Iron absorption in adults with sickle cell anemia: a stable-isotope approach

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Abstract

Purpose Iron absorption in sickle cell anemia (SCA) remains unclear and studies in adults with SCA are scarce. The aim of this study was to evaluate the iron absorption SCA adults and its association with iron status and hepcidin concentration.

Methods SCA patients ($n = 13$; SCA_{total}) and control participants ($n = 10$) ingested an oral stable iron isotope (^{57}Fe). Iron absorption was measured by inductively coupled plasma mass spectrometry (ICP-MS) 14 days after isotope administration. Patients with ≥ 1000 ng/mL serum ferritin were considered to present iron overload (IO) (SCAio+; $n = 3$) and others classified without IO (SCAio-; $n = 10$).

Results Iron absorption in the control group ranged from 0.3 to 26.5% (median = 0.9%), while it varied from 0.3 to 5.4% in SCAio+ (median = 0.5%) and from 0.3 to 64.2% in the SCAio- (median = 6.9%). Hepcidin median values were 14.1 ng/mL (3.0–31.9 ng/mL) in SCAio-, 6.2 ng/mL (3.3–7.8 ng/mL) in SCAio + and 6.2 ng/mL (0.6–9.3 ng/mL) in control. Iron absorption was associated with ferritin level ($r = -0.641$; $p = 0.018$) and liver iron concentration (LIC; $r = -0.786$; $p = 0.036$) in the SCA_{total} group.

Conclusion Our data suggest that SCAio- individuals may be at risk of developing primary IO. Simultaneously, secondary IO may induce physiological adaptation, resulting in reduced iron absorption. Further studies evaluating intestinal iron absorption using larger sample sizes should be conducted to help establish a safe nutrition approach to be adopted and to ensure the security of food-fortifying public policies for these patients.

Trial registration This trial was registered at www.ensaiosclinicos.gov.br (Identifier RBR-4b7v8pt).

Keywords Iron overload · Liver iron concentration · Hepcidin · Ferritin · Bioavailability

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Introduction

Sickle cell disease (SCD) is an inherited hemoglobinopathy caused by a genetic mutation that leads to the substitution of glutamic acid with valine in the beta-globin chain of hemoglobin. This mutation results in the production of hemoglobin S (Hb S) [1]. The term SCD refers to all the different Hb S genotypes, whereas sickle cell anemia (SCA) refers specifically to the homozygosity of the βS gene [2]. Approximately 275,000 children are born annually with SCD worldwide [3], and 30,000 individuals are estimated to have hemoglobinopathy in Brazil [4].

Blood transfusion is administered to treat acute conditions in SCA [5, 6] or prevent complications of the disease [6–8]. Despite their benefits, regular blood transfusions can lead to secondary iron overload (IO) [9, 10]. Chronically transfused patients have an excess of 0.3 to 0.5 mg/kg/day of iron [11, 12] and can store approximately 5 to 10 g of iron per year [13]. Iron becomes potentially toxic with the progression of IO, causing oxidative stress and cellular damage from free radical release [14].

Iron regulation occurs by a fine mechanism, which promotes the balance between its absorption and storage. Hepcidin – a hormone produced and secreted mainly by the liver – exerts its function by binding to ferroportin, a transmembrane cellular iron exporter expressed in various cell types [15]. The hepcidin-ferroportin complex controls the main inflows of iron into plasma: from duodenal enterocytes absorbing dietary iron, from macrophages of the reticuloendothelial system involved in the recycling of iron from senescent red blood cells (RBC), and from hepatocytes involved in iron storage. When hepcidin concentrations are high, the hepcidin-ferroportin complex is internalized and ferroportin undergoes degradation, resulting in a reduction in the absorption of intestinal iron and its bioavailability [16]. Therefore, hepcidin is induced to decrease the levels of iron circulating in blood plasma [17]. Other proteins, such as ferritin and transferrin, play essential roles in iron metabolism. Ferritin is responsible for iron storage, while transferrin facilitates iron transport. Therefore, the total iron-binding capacity (TIBC) indicates the overall amount of iron that can be bound by transferrin [18].

In SCA, the intense erythropoiesis and anemia can reduce hepcidin concentration [19]. On the other hand, the inflammatory characteristic of this disease and the iron overload due to blood transfusions can induce the transcription of hepcidin [20]. These disorders on iron metabolism can impact molecular and biochemical parameters, altering not only the expression of hepcidin, but also affecting the other iron biomarkers levels [21].

In recent studies [22, 23] that evaluated the concentration of hepcidin in SCA, the authors found that the levels of this hormone differed according to the presence or absence of IO. Hepcidin acts by reducing iron bioavailability [15]. Patients with SCA without IO had lower hepcidin concentrations compared with IO and control groups [22]. Conversely, patients with secondary IO and SCA had higher concentrations of hepcidin compared with other groups [22, 23]. These data support the hypothesis that patients with SCA may have higher iron absorption compared to individuals without the disease unless they present IO. The estimation of iron absorption is necessary to establish an adequate dietary guideline of iron intake for this population.

To our knowledge, this is the first study to evaluate iron absorption, using a stable isotope approach, in adults with SCA. In addition, it aimed to explore associations between iron absorption and markers of iron status and hepcidin concentration.

Methods

Participants

This is a pilot study and the sample size of 22 subjects (11 for each group) was calculated to test whether the mean iron absorption of the SCA group was greater than that of the control group by 22% (with an additional 10% for potential losses and refusals, making the total 26). The calculation considered a power of 80%, a significance level of 5%, and a standard deviation of 20, based on previous iron absorption data in SCA [24]. This calculation was performed using the PSS Health online version tool [25]. Male and female patients aged 19–50 years old, with SCA (Hb SS genotype), were recruited between October 2019 and March 2020 from the State Institute of Hematology Arthur Siqueira de Cavalcanti (Hemorio, Rio de Janeiro, Brazil). Individuals who met the inclusion criteria and agreed to participate in the study were included. The control group was composed of healthy volunteers without SCA with matched sex, age, and race/ethnicity. Patients with SCA who had been hospitalized for 30 days and/or had received blood transfusions 15 days before the first participation in this study were not included. Patients with SCA who had received blood transfusions during the iron absorption period were excluded. In the control group, participants with autoimmune diseases or who used medications for treatment of diabetes were not included. Women who were pregnant or experiencing menopause and/or participants who underwent bariatric surgery and/or used erythropoietin were not included. Participants were divided into two groups: the control group, consisting of individuals without SCA; and the sickle cell anemia group (SCA$_{total}$). We additionally compared the results from two subgroups according to the presence (SCAio+) or absence

(SCAio-) of IO. IO was diagnosed by using the cut-off point (serum ferritin ≥ 1,000 ng/mL) for SCD [26]. The study was conducted in accordance with the Declaration of Helsinki principles. The Ethical Committee of Hemorio (419/17; 2.788.659) and Pedro Ernesto University Hospital (2.695.418) approved the study protocol. Written informed consent was obtained from each participant.

Isotope enriched ferrous sulfate preparation

There are four naturally occurring iron stable isotopes at abundances of 5.8% (^{54}Fe), 91.8% (^{56}Fe), 2.1% (^{57}Fe) and 0.3% (^{58}Fe) [27]. The lowest abundant isotopes – ^{58}Fe and ^{57}Fe – are commonly used in nutritional research being usually provided as iron metal and converted to ferrous sulfate before administration [28]. Therefore, it was decided to use the iron isotope ^{57}Fe, as it is more financially viable compared to ^{58}Fe.

Elemental ^{57}Fe was purchased from Cambridge Isotope Laboratories (Massachusetts, USA). At Laboratory of Analytical Development (LaDA) (Institute of Chemistry, Federal University of Rio de Janeiro, Brazil), 0.50079 g of ^{57}Fe and 490 µL of sulfuric acid was mixed and this mixture was left in an inert atmosphere with gentle heating until the complete dissolution of metal. After complete dissolution, 50 µL of nitric acid was added to stabilize the solution and brought to a volume of 100 mL with water. The solution was parted into 1 mL aliquots and kept in an inert atmosphere until use with the participants.

Study procedure and isotope dosing

To define the dose of ^{57}Fe to be administered, a literature review was carried out in studies with healthy adults using this stable isotope. It was observed that in different publications, ^{57}Fe dosages varied between 5 mg and 7.5 mg [29–31]. In order to provide the smallest possible amount of stable iron isotope to participants with SCA, as there are no studies carried out with this approach in this hemoglobinopathy, we opted for the lowest dose (5 mg).

Participants were evaluated at day 1, with blood collection for baseline analyses and oral administration of ^{57}Fe, and at day 14, with blood collection for measurement of the incorporation of ^{57}Fe into erythrocytes [32–34]. Volunteers were instructed to fast for a period of 10 h. After blood collection at baseline, 5 mg of ^{57}Fe, in the form of ferrous sulfate, was diluted in 100 mL of unsweetened and chemical food preservative-free orange juice (Do Bem®). The labeled juice was ingested under close supervision. Subsequently, each participant received a 40 g packet of starchy snack (cassava starch, eggs, vegetable oil and salt) without chemical food preservatives (Líder de Minas®). No other foods or liquids, except water, were allowed to be ingested for a period of at least 2 h following isotope administration.

Blood collection and isotope administration were performed at Hemorio (patients with SCA); and at Laboratory of Pathophysiology and Nutritional Biochemistry (Nutrition Institute, Rio de Janeiro State University, Brazil) (control group).

Iron isotope analysis in blood

First, 1 mL of blood collected in a heparin tube was transferred to Falcon tubes and centrifuged at 25°C for 10 min at $700 \times g$. Subsequently, the plasma and the white cells in the sample were removed and 2 mL of double sub-boiling distilled nitric acid (65% m/m) was added to the packed red blood cells to preserve the samples. At LaDA, packed red blood cells were transferred to microwave-sealed vessels and 1 mL of hydrogen peroxide (30%) and 1 mL of double sub-boiling distilled nitric acid were added to the RBC concentrate. The resulting mixture was placed in a microwave oven at 200 ºC for 10 min, and then cooled at 25°C. After this procedure, ultrapure water was added to this mixture up to 5 mL. Finally, the resulting extract was analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Fisher Scientific iCAP Qc, Bremen, Germany) and the ^{57}Fe/^{56}Fe ratio was determined.

Calculation of ^{57}Fe incorporation into red blood cells and estimation of iron absorption

Fractional iron absorption was estimated based on the measured erythrocyte incorporation of the isotopic oral label (^{57}Fe) and the estimated total amount of circulating iron in blood, was expressed as a percentage of the administered oral ^{57}Fe dose [32].

The quantification of administered ^{57}Fe incorporated into total red blood cells (^{57}Fe$_{inc}$) 14 days after isotope administration was calculated as follows [32]:

$$^{57}Fe_{inc} = \frac{R^t - R^0}{R^0} \times \frac{\text{circulating Fe (mg)}}{W_{\text{natural Fe}}} \times \frac{NA^{57}Fe}{100} \times m^{57}Fe$$

$R^0 W_{\text{natural Fe}}$ 100.
where ^{57}Fe$_{inc}$ is expressed in mg, R^t is the ^{57}Fe/^{56}Fe ratio at a time after dosing (14 days), R^0 is the ^{57}Fe/^{56}Fe ratio at baseline, circulating Fe is the total circulating iron (mg), $W_{\text{natural Fe}}$ is the average atomic weight of Fe (55.845 mg/mmol), NA^{57}Fe is the natural abundance of ^{57}Fe (2.1191%), and m^{57}Fe is the atomic mass of ^{57}Fe (56.935 mg/mmol).

The total circulating Fe (mg) was estimated as follows:

$$\text{Circulating Fe} = Hb \times BV \times 3.47 \times 0.001$$

where Hb is blood hemoglobin concentration in g/L, BV is blood volume in liters (assumed to be 65 mL/kg body weight for adults) and 3.47 is the concentration of iron in hemoglobin (mg/g) [32].

Finally, percent iron absorption was calculated as follows:

$$\text{Iron absorption } (\%) = \frac{^{57}\text{Fe}_{inc}}{^{57}\text{Fe}_{dose}} \times \frac{100}{80} \times 100$$

where $^{57}\text{Fe}_{inc}$ assumed incorporation of the absorbed isotope into red blood cells is 80% [32]. The limit of detection for iron absorption was 0.5%, and values < 0.5% were classified as undetectable. For statistical purposes, a value of 0.3% was assigned for values < 0.5%.

Hematological and biochemical analysis

Blood samples (days 1 and 14) were collected in EDTA-containing, heparin-containing, and clot activator gel tubes. Except for the samples collected in EDTA tubes, the others were centrifuged at $700 \times g$ for 10 min at 25ºC. Serum samples collected at day 1 were aliquoted into microtubes and stored at −80ºC until analyses were performed. A complete blood count was performed using an automated counter Sysmex XP-300 (Sysmex, Kobe, Japan). Serum iron was analyzed using the modified Goodwin test (Bioclin, Belo Horizonte, Brazil) and total iron-binding capacity (TIBC) analysis was performed using a colorimetric method (Bioclin, Belo Horizonte, Brazil). Transferrin was measured using an immunoturbidimetry assay (Bioclin, Belo Horizonte, Brazil). The transferrin saturation index (TS) was calculated as follows: (serum iron/TIBC) × 100. Serum ferritin, bioactive hepcidin-25, and C-Reactive Protein (CRP) analyses were performed using a commercially available ELISA kit (Elabscience, Wuhan, China). In addition, Interleukin-6 (IL-6) (Merck Millipore, Darmstadt, Germany) was analyzed using ELISA.

Assessment of liver iron concentration (LIC)

Magnetic resonance imaging (MRI) procedures were conducted at the Radiology Department of Pedro Ernesto University Hospital. To evaluate liver IO, upper abdomen MRI was performed on a 1.5 Tesla Optima MR 360 (GE Healthcare, Chicago, USA), using an MRI T2* established method [35–37], without administration of contrast or sedation. LIC was quantified, as described, in micromoles of iron per gram of dry weight (μmol/g) of the liver with normal values being under 40 μmol/g [38].

Assessment of iron intake

For iron intake assessment, two 24-hour dietary recalls of each participant were evaluated, in which all foods and beverages consumed in the 24-hour period prior to participation in the study were reported. The software Avanutri (available at: https://www.avanutri.com.br/software_avanutri_online/) was used to estimate the daily iron intake. The median iron intake of the two 24-hour recalls was calculated, and it was analyzed whether the intake of this micronutrient reached the recommended for sex and age according to the Dietary Reference Intake recommendations [39].

Statistical analysis

The distribution of variables was analyzed for normality using the Shapiro–Wilk test. Measures of central tendency and dispersion were expressed as median and minimum and maximum values for continuous variables. For categorical variables, data were expressed as frequencies (n) and percentages (%). A Mann–Whitney U test was performed to assess the differences in continuous variables between SCA_{total} and control groups. A Kruskal–Wallis test was performed on the three study groups (SCAio+, SCAio-, and control groups). Categorical variables were compared using the chi-square test (χ^2) or Fisher's exact test. Spearman´s correlation coefficient was used to measure the association between continuous variables. P values < 0.05 were considered significant. The statistical analysis was performed using the Statistical Package for Social Science software, version 22.0 (IBM SPSS, New York, USA) and graphics were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, USA).

Results

A total of 34 individuals were recruited in this study (Figs. 1), 11 for the control group, and 23 for the SCA_{total} group. After blood collection and isotope administration, one participant in the control group was excluded owing to iron deficiency anemia. In the SCA_{total} group, eight participants did not return for the next steps and two participants were excluded for undergoing blood transfusion. Thus, the final study population consisted of 23 participants, of whom 10 were included as a control group (60.0% females) while 13 were included in the SCA_{total} group (53.8% females). The percentage of women, self-reported race, and median age of the participants did not differ between the groups, indicating homogeneity (Table 1). Among participants with SCA, six (46.2%) were using hydroxyurea.

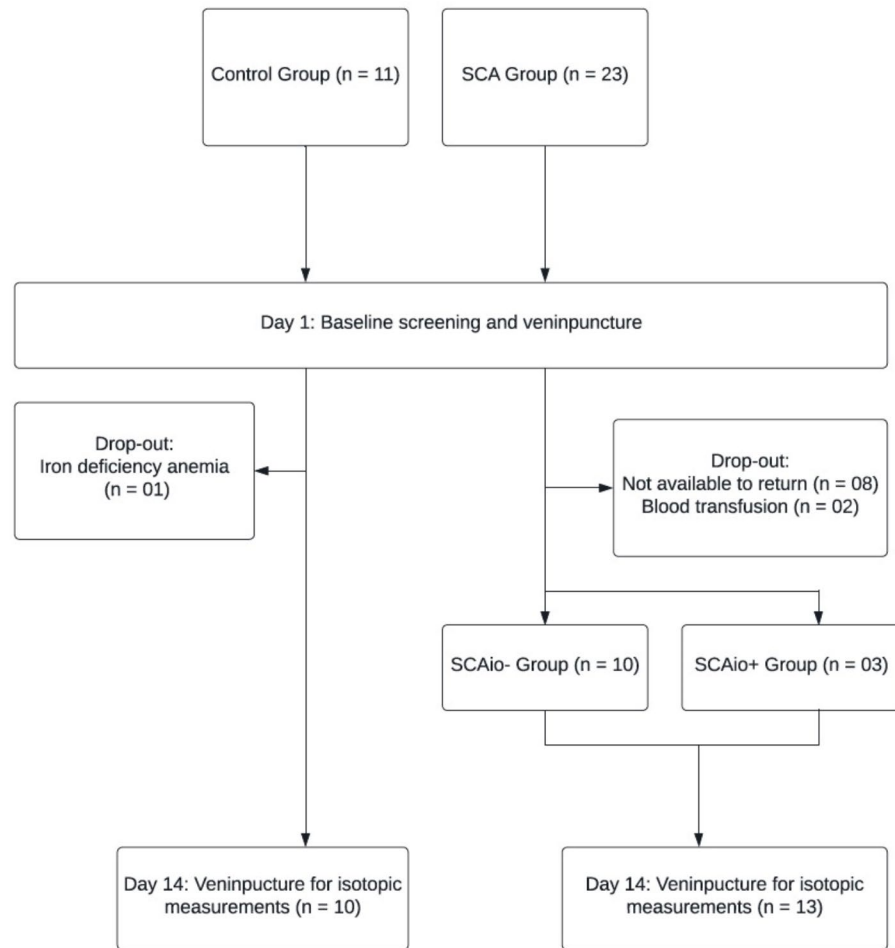**Fig. 1** Flowchart of participants' enrollment

Table 1 Frequency of general characteristics of the control group and patients with SCA

Variables	Control (n = 10)	SCA$_{total}$ (n = 13)	p value (*)
Age, years, median (range)	28.5 (23.0–46.0)	31.0 (22.0–40.0)	1.000
Sex, No (%)			
Male	4 (40.0)	6 (46.2)	0.768
Female	6 (60.0)	7 (53.8)	
Race/Ethnicity, No (%)			
White	0 (0.0)	0 (0.0)	
Black	10 (100.0)	13 (100)	1.000
Other	0 (0.0)	0 (0.0)	

Differences between two groups were determined using the Mann–Whitney U Test (for continuous variables) and the chi–squared test or Fisher's exact test (for categorical variables). The asterisk indicates statistical difference ($p < 0.05$) between groups.

Abbreviations: *SCA:* Sickle Cell Anemia;*N*: Number of participants

Serum ferritin and IL-6 concentrations were higher in SCA$_{total}$ group than in control participants ($p = 0.008$ and $p = 0.042$, respectively – data not shown). Considering the participants with SCA divided into SCAio- and the SCAio + groups, as expected, both groups had lower red blood cell count and hematocrit and higher leukocytes when compared with the control group. Hepcidin did not present statistical differences among the groups, while TIBC and transferrin levels were lower in SCAio + than in the control group. Regarding inflammation markers, no significant differences were found in CRP and IL-6 levels among the three groups (Table 2).

Iron absorption was not different between control, SCAio- and SCAio + groups (Fig. 2). The SCAio + group had low iron absorption values (median: 0.5%), close to median values found in the control group (median: 0.9%). On the other hand, the SCAio- group presented an iron absorption median of 6.9%, but with a large scatter around this value.

The hepcidin: ferritin ratio of the SCAio + group is also shown in Fig. 2. The hepcidin: ferritin ratio was lower in the SCAio + group compared to SCAio- and presented a trend if compared to the control group.

Table 3 shows the iron absorption, LIC, and ferritin values for all participants. Of the 23 participants, only 7 performed MRI to assess the LIC, 5 SCAio-, and 2

Table 2 Comparison of hematological and biochemical measurements among control, SCAio- and SCAio + groups

Parameters	Control (n = 10)	SCAio- (n = 10)	SCAio+ (n = 03)	p value (*)
RBC, x 10^6 μ/L	4.7 ˣ (4.0–5.5)	2.9 ʸ (1.7–4.3)	2.4 ʸ (2.3–3.6)	**0.001 ***
Hemoglobin, g/dl	12.5 ˣ (11.6–15.0)	8.1 ʸ (6.2–9.7)	7.6 ˣʸ (6.3–12.2)	**0.001 ***
Hematocrit, %	39.0 ˣ (36.4–45.8)	23.7 ʸ (17.9–30.9)	22.9 ʸ (20.9–35.8)	**< 0.0001 ***
RDW, %	13.3 ˣ (12.3–16.4)	19.8 ʸ (14.5–24.7)	17.5 ˣʸ (17.5–19.9)	**0.001 ***
Leukocytes, x 10^3 μ/L	5.7 ˣ (3.5–10.2)	9.7 ʸ (7.8–16.9)	12.7 ʸ (10.7–17.1)	**0.001 ***
Ferritin, ng/mL	93.3 ˣ (14.9–262.3)	122.8 ˣʸ (75.7–467.0)	1339.0 ʸ (1242.9–2908.7)	**0.005***
Hepcidin, ng/mL	6.2 (0.6–9.3)	14.1 (3.0–31.9)	6.2 (3.3–7.8)	0.119
Serum iron, μg/dL	99.0 (53.0–138.0)	86.0 (52.0–162.0)	84.0 (75.0–100.0)	0.865
TIBC, μg/dL	372.9 ˣ (341.4–575.7)	347.1 ˣʸ (198.6–448.6)	225.7 ʸ (211.4–278.6)	**0.014***
Transferrin, mg/dL	261.0 ˣ (239.0–403.0)	243.0 ˣʸ (139.0–314.0)	158.0 ʸ (148.0–195.0)	**0.015***
TS,%	23.7 (15.0–32.7)	29.2 (15.2–77.1)	35.9 (33.2–39.7)	0.087
IL-6, pg/mL	0.1 (0–1.0)	2.5 (0–24.5)	4.0 (0.3–10.8)	0.113
CRP, ng/mL	0.5 (0.1–2.1)	0.9 (0.2–1.6)	1.8 (1.1–1.9)	0.219

Values are expressed as median (range). Differences between groups were performed Kruskal−Wallis. Post hoc analysis was performed using Dunn's Test for multiple pairwise comparisons ($p < 0.05$). Different superscript letters indicate statistical difference between groups.

Abbreviations: *SCA*io−: sickle cell anemia without iron overload; SCAio+: sickle cell anemia with iron overload; RBC: Red Blood Cells; RDW: Red Cell Distribution Width; TIBC: Total Iron−Binding Capacity; TS: Transferrin Saturation; IL−6: Interleukin−6.; CRP: C−Reactive Protein
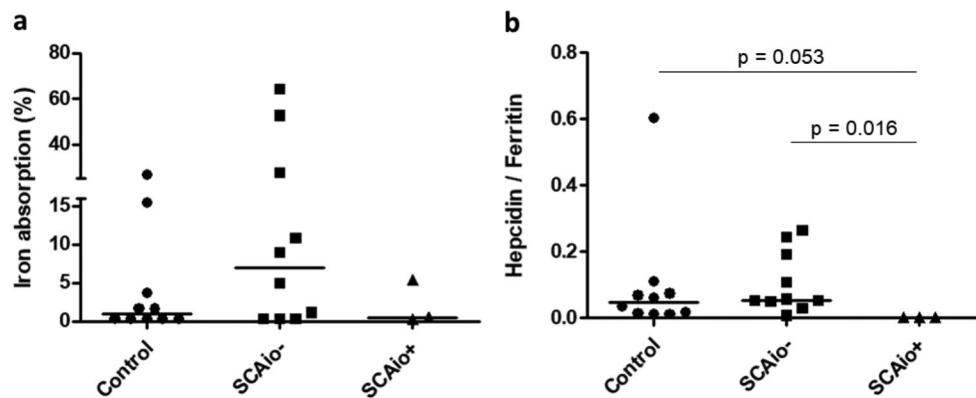

Fig. 2 Comparison of iron absorption (**a**) and hepcidin: ferritin ratio (**b**) among controls, SCAio− and SCAio+ groups. Differences among the three groups were tested using Kruskal−Wallis. Post hoc analysis was performed using Dunn's Test for multiple pairwise comparisons. The asterisk indicates statistical significance (significant when $p < 0.05$). Iron absorption was not different among control, SCAio− and SCAio+ ($p = 0.372$). Abbreviations: SCAio−: sickle cell anemia without iron overload; SCAio+: sickle cell anemia with iron overload

SCAio + individuals. The two participants with IO had LIC above the reference range, with the diagnosis of liver IO (mild grade). The SCAio- participants presented LIC below the cut-off point.

Figure 3 shows the results of Spearman's correlation analysis between serum ferritin, LIC, hepcidin, and iron absorption in the control individuals and patients with SCA. Iron absorption was inversely associated with serum ferritin level and LIC in the SCA$_{total}$ group. However, there was no significant correlation between ferritin level and iron absorption when adjusted for inflammation markers (IL-6: $r = -0.509$; $p = 0.198$; PCR: $r = -0.378$; $p = 0.225$; data not shown). Serum hepcidin level was not significantly correlated with iron absorption.

There were strong positive correlations between LIC and serum ferritin level in SCA$_{total}$ and in SCAio- patients. No

significant correlations were found between serum hepcidin level and LIC. The lowest detectable absorption rate was 0.5% in a participant of the SCAio + group, whose serum ferritin level was 1339.0 ng/mL, while the highest iron absorption was found in the SCAio- group.

Discussion

The heightened production of RBC and the presence of hypoxia, both inherent in SCA, could potentially reduce hepcidin synthesis and contribute to elevated iron absorption [16, 19]. Conversely, the inflammatory nature of the disease, along with secondary iron overload, may hinder intestinal iron absorption [20]. These factors often coexist in individuals with SCA, underscoring the importance of

Table 3 Iron absorption, liver iron concentration (LIC), serum ferritin and hepcidin concentrations of control, SCAio- and SCAio + groups
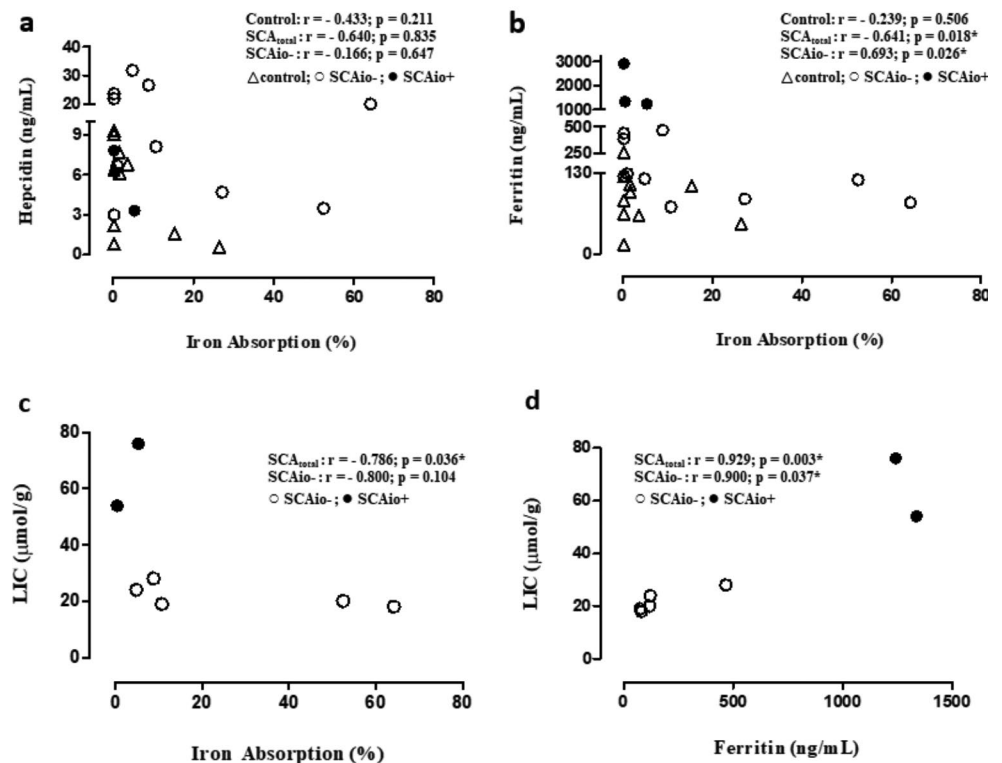
Participants	Group	Iron Absorption (%)	LIC (μmol/g)	Ferritin (ng/mL)
1	Control	0.3	NM	86.1
2		3.7	NM	62.0
3		0.3	NM	64.0
4		26.5	NM	48.7
5		1.7	NM	100.4
6		0.3	NM	124.2
7		0.3	NM	14.9
8		15.4	NM	108.5
9		1.6	NM	112.0
10		0.3	NM	262.3
11	SCAio-	0.3	NM	386.5
12		0.3	NM	436.4
13		52.5	20.0	119.1
14		64.2	18.0	82.5
15		4.9	24.0	121.0
16		0.3	NM	124.7
17		8.9	28.0	467.0
18		10.8	19.0	75.7
19		27.3	NM	88.3
20		1.1	NM	128.8
21	SCAio+	0.5	54.0	1339.0
22		5.4	76.0	1242.9
23		0.3	NM	2908.7

Abbreviations *SCA*io−: patients with sickle cell anemia without iron overload; SCAio+: patients with sickle cell anemia and iron overload; LIC: Liver iron concentration. NM: Not measured. LIC cut−off point for normal values: < 40 μmol/g

Fig. 3 Spearman correlations of iron absorption with hepcidin (*a*), ferritin (*b*) and LIC (*c*) and ferritin with LIC (*d*). All correlations were performed using Spearman´s correlation coefficient (significant when *p* < 0.05). The asterisk indicates statistical significance. Spearman´s correlation (r) and p values for SCAio+ group were not presented owing to the small sample size. Abbreviations: SCAio−: sickle cell anemia without iron overload; SCAio+: sickle cell anemia with iron overload; SCAtotal, patients with and without iron overload

comprehending iron metabolism and assessing iron absorption in these patients. Although critical for health monitoring purposes, iron absorption by the stable-isotope approach has never been evaluated in SCD. Furthermore, the very few studies that investigated iron absorption did not carry out the diagnosis of IO, in contrast to the methodology applied in the current study. Here, iron absorption was not significantly different between SCA and control groups, probably owing to the small number of individuals. Nevertheless, even though it was not significantly different compared to the control group, the iron absorption median value in the SCAio- group was almost 7 times higher than that of other groups and the highest iron absorption values (52.5% and 64.2%) were found in this group. Since SCA is a hemolytic disorder with intense erythropoietic activity, it was expected that individuals without IO would have greater iron absorption values. Despite the relatively low consumption of iron by individuals with SCA [40] in Brazil, the increasing iron fortification of foods, as well as iron supplementation, are worldwide conducted. These patients may therefore be at risk of progressively developing primary IO. Evidence from studies in experimental animal models shows that dietary iron restriction protects against both SCD vaso-occlusion and organ damage [41]. However, the effects of this restriction have not been tested in humans.

In our study, the median iron intake was 12.1 mg (11.8 mg in controls and 12.3 in the SCA$_{total}$ group), with 43.5% of all participants meeting Dietary Reference Intakes

(DRI) recommendations. There was no correlation observed between iron absorption and the iron intake (data not shown). Regarding sources of dietary iron, this study corroborates with other study [40], which found that the primary sources of iron were culinary preparations based on beans and meat (beef, poultry, and pork), along with the consumption of bread and foods made from wheat and corn flour, which are mandatorily fortified with iron in Brazil. Therefore, even if the iron absorption in the SCAio- group could be considered higher than that observed in the other groups, the iron intake of the individuals analyzed here and in the study by Teixeira et al. (2023) [40] suggests that the low observed dietary iron intake does not pose a risk of primary overload.

The SCAio + group presented low iron absorption values: the lowest detectable absorption rate was 0.5%, while the highest absorption value was only 5.4%. These values are close to the median found in the control group (0.9%), suggesting that, in this group, the presence IO is not increasing intestinal iron absorption.

In 1970, an iron absorption test was performed by administering ^{59}Fe to six adults with SCD (SS and SC genotypes were included), with iron absorption values ranging from 27.2 to 98%. Given the large scatter around the mean in all groups, no statistically significant differences were found between groups although the authors observed a tendency towards higher percentages of absorbed ^{59}Fe in the SCA group (SS genotype) compared to SC genotype and controls [24]. This unique study [24] that evaluated iron absorption in SCD adults, used the fecal recovery method, which usually presents overestimated results [42] and did not determine whether the participants had IO.

Ferritin is widely used as an indicator of iron nutritional status, but its concentration is influenced by inflammation [43]. For this reason, the cut-off value for the classification of IO adopted in SCA is 1,000 ng/mL [26] and we also conducted a single-arm analysis involving a limited number of SCA individuals to assess LIC by MRI. In the present study, the percentage of iron absorption was negatively associated with ferritin level and LIC in SCA. Similar associations between serum ferritin level and iron absorption were found in earlier studies with normal subjects [44] and with idiopathic hemochromatosis patients [45]. Serum hepcidin levels could be higher in the SCAio + group and, consequently, these individuals could absorb less dietary iron. However, hepcidin levels did not differ between controls and the SCA-total group and, when separated accordingly to the absence or presence of IO, the values remained without differences among all groups. Unlike ferritin, no relationship was found between serum hepcidin and iron absorption percentage in the study SCA groups. The correlation between hepcidin level and iron absorption has not been previously investigated in SCA. Previous studies determined whether serum

ferritin and hepcidin levels can predict dietary iron absorption in healthy and iron-deficient individuals [46, 47]. Using a multiple linear regression model, a study in 2009 found that hepcidin level is only a modest predictor of dietary iron bioavailability in iron deficient humans [47]. The model that best-predicted iron absorption contained age, hemoglobin content, and serum ferritin level and explained 43% of the variance in iron absorption; when ferritin was excluded from the model, hepcidin level and age were predictors, but explained only 28% of the variance of iron absorption. Thus, serum ferritin seemed to be a better predictor of iron absorption than hepcidin.

A limitation of our study is the small sample size, particularly in the SCAio + group. However, the scarcity of larger samples is a limitation shared by other studies on sickle cell disease [24, 48]. Furthermore, iron absorption could not be detected in some participants. This may be attributed to the absence of high-resolution ICP-MS (HR-IC-MS) in our analysis, and the use of a 5 mg dose of ^{57}Fe might have been insufficient for certain participants, resulting in reduced detection power. Nevertheless, the homogeneity of the participants' characteristics is a strength of the study, as all were adults and exhibited homozygosity for the HbS gene. It is worth noting that we observed little variation in iron absorption values among people with iron overload.

Conclusions

High erythropoietic activity, which is characteristic of SCA, seems to contribute to increased intestinal iron absorption. SCAio- individuals may, therefore, be at risk of developing primary IO. Simultaneously, secondary IO may induce physiological adaptation, resulting in reduced iron absorption. Thus, the iron intake of SCAio- individuals must be carefully monitored by health professionals, as well as taken into account in the development of public policies that aim at iron fortification/supplementation. On the other hand, SCAio + individuals may not need to reduce their intake of iron-rich foods since they did not show higher iron absorption compared to the control group. Further studies evaluating intestinal iron absorption using larger sample sizes should be conducted to help establish a safe nutrition approach to be adopted and to ensure the security of food-fortifying public policies for these patients.

Author contributions MC, JO, FFB, and CSCR designed the research; JO, MC, and VMV conducted the research; JO, VMV, MC, BFB and

RES conducted the laboratory analysis; FFB and CMD helped to interpret the data and provided critical suggestions and comments; GFJ and ASR conducted the MRI procedures; JO, FFB and MC performed the statistical analysis, wrote the manuscript, and had primary responsibility for the final content. All authors read, contributed and approved the final manuscript. None of the authors declared any personal or financial conflict of interest.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The study was conducted in accordance with the Declaration of Helsinki principles. The Ethical Committee of Hemorio (419/17; 2.788.659) and Pedro Ernesto University Hospital (2.695.418) approved the study protocol. Written informed consent was obtained from each participant.

Conflict of interest The authors declare no conflicts of interest.

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