variability; following this, V0 data (under fasting conditions) were analyzed to estimate the difference between the pathologies in the fasting state (basal values), followed by the evaluation of the meal and treatment. To identify the effect of procyanidin supplementation, we used a two-step correction of the mean iron concentrations. First, we corrected the value to the endogenous serum iron level collected during visit V0 under fasting conditions to calculate the added iron due to the meal replacing endogenous basal conditions. In the second step, we corrected the T0 values in order to scale all individual profiles to the same starting point, allowing a valid comparison between only the iron-meal induced variations in serum iron. In cases with missing data at T0 (before meal and treatment), the mean of all values observed for the visit was used because no treatment and no meal were given (mean bias). The other missing data were not replaced and were left missing to avoid any bias.

For the non-crossover comparisons, the usual statistical tests were performed for quantitative parameters: an independent Student *t*-test or Mann–Whitney test (when the assumptions of the t-test, normality and homoscedasticity, were not met).

3. Results

Between March and July 2018, 91 patients were screened for participation in Clermont-Ferrand University hospital and 41 were recruited (Fig. 1). The primary reasons for declining to participate

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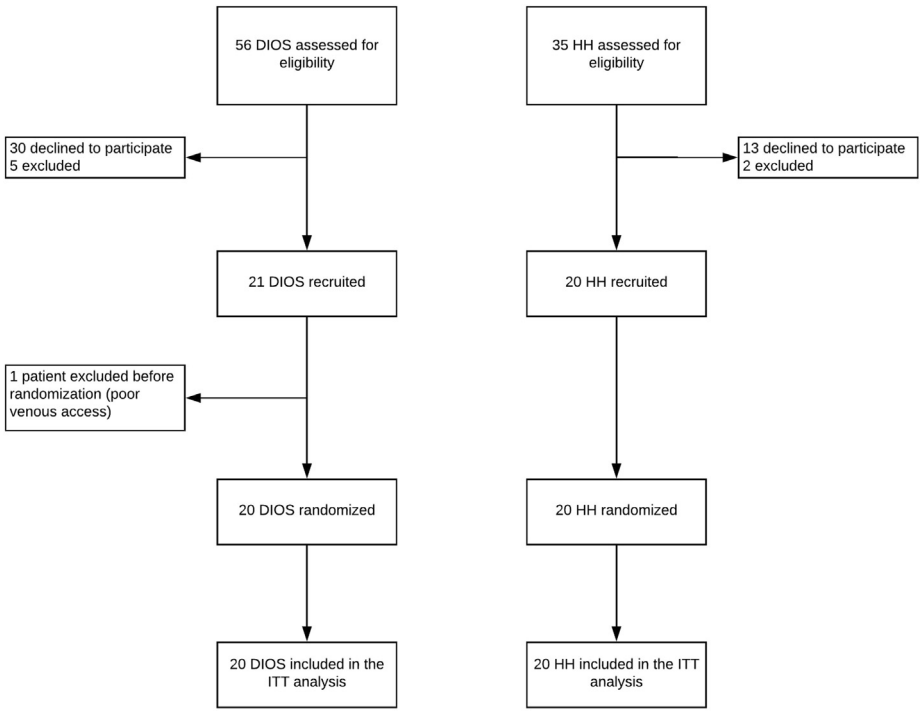

Fig. 1. Flowchart. In the dysmetabolic iron overload syndrome (DIOS) group, five patients were excluded: two because of abdominal surgery that could cause malabsorption, and three because of excessive alcohol consumption. In the type I hereditary hemochromatosis (HH) group, two patients were excluded because they were receiving chemotherapy for hematological malignancies.

were time and distance constraints. One patient in the DIOS group was excluded at the beginning of V0 because of poor venous access. Finally, 20 patients in each group were randomized; no patient was lost to follow-up, allowing 20 patients to remain in each group for the intention-to-treat analysis. As recruitment was completed, we stopped the study in July 2018.

Table 3
Clinical and demographic baseline characteristics of the patients.

	HH n = 20	DIOS n = 20
Age (years)	55.4 ± 15.3	61 ± 7.5
Sex male, n (%)	10 (50)	19 (95)
BMI (kg/m^2)	24.9 ± 3.5	27.8 ± 3.0
Hepatic iron content (μmol/g)	NA	83 [63; 92]
Number of metabolic syndrome criteria, n (%)	NA	
1		1 (5%)
2		2 (10%)
3		4 (20%)
4		8 (40%)
5		5 (25%)
Ferritin (μg/L)	54 [31; 87]	508 [267; 597]
Hemoglobin (g/L)	14.8 ± 1.0	15.6 ± 1.3
CRP (mg/L)	3.3 ± 1.1	3.2 ± 0.9
Venous glycemia (μmol/L)	4.8 ± 0.6	5.5 ± 2.2
Haptoglobin (g/L)	1.2 ± 0.4	1.2 ± 0.5
LDH (IU/L)	224.7 ± 41.1	221.6 ± 36.2
AST (IU/L)	22.4 ± 6.8	25.3 ± 6.5
ALT (IU/L)	29.1 ± 12.9	42.4 ± 15.4
GGT (IU/L)	33.1 ± 21.3	44.2 ± 17.5
ALP (IU/L)	61.2 ± 14.0	68.0 ± 19.1
Total bilirubin (μmol/L)	9.9 ± 5.3	10.5 ± 3.6

Data are presented as mean ± standard deviation; ferritin and LDH are presented as median and inter-quartile range. BMI: body mass index. CRP: C-reactive protein; LDH: lactate dehydrogenase; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transpeptidase; ALP: alkaline phosphatase.

Baseline characteristics are presented in Table 3. In the DIOS group, the mean age was 61 ± 7.5 years, with a male:female ratio of 19:1. In the HH group, the mean age was 55.4 ± 15.3 years, with a sex ratio of 1:1. As expected, ferritin levels at inclusion were significantly higher in the DIOS group compared with the HH group (median [IQR]: 508 μg/L [267; 597] vs. 54 μg/L [31; 87], $p < 0.001$). Only two participants (one in each group) had a mildly increased C-reactive protein level (6.8 mg/L in DIOS and 7.3 mg/L in HH). No patient in the DIOS group was undergoing therapeutic phlebotomy, whereas 18 (80%) of the HH patients were undergoing phlebotomy. The two remaining HH patients were in the grade 2 preclinical phase of the disease: elevation of serum transferrin saturation and ferritin levels with no impairment of quality of life or life-threatening clinical complications [26]. Three patients in the HH group were undergoing phlebotomy induction therapy. The delay between the phlebotomy and each visit for these patients ranged from 1 to 8 days. The 15 remaining HH patients underwent no phlebotomy during the study; the delay between their last phlebotomy and V0 ranged from 6 to 107 days (median 43 days ± 28). In the DIOS group, two patients were regular blood donors but neither made a blood donation during the study.

Two patients in the DIOS group had type 2 diabetes mellitus. One patient in the HH and two in the DIOS group received proton pump inhibitors since several years as usual treatment (4 and 10 years for DIOS patients, 12 years for HH patient). Only one patient underwent modification of their usual treatment: a woman in the HH group received a single dose of 200 mg doxycycline for a tick bite, with no sign of early Lyme's disease, 5 days before the third visit.

Altogether, 11 points of serum iron concentration data were missing (1.5% of the data). Noticeably, four of the missing points were for one DIOS patient, who had poor venous access causing hemolysis that led to uninterpretable serum iron concentrations.

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3.1. Iron value in the fasting condition

We found no significant effect on the variability of iron value in fasting condition at T0 regardless of the randomized sequence of polyphenol or placebo administration in either disease ($p = 0.7463$ in the HH group, $p = 0.169$ in the DIOS group). The mean value of the serum iron concentration in the DIOS group at each timepoint was 19.7–21% lower than in the HH group ($p < 0.001$). Interindividual variation was overall 48% (54% in the HH group and 26% in the DIOS group) and was associated with intraindividual variability of around 23% in both groups.

3.2. Effect of the meal on serum iron concentration

In the DIOS group, after the meal, mean serum iron was significantly increased ($p < 0.005$) compared with the fasting condition at T120 (20.87 ± 4.39 vs 19.2 ± 4.81 μmoL/L, 8.7% increase, $p = 0.002$), T180 (21.84 ± 4.61 vs 20.17 ± 4.79 μmoL/L, 9.1% increase, $p = 0.001$) and T240 (21.91 ± 4.43 vs 20.49 ± 4.67 μmoL/L; 8% increase, $p = 0.003$) (Fig. 2). The increase in the mean serum iron was twofold higher in the HH compared with the DIOS group ($p < 0.001$). In the HH group, after the meal, mean serum iron was significantly increased ($p < 0.001$) compared with the fasting condition at T120 (28.64 ± 8.7 vs 24.73 ± 9.94; 15.8% increase), T180 (31.02 ± 9.09 vs 24.96 ± 10.17 μmoL/L; 24.2% increase) and T240 (31.76 ± 8.06 vs 25.25 ± 10.20 μmoL/L; 25.7% increase). At T0, when no meal or treatment had been given, there was also a significant difference in mean serum iron (24.83 ± 10.23 vs 26.48 ± 10.28 μmoL/L, $p = 0.008$) that led us to proceed to a correction of T0 values as described in "Statistical analysis" paragraph, to assess only the added iron, allowing comparison of the results.

3.3. Effect of polyphenol supplementation

Immediate tolerance of the procyanidins was excellent, and no adverse effect was noted during the entire study. As shown in Fig. 3 (corrected values of iron absorption), in the DIOS group, procyanidin supplementation during the test meal did not significantly modify the mean increase in serum iron when compared with placebo (1.46 vs 1.15 μmoL/L, $p = 0.241$). The same results were found in the HH group (mean serum iron increase 4.12 vs 4.02 μmoL/L, $p = 0.706$). These results were confirmed by the corrected serum-iron AUC analysis that reflected the extent of

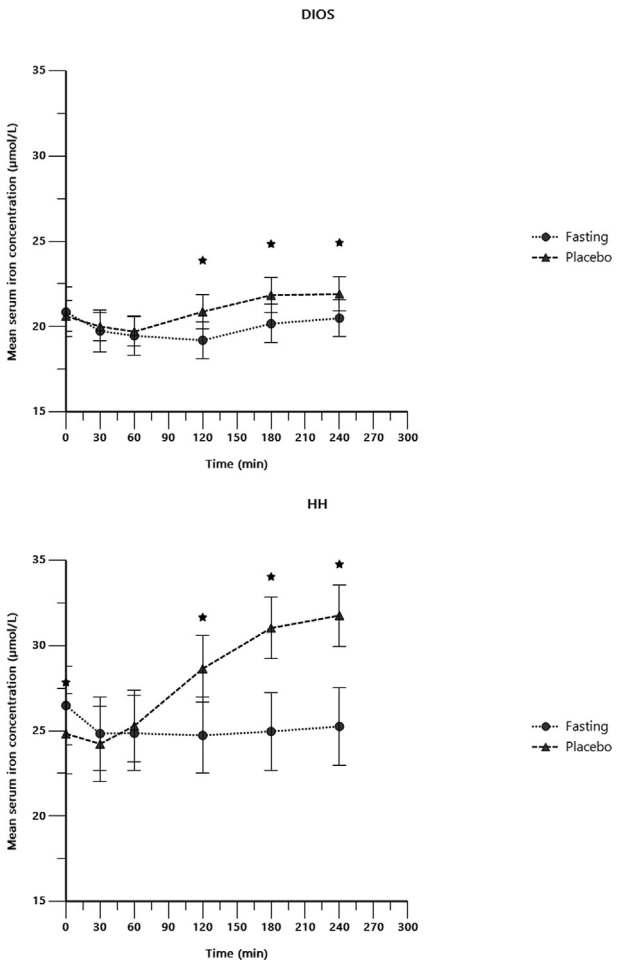

Fig. 2. Variation in mean ± standard deviation (SD) serum iron (μmol/L) in the fasting condition and after the test meal, by time and by disease. ○ and small dotted line show mean ± SD serum iron values in fasting condition. Δ and large dotted line show mean ± SD serum iron values after the iron-rich meal associated with placebo. *shows significant difference between fasting and after-meal mean serum iron concentration at each time. In DIOS, at T120 ($p = 0.002$), T180 ($p = 0.001$) and T240 ($p = 0.003$). In HH, $p < 0.001$ at T120, T180 and T240.

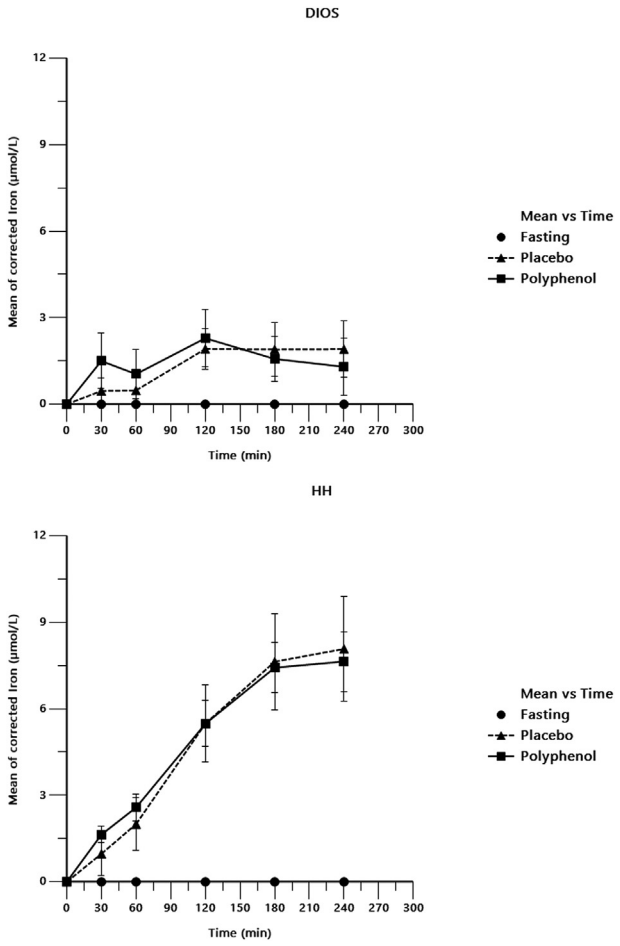

Fig. 3. Variation in corrected mean ± standard deviation (SD) serum iron concentration (μmol/L) after iron-rich meal, by disease and by time. □ and solid line show corrected mean ± SD serum iron values after iron-rich meal associated with polyphenol. Δ and dotted line show corrected mean ± SD serum iron values after iron-rich meal associated with placebo. ○ shows mean corrected serum iron values in the fasting condition (equal to 0 owing to the correction for basal endogenous serum iron). No significant difference was found in mean corrected serum iron when the iron-rich meal was associated with placebo or polyphenol, in DIOS ($p = 0.241$) or in HH ($p = 0.706$).

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added iron. AUC after the test meal was not significantly different with procyanidin supplementation compared with placebo in the DIOS group (332.87 ± 649.55 vs 312.61 ± 678.61 μmol.h/L, p = 0.916) or in the HH group (1168.63 ± 652.87 vs 1148.55 ± 1290.05 μmol.h/L, p = 0.917) (Supplemental Fig. 1). The AUC values were 3.5-fold higher in the HH than DIOS group, with both procyanidin supplementation and placebo ($p < 0.001$). Other pharmacokinetics parameters ($C\text{-}_{max}$ and $C\text{-}_{last}$) were not significantly modified by the procyanidin supplementation in either group (Supplemental Figs. 2 and 3).

4. Discussion

This study was designed to investigate the effect of procyanidin supplementation on iron absorption during an iron-rich meal, in 20 patients with DIOS and 20 with HH. To the best of our knowledge, this is the first study to investigate the effect of supplemental polyphenol on iron absorption in iron-overload diseases. Moreover, this is also believed to be the first study to investigate iron absorption by measurement of serum iron kinetics after iron loading by an iron-rich meal. We showed: (i) a significant increase in serum iron after the high-iron meal compared with the fasting condition; and (ii) higher dietary absorption of iron in HH than in DIOS. In answer to the primary objective of the study, dietary polyphenol (procyanidin) supplementation during a meal does not modify dietary-iron absorption in HH or in DIOS.

Iron absorption has been investigated using iron radioisotopes [27] or a stable iron isotope [28], but these methods are not available in routine clinical practice. Several alternative methods have been developed using serum iron increase after oral iron loading [29–32], with a good correlation between absorption of radiolabeled iron and serum iron increase. In those studies, a significant increase in serum iron and transferrin saturation was observed 180 min after oral administration of 50–200 mg of diverse iron pharmaceutical salts. In our study, we decided to use a standardized iron-rich test meal to get as close as possible to normal nutritional conditions while providing a significant increase in serum iron. Similar to the recommended methodology to assess the bioequivalence of endogenous substances [33], we used a crossover study versus placebo, with measurement of basal iron serum level in the same patients as part of the main study, to remove the effect of endogenous iron as well as any chronobiological effect, in order to assess only iron absorption linked to the test meal. In both diseases, the iron-rich meal showed good efficacy in increasing serum iron, reflecting iron absorption. Another limitation of our results is the wide range of time between each visit and phlebotomy in HH subjects undergoing maintenance therapy, that could have influenced iron absorption. Variation of hepcidin after blood donation has been barely reported [34]: hepcidin level decreases while erythroferrone decreases significantly from 2.5 to 14 days after blood donation; baseline levels are recovered 16 weeks later.

Although our study was not designed to compare iron absorption between the two diseases, iron absorption was significantly lower in DIOS than in HH. To the best of our knowledge, this is the first study assessing dietary iron absorption in both diseases with dietary iron tolerance test. This result is in line with those obtained by a stable isotope method [10] and an oral iron tolerance test using 105 mg ferrous sulfate [35] and consistent with the mild increase of iron stores in DIOS compared to the marked increase in HH. As such, the underlying mechanisms resulting in iron overload in DIOS remain challenging. As hepcidin is synthetized in the liver, we can hypothesize that transient impaired hepatic function could induce decreased hepcidin levels leading to transient enhanced iron absorption. Another hypothesis is a transient decreased hepcidin production due to enhanced erythroferrone during the phase of weight gain to increase erythropoiesis. In our study, procyanidin supplementation did not reduce iron absorption in DIOS or HH. However, several limitations of our study can be raised. First, the duration of the kinetics study (4 h) could have been too short to show a difference in AUC; however, most of the oral iron tolerance tests established previously were performed for up to 4 h. In addition, the availability of polyphenol to chelate iron could have been counteracted by the proportion of heme-iron in our meal. Indeed, formation of a polyphenol–iron complex in the intestinal tract is believed to limit iron absorption, which may be reduced with heme-iron. Moreover, the dosage of 100 mg procyanidin could have been too low to make an impact on the amount of iron absorbed. Indeed, Wu and colleagues [16] showed *in vitro* that procyanidin at 300 μg/mL chelate 30% of a 50 μM solution of ferrous sulfate. Therefore, 100 mg procyanidin could have bind about 1% of the iron content of the meal. However, *in vitro* experimental conditions are quite different from normal digestion and as we do not have *in vivo* polyphenol-to-iron molar ratio, we are unable to establish a relevant comparison. These parameters could explain the contradictory results of human studies. Furthermore, iron content of the capsules was not measured, however iron content of a very similar product (Pycnogenol®, extract from the bark of *Pinus maritima*) is very low (65 μg/g of dry product) [36]. Finally, the enhanced iron absorption in patients with HH could have exceeded the inhibitory iron absorption of the polyphenols.

Delimont and colleagues reviewed several studies of the impact of tannin consumption on iron bioavailability [37]: they emphasized the use of tannic acid in most of the studies that showed a reduction of iron absorption but these compounds are virtually absent in our diet. Furthermore most of the studies using condensed tannins (flavonoid oligomers, of which procyanidin are a subgroup) did not support their ability to reduce iron absorption [38–40]. Although our work was designed to assess only the acute effect of procyanidin supplementation on dietary iron absorption, our results does not support the relevance of a long-term supplementation study.

Low-grade inflammation is usually associated with insulin-resistance in patients with DIOS [41], and iron overload is associated with oxidative stress and DNA damage [42]. In line with these data, another potential use of polyphenols in DIOS or HH could be to reduce or modulate the oxidative stress and inflammation caused by iron overload. Our previous studies have shown an antioxidant action of the tested procyanidins on LDL oxidation *in vitro* [43] and in non-human animals *in vivo* [44]. Although C-reactive protein did not differ between the groups in the current study, it would be interesting to assess the inflammatory and oxidative status of DIOS and HH patients using lipidomic profiling of oxylipins, which would allow subtle and integrative characterization of inflammation and oxidative stress [45].

In summary, acute nutritional supplementation with procyanidins in patients with DIOS or HH does not significantly reduce dietary iron absorption during an iron-rich meal, in a double-blind randomized controlled study. This standardized-meal method could be used to assess the efficacy of other nutritional and pharmacological iron-absorption modulators.

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Statement of authorship

The authors' responsibilities in this work are as follows: MR and HL proposed the work; MR, HL, BP, JMC, AM and CG designed the

protocol trial; CD and HL contributed to data collection; HL and JMC designed the figures; HL wrote the manuscript. All authors contributed to data interpretation, reviewed and approved the final manuscript.

Conflict of interest

The research was supported by Clermont-Ferrand University Hospital. DRT society provided Oligopin® and placebo capsules free of charge and made a grant to support the research.

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2019.02.012.

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