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RESEARCH ARTICLE

Nutrient Sensing, Nutrition, and Metabolism

Absorption of nonheme iron during gastric acid suppression in patients with hereditary hemochromatosis and healthy controls

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Abstract

Phlebotomies are performed in hereditary hemochromatosis (HH) to maintain normal iron concentrations. Proton-pump inhibitors (PPIs) can reduce the number of phlebotomies in patients with HH. However, in patients without HH, the iron concentrations do not appear to be compromised when using PPIs. Therefore, we aim to explain the differences in iron absorption between patients with and without HH. In 10 p.cysteine282tyrosine (p.C282Y) homozygous HH patients with normalized iron stores and 10 healthy control subjects (HCs), the iron parameters and hepcidin concentrations were determined before ingestion of a pharmacological dose of 50 mg iron [ferric iron (Fe^{3+})] polymaltose and hourly for 4 h afterward. This was repeated after 7 days of treatment with pantoprazole 40 mg once daily. Serum iron concentrations and transferrin saturation percentages dropped significantly during PPI use in the patients with HH, whereas no changes were observed in the HCs. Hepcidin concentrations were lower in the patients with HH compared with the HCs both before and during PPI use. In both groups, hepcidin levels did not significantly decrease during the treatment. Seven-day PPI use significantly reduces iron absorption in patients with HH but not in HCs. Changes in hepcidin concentrations could not explain these different PPI effects on iron absorption probably due to a small sample size.

NEW & NOTEWORTHY This study confirms that lowering gastric acidity by proton pump inhibitors results in a reduction in iron absorption in patients with hemochromatosis and not in healthy control subjects. The presupposition that a decrease in hepcidin concentration in healthy control subjects in response to lowering gastric acidity can explain the difference in iron absorption between these groups could not be confirmed probably because of a small sample size.

hepcidin; hereditary hemochromatosis; iron absorption; iron overload; proton-pump inhibitors

INTRODUCTION

The most prevalent form of hereditary hemochromatosis (HH) is homozygosity for the p.cysteine282tyrosine (p.C282Y) variant in the *HFE* gene (1). This condition is characterized by an ineffective regulatory feedback mechanism in which circulating hepcidin concentrations are disproportionally low for body iron stores. Since hepcidin is the key regulator of systemic iron metabolism, persistently low hepcidin concentrations will result in excessive iron absorption leading to iron accumulation (1–3). Iron overload causes damage to parenchymal tissues and can lead to liver cirrhosis, severe arthropathy, diabetes mellitus, cardiac disease, and premature death (4). The standard therapy for iron overload in HH is phlebotomy. However, patients can experience side effects with significant burden, reducing the quality of life and requiring additional hospital visits (5, 6).

Proton-pump inhibitors (PPIs) have been suggested as an attractive additional therapy to reduce the need for phlebotomies by reducing gastric acid secretion, which results in decreased iron absorption (7, 8). Studies about the occurrence of anemia during long-term use of PPIs in patients without HH are contradictory (9, 10). Furthermore, the study describing PPI-associated anemia in patients without HH

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did not rule out a preexistent iron-deficient state or possible upper gastrointestinal blood loss (10). To date, it is unclear via which mechanism PPIs appear to have a different influence on iron absorption in patients with HH and without HH. We hypothesized that patients with HH benefit from a reduction in bioavailable iron via gastric acid inhibition because their hepcidin levels are already disproportionally low. Whereas, in patients without HH, the use of PPI will not result in a reduction in iron absorption because their hepcidin concentrations will decrease in response to gastric acid inhibition and the accompanying reduction in bioavailable iron.

METHODS

Patients

We conducted a nonrandomized proof-of-concept study, between January 2015 and January 2016, with 10 p.C282Y homozygous HH patients with normalized iron stores and 10 gender-matched healthy control subjects (HCs). The patients with HH were recruited from the outpatient department of the Zuyderland Medical Center in Heerlen/Brunssum, The Netherlands. The HCs were recruited among personnel of the medical center and their acquaintances. All participants gave written informed consent, in keeping with the Declaration of Helsinki. The regional ethics committee Atrium-Orbis-Zuyd approved the study. In both groups, men and menopausal women between the age of 18 and 65 yr with ferritin concentrations <400 μg/L for at least 3 mo were included. Patients with HH needed to be homozygous for p.C282Y, to be on maintenance treatment for at least 1 yr, and to have had their last phlebotomy ≥6 wk before entering the study. HCs did not have *HFE* mutations [(compound) heterozygosity or homozygosity for p.C282Y or p.H63D]. Exclusion criteria for both groups were coexistence of acute or chronic inflammatory disorders, such as inflammatory bowel disease or rheumatoid arthritis, hepatitis B, hepatitis C, or HIV infection. Also, anemia, an active malignancy, alcohol intake of >21 units a week for men and >14 units a week for women, and present PPI treatment or other gastric acid-suppressing medication were criteria to exclude patients from the study. The same goes for the use of medication that interfered with PPIs, e.g., vitamin C supplements; the use of iron supplements; and previous side effects of PPIs.

Study Design

On the first test day, after an overnight fast, baseline blood samples were drawn between 7.30 and 8.00 AM (*T0*), after which the participants ingested iron polymaltose (Ferrum Hausman, Vifor, Germany) containing a pharmacological dose of 50 mg of ferric iron (Fe^{3+}) on a small piece of white bread. After the iron polymaltose was ingested, blood samples were drawn hourly for 4 h (*T1–T4*). The participants did not receive breakfast until the third blood sample (*T2*) was drawn, to reduce the chance of an interference with the outcome (Fig. 1). They all consumed the same breakfast of ±300 kcal consisting of bread, coffee, and water. The following day, the participants started using 40 mg pantoprazole orally once daily, before breakfast, for 7 days. On the seventh day, we repeated the oral iron challenge and blood sample collection.

Laboratory Analyses

Blood samples for hepcidin analysis were stored at −80°C. Serum iron and transferrin were determined on the days of the test by standard laboratory analysis. Transferrin saturation was calculated from serum iron and transferrin: iron (μmol/L) × 4.5/transferrin (g/L).

Hepcidin measurements were performed in freshly thawed serum samples by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry using an internal standard for quantification (11, 12). Hepcidin-25 concentrations were expressed as nmol/L (nM). The median reference concentrations for serum/plasma hepcidin-25 (Dutch population) are 4.5 nM for men, 2.0 for premenopausal women, and 4.9 nM for postmenopausal women (13). The hepcidin test lower limit of detection was 0.5; in case of a hepcidin concentration below 0.5, the result is shown as <0.5.

Statistical Analysis

Statistical analyses were carried out using SPSS version 23 for Windows (IBM Statistics for Macintosh, Chicago, IL). A power calculation was not possible since this is an

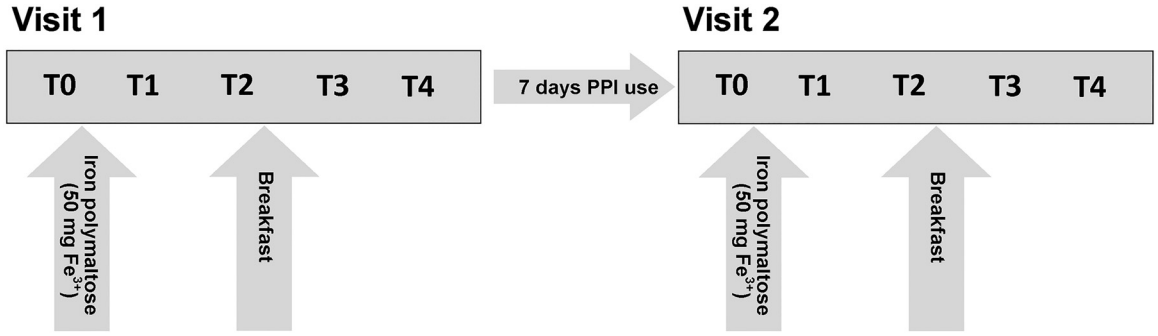

Figure 1. Study design. The baseline blood samples were drawn between 7.30 and 8.00 AM (*T0*), after which the participants ingested iron polymaltose (Ferrum Hausman, Vifor, Germany) containing a pharmacological dose of 50 mg of Fe^{3+} iron on a small piece of white bread. After the iron polymaltose was ingested, blood samples were drawn hourly for 4 h (*T1–T4*). All patients consumed the same breakfast of ±300 kcal consisting of bread, coffee, and water after the third blood sample (*T2*). The following day, the participants started using 40 mg pantoprazole orally once daily, before breakfast, for 7 days. On the seventh day, we repeated the oral iron challenge and blood sample collection. In all blood samples, serum iron, transferrin, transferrin saturation, and hepcidin concentrations were measured. Fe^{3+}, ferric iron; PPI, proton-pump inhibitor.

exploratory study and no previous data regarding hepcidin concentrations in hemochromatosis were available. Data are expressed as means (SD) for continuous variables and frequency (%) for categorical variables. Comparison of baseline values between the HH group and the HC group was performed using the independent *t* test in case of continuous variables and using the Fisher's exact test for categorical variables.

The comparison of repeated measurements, before and during PPIs, within groups and between groups (patients with HH and HCs), was performed using a linear mixed model with a first-order autoregressive (AR1) covariate type for the repeated measurements. Fixed factors included the following variables: group (patient with HH vs. HC), PPI use (before vs. during PPI use), and the time points of the blood sample collection (*T0*, *T1*, *T2*, *T3*, and *T4*). The random factor was the participant ID. The repeated variables included the test days (before vs. during PPI use) and the time points of the blood sample collection. Furthermore, estimated measures means were calculated. In case of hepcidin concentrations < 0.5, we performed statistical analyses with hepcidin concentrations of 0.5, 0.25, and 0.01 to test if this would lead to different outcomes. This was not the case; therefore, in this paper, we reported the value 0.25 when hepcidin analysis showed <0.5. A Bonferroni correction was used to correct for multiple testing. *P* values were considered significant when ≤ 0.05.

RESULTS

Patient Baseline Characteristics

Of the 126 p.C282Y homozygous HH patients regularly visiting the outpatient clinic, 24 matched the inclusion criteria. Of these 24 patients with HH, 10 agreed to participate in the study.

Twenty-three HCs were screened for the *HFE* mutations p.C282Y and p.H63D, and their ferritin concentrations were checked. In 13% (3/23), hyperferritinemia was observed, and in 43.5% (10/23), heterozygosity for one of the *HFE* mutations was found (2/10 p.C282Y and 8/10 p.H63D). These HCs were then excluded, leaving 10 subjects who fulfilled the inclusion criteria. Baseline characteristics are summarized in Table 1.

In this gender-matched study, the majority of participants were male (70%). Age and body mass index were not significantly different between groups.

Serum Iron and Transferrin Saturation

In patients with HH, both serum iron concentrations before and during PPI treatment were significantly higher than those obtained from HCs at all time points, with a mean difference between the two groups of 14.2 μmol/L ($P = 0.001$) before PPI use and 9.9 μmol/L ($P = 0.013$) during PPI use (Fig 2*A*).

In the HC group, serum iron concentrations before and during PPI use showed no significant difference (mean difference of 0.02 μmol/L, $P = 0.985$). In the patients with HH group, lower serum iron concentrations were found during PPI use compared with before PPI use with a mean difference of 4.35 μmol/L ($P < 0.001$) (Fig 2*A*).

Similar results were obtained for transferrin saturation (T_{sat}) (Fig 2*B*). Patients with HH had higher T_{sat} percentages at all time points both before (mean difference of 39.18%, $P < 0.001$) and during PPI use (mean difference of 28.25%, $P = 0.001$) compared with HCs. In the patients with HH group, significantly lower T_{sat} percentages were seen during PPI use compared with before PPI use (mean difference of 10.33%, $P < 0.001$). In the HC group, no significant difference was found in T_{sat} comparing before and during PPI use (mean difference of 0.60, $P = 0.760$).

Hepcidin

Patients with HH had significantly lower hepcidin concentrations at all time points compared with HCs, both before (mean difference of 2.46 nmol/L, $P = 0.002$) and during PPI use (mean difference of 1.69 nmol/L, $P = 0.029$) (Fig. 3, *A* and *B*). The hepcidin concentration in the HC group was lower during PPI use compared with before PPI use; however, this difference was not statistically significant (mean difference of 0.54 nmol/L, $P = 0.166$). Also, in the patients with HH group, no statistically significant difference in hepcidin concentrations was found during PPI use compared with before PPI use (mean difference of 0.23 nmol/L, $P = 0.549$) (Fig. 4).

Table 1. *Baseline characteristics of the study population*

Baseline Features	Hemochromatosis Patients	Healthy Control Subjects	*P* Value
n	10	10	
Age, yr	55.3 ± 8.2	50.1 ± 9.4	0.205
Gender (male)	7 (70%)	7 (70%)	>0.999
BMI, kg/m^2	26.8 ± 2.8	24.5 ± 3.0	0.100
Smoking (yes)	2 (20%)	1 (10%)	>0.999
Alcohol (yes)	10 (100%)	7 (70%)	0.211
CRP, mg/L (0–10)	1.8 ± 2.6	0.8 ± 1.1	0.282
ALT, U/L (0–40)	25.8 ± 8.8	26.7 ± 8.8	0.821
GGT, U/L (0–40)	52 ± 29.0	47 ± 42.2	0.761
Hb, mmol/L (8.5–11 ♂; 7.5–10 ♀)	9.7 ± 0.8	9.3 ± 0.7	0.258
Ht, L/L (0.41–0.51 ♂; 0.36–0.47 ♀)	0.45 ± 0.04	0.44 ± 0.03	0.500
SF, μg/L (30–400)	98.3 ± 110.0	153.1 ± 91.6	0.242
T_{sat}, % (16–45)	61.2 ± 18.2	26.7 ± 10.6	0.000
Transferrin, g/L (2–4.1)	2.0 ± 0.2	2.5 ± 0.3	0.000

Data are expressed as absolute numbers (percentage), means ± SD; *n*, number of subjects. ALAT, alanine aminotransferase; BMI, body mass index; CRP, C-reactive protein; GGT, γ-glutamyl transferase; Hb, hemoglobin; Ht, hematocrit; SF, serum ferritin; T_{sat}, transferrin saturation.

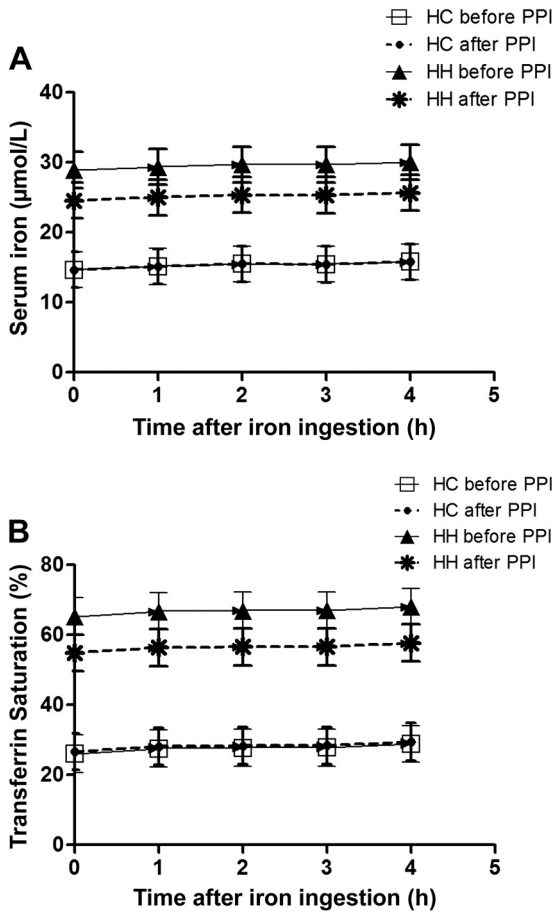

Figure 2. Effect of PPI on serum iron concentrations (*A*) and transferrin saturation (*B*) over time in patients with HH ($n = 10$) and HC subjects ($n = 10$) after oral iron supplementation the day before and on *day 7* of PPI administration. *Values represent estimated means with a standard error of the mean calculated by mixed-model analysis. HC, healthy control subjects; HH, hereditary hemochromatosis; PPI, proton-pump inhibitor.

DISCUSSION

This proof-of-concept study shows that short-term use of PPIs leads to significantly lower circulating iron concentrations in iron-depleted HH patients, whereas it has no effect on serum iron concentrations in HCs. These results support the finding that reducing the acidity of the gastric content with PPIs leads to a decrease in iron absorption in patients with HH (7, 8).

Iron in the food can be present in the nonheme and/or heme-bound form. Iron is absorbed mainly in the duodenum and the upper jejunum. In persons who eat meat, heme iron may contribute to 10%–15% of the daily iron intake. Heme iron is absorbed to a higher extent than nonheme iron. In contrast to nonheme iron, heme iron is less influenced by dietary constituents and the higher pH of the small intestine (14, 15). Heme iron is absorbed into the enterocyte through the heme carrier protein 1. Inside the cell, iron is released from heme by heme oxygenase (2). Nonheme iron absorption takes place mainly on the apical membrane of the enterocyte via the divalent-metal transporter-1 (DMT-1) (2, 16). This transporter is selective for ferrous (Fe^{2+}) iron. Since ferric (Fe^{3+}) iron is the predominant form present in the diet, a reduction step of ferric iron to ferrous iron is necessary for absorption. The reduction is catalyzed by duodenal cytochrome-b, a major intestinal ferrireductase (2, 17, 18). DMT-1 is a H^+-coupled cotransporter, and it functions optimally at an acidic pH (19). Proton-pump inhibitors reduce the acid content of gastric secretions, resulting in higher pH up to 6. As a result, the DMT-1 function will decrease and the reduction of ferric iron will be diminished, with less ferrous iron available for absorption via the DMT-1 (20). In patients with HH, intestinal DMT-1 is upregulated, which may result in increased iron absorption into the enterocyte (20, 21). Results of a mice study suggested that in case of a more acidic pH, the ferrireductase activity is lower (22). This mechanism suggests that patients with HH on PPIs would have increased iron absorption through upregulated DMT-1 and increased ferrireductase activity. However, in patients with HH treated with PPI, fewer phlebotomies were needed to maintain a stable serum ferritin concentration. This

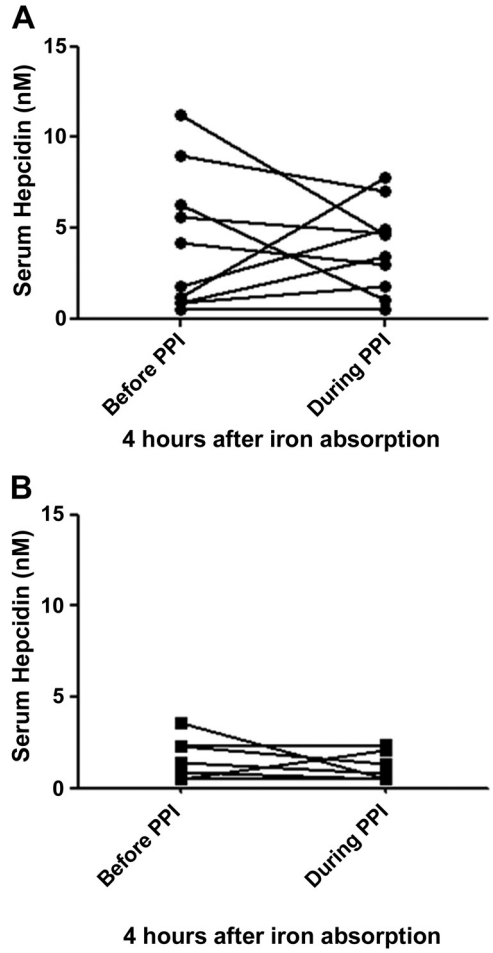

Figure 3. Serum hepcidin concentrations, measured 4 h (*T4*) after the intake of iron polymaltose, displayed for each individual patient/healthy control subject before and during PPI. *A*: the serum hepcidin concentrations for each healthy control subject ($n = 10$); in 5 subjects, the hepcidin level decreased ($n = 4$) or stayed unchanged ($n = 1$) comparing before versus during PPI. *B*: the serum hepcidin concentrations for each hemochromatosis patient ($n = 10$); in 7 patients, the hepcidin level decreased ($n = 5$) or stayed unchanged ($n = 2$) comparing before versus during PPI use, and in 2 other patients, the measured increase was not more than 0.1 nM. PPI, proton pump inhibitor.

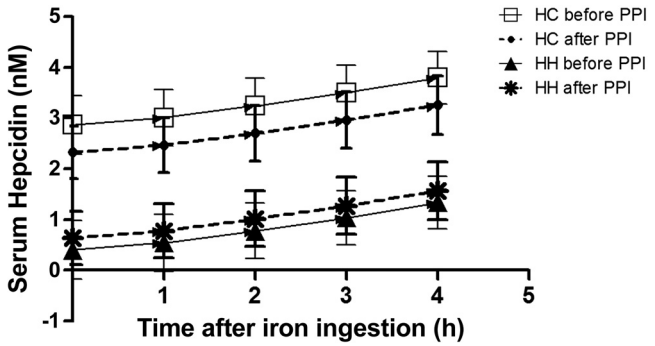

Figure 4. No significant differences were seen between the hepcidin concentrations before and during PPI use within HCs ($n = 10$) and within patients with HH ($n = 10$) (HC group mean difference of 0.54, $P = 0.166$; HH group mean difference of 0.23, $P = 0.549$). Between-group analysis showed a statistically significantly lower hepcidin concentration in patients with HH for all time points (before PPI use mean difference of 2.46, $P = 0.002$; during PPI use mean difference of 1.69, $P = 0.029$). *Values are estimated means with a standard error (SE) of the mean calculated by mixed-model analysis. HCs, healthy control subjects; HH, hereditary hemochromatosis; PPI, proton-pump inhibitor.

indicates that the inhibitory effects of PPI use, lowering gastric acid secretion, on the uptake of iron overruled the promoting effects (upregulated DMT-1 and increased ferrireductase activity) seen in the intestinal cells in hemochromatosis (7, 8, 23). Our results also show significantly lower circulating iron concentrations in patients with HH after PPI use. These results are in line with Hutchinson et al. (23), who previously reported a significant reduction in increase of iron concentrations following an iron challenge, after 7 days of PPIs, in patients with HH. Furthermore, our study showed no decrease in circulating iron concentrations after PPI use in HCs. An explanation for this might be the fact that iron, whether originating from heme or nonheme sources, can only leave the enterocyte via ferroportin, a process that is regulated by hepcidin (24). This protein is disproportionally low in patients with hereditary hemochromatosis but not in HCs, suggesting that in HCs, hepcidin concentrations could be lowered to ensure enough iron uptake from the enterocyte, whereas patients with HH cannot use hepcidin to regulate their iron concentrations.

We did not observe a significant decrease in serum hepcidin concentrations after 1 wk of PPIs in HCs. As shown in Fig. 3, there was a wide variation in hepcidin concentrations in HCs both before and after PPI administration. Because of these wide variations and our small sample size in this study, it is not possible to determine the possible effect of hepcidin on the differences in iron absorption.

It should be noted that an increase in iron concentrations after the iron challenge was not seen in our study. Previous studies measuring iron concentrations following an oral iron challenge used ferrous sulfate. These studies did observe an increase in iron parameters (25, 26). We aimed to measure the effect of gastric acid inhibition, so in our study, the use of a ferric preparation was essential. Furthermore, most of the iron in our food is in the ferric form. However, the lack of rise in iron concentrations after iron administration cannot solely be related to the administration of ferric iron compared with ferrous iron (27). Hutchinson et al. (23) also used ferric chloride and showed an increase in iron concentrations. A possible explanation for the lack of iron increase in our study could be that absorption of ferric preparations is up to seven times better when taken with food, and our patients were fasted, whereas the patients in the Hutchison et al. study received their ferric chloride combined with an iron-enriched meal (28).

Strengths of this study include the accurate study protocol, including an hourly monitoring of serum iron parameters and the accurate selection process of the HCs to exclude acquired or genetic confounding factors, including *HFE* genotypes susceptible for iron overload. Moreover, using the stable portion of iron polymaltose instead of an iron-enriched meal allowed an exact amount of Fe^{3+} to be ingested. Furthermore, to our knowledge, the effect of PPI use on hepcidin concentrations after an oral iron challenge has never been studied before. We recognize that the current study has limitations. First, the period of PPI use was short and no gastric pH measurements were done to check the effect of PPI. However, continually measuring gastric pH can be experienced as invasive, and the decrease in acidity following use of PPI has already been clearly documented, even after 7 days of PPI (29).

Furthermore, the study population was small. The sample size was based on previous studies researching serum ferritin concentrations. Because of the lack of data on hepcidin concentrations in patients with hemochromatosis using PPI, a power calculation for hepcidin as outcome measure could not be performed. Therefore, the results on hepcidin should be interpreted with caution, and it is necessary for future studies on hepcidin to include a larger study population.

In conclusion, our proof-of-concept study has shown that PPI use significantly reduces serum iron concentrations in patients with HH but not in HCs, indicating that PPI use reduces iron absorption only in patients with HH. The presupposition that PPI use in HCs will not result in a reduction in iron absorption because their hepcidin concentrations will decrease in response to lowering gastric acidity could not be confirmed. However, it should be noted that the sample size was small.

Future studies should include a larger study population and preferably also different doses of PPIs to unravel the pathophysiological mechanisms.

ACKNOWLEDGMENTS

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DISCLOSURES

Coby Laarakkers and Dorine Swinkels are employees of Radboudumc that via its Hepcidinanalysis.com initiative offers high-quality hepcidin measurements to the scientific, medical, and pharmaceutical communities at a fee-for-service basis. Our study aim was purely scientific, and no commercial factors or intentions were involved. We have paid for the hepcidin assays. A. M. Masclee has received a consultancy fee from Allergan, Takeda, and Kyowa Kirin. A. M. Masclee has received a ZonMw [The Netherlands Organisation for Health Research and Development (Dutch Government)] health-care efficiency grant. A. M. Masclee has received an unrestricted research grant from Will Pharma SA, and he has received research funding from Allergan and Grünenthal. A. M. Masclee has received funding

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AUTHOR CONTRIBUTIONS

W.M., G.H.K., and C.T.B.M.v.D. conceived and designed research; W.M., and P.L.M.V. analyzed data; W.M., and P.L.M.V. interpreted results of experiments; W.M. prepared figures; W.M. drafted manuscript; W.M., P.L.M.V., J.V., D.W.S., C.M.L., A.A.M.M., G.H.K., and C.T.B.M.v.D. edited and revised manuscript; W.M., J.V., D.W.S., C.M.L., A.A.M.M., G.H.K., and C.T.B.M.v.D. approved final version of manuscript.

REFERENCES

1. **Brissot P**, **Pietrangelo A**, **Adams PC**, **de Graaff B**, **McLaren CE**, **Loreal O.** Hemochromatosis. *Nat Rev Dis Primers* 4: 18016, 2018. doi:10.1038/nrdp.2018.16.
2. **Evstatiev R**, **Gasche C.** Iron sensing and signalling. *Gut* 61: 933–952, 2012. doi:10.1136/gut.2010.214312.
3. **Bridle KR**, **Frazer DM**, **Wilkins SJ**, **Dixon JL**, **Purdie DM**, **Crawford DH**, **Subramaniam VN**, **Powell LW**, **Anderson GJ**, **Ramm GA.** Disrupted hepcidin regulation in HFE-associated hemochromatosis and the liver as a regulator of body iron homoeostasis. *Lancet* 361: 669–673, 2003. doi:10.1016/S0140-6736(03)12602-5.
4. **Pietrangelo A.** Hereditary hemochromatosis–a new look at an old disease. *New Eng J Med* 350: 2383–2397, 2004. doi:10.1056/NEJMra031573.
5. **Brissot P**, **Ball S**, **Rofail D**, **Cannon H**, **Jin VW.** Hereditary hemochromatosis: patient experiences of the disease and phlebotomy treatment. *Transfusion* 51: 1331–1338, 2011. doi:10.1111/j.1537-2995.2010.02997.x.
6. **Newman B.** Arm complications after manual whole blood donation and their impact. *Transfus Med Rev* 27: 44–49, 2013. doi:10.1016/j.tmrv.2012.05.002.
7. **Vanclooster A**, **van Deursen C**, **Jaspers R**, **Cassiman D**, **Koek G.** Proton pump inhibitors decrease phlebotomy need in HFE hemochromatosis: double-blind randomized placebo-controlled trial. *Gastroenterology* 153: 678–680, 2017. doi:10.1053/j.gastro.2017.06.006.
8. **van Aerts RM**, **van Deursen CT**, **Koek GH.** Proton pump inhibitors reduce the frequency of phlebotomy in patients with hereditary hemochromatosis. *Clin Gastroenterol Hepatol*14: 147–152, 2016. doi:10.1016/j.cgh.2015.06.043.
9. **Stewart CA**, **Termanini B**, **Sutliff VE**, **Serrano J**, **Yu F**, **Gibril F**, **Jensen RT.** Iron absorption in patients with Zollinger-Ellison syndrome treated with long-term gastric acid antisecretory therapy. *Aliment Pharmacol Ther* 12: 83–98, 1998. doi:10.1046/j.1365-2036.1998.00274.x.
10. **Tran-Duy A**, **Connell NJ**, **Vanmolkot FH**, **Souverein PC**, **de Wit NJ**, **Stehouwer CDA**, **Hoes AW**, **de Vries F**, **de Boe A.** Use of proton pump inhibitors and risk of iron deficiency: a population-based case-control study. *J Intern Med* 285: 205–214, 2019. doi:10.1111/joim.12826.
11. **Kroot JJ**, **Laarakkers CMM**, **Geurts-Moespot AJ**, **Grebenchtchikov N**, **Pickkers P**, **van Ede AE**, **Peters HPE**, **van Dongas-Lases E**, **Wetzels JFM**, **Sweep FCGJ**, **Tjalsma H**, **Swinkels DW .** Immunochemical and mass-spectrometry-based serum hepcidin assays for iron metabolism disorders. *Clin Chem* 56: 1570–1579, 2010. doi:10.1373/clinchem.2010.149187.
12. **Swinkels DW**, **Girelli D**, **Laarakkers C**, **Kroot J**, **Campostrini N**, **Kemma EHJM**, **Tjalsma H.** Advances in quantitative hepcidin measurements by time-of-flight mass spectrometry. *PloS One* 3: e2706, 2008. doi:10.1371/journal.pone.0002706.
13. **Galesloot TE**, **Vermeulen SH**, **Geurts-Moespot AJ**, **Klaver SM**, **Kroot JJ**, **van Tienoven D**, **Wetzels JFM**, **Kiemeney LALM**, **Sweep FC**, **den Heijer M**, **Swinkels DW.** Serum hepcidin: reference ranges and biochemical correlates in the general population. *Blood* 117: e218–e225, 2011. doi:10.1182/blood-2011-02-337907.
14. **Lynch S**, **Pfeiffer CM**, **Georgieff MK**, **Brittenham G**, **Fairweather-Tait S**, **Hurrell RF**, **McArdle HJ**, **Raiten DJ.** Biomarkers of nutrition for development (BOND)-iron review. *J Nutr* 148: 1001S–1067S, 2018. doi:10.1093/jn/nxx036.
15. **Conrad ME**, **Cortell S**, **Williams HL**, **Foy AL.** Polymerization and intraluminal factors in the absorption of hemoglobin-iron. *J Lab Clin Med* 68: 659–668, 1966.
16. **Shawki A**, **Anthony SR**, **Nose Y**, **Engevik MA**, **Niespodzany EJ**, **Barrientos T**, **Ohrvik H**, **Worrell RT**, **Thiele DJ**, **Mackenzie B.** Intestinal DMT1 is critical for iron absorption in the mouse but is not required for the absorption of copper or manganese. *Am J Physiol Gastrointest Liver Physiol* 309: G635–G647, 2015. doi:10.1152/ajpgi.00160.2015.
17. **Milman NT.** A review of nutrients and compounds, which promote or inhibit intestinal iron absorption: making a platform for dietary measures that can reduce iron uptake in patients with genetic hemochromatosis. *J Nutr Metab* 2020: 7373498, 2020. doi:10.1155/2020/7373498.
18. **Sharp PA.** Intestinal iron absorption: regulation by dietary & systemic factors. *Int J Vitam Nutr Res* 80: 231–242, 2010.
19. **Knutson MD.** Iron transport proteins: Gateways of cellular and systemic iron homeostasis. *J Biol Chem* 292: 12735–12743, 2017. doi:10.1074/jbc.R117.786632.
20. **Shawki A**, **Knight PB**, **Maliken BD**, **Niespodzany EJ**, **Mackenzie B.** H(+)-coupled divalent metal-ion transporter-1: functional properties, physiological roles and therapeutics. *Curr Top Membr* 70: 169–214, 2012. doi:10.1016/B978-0-12-394316-3.00005-3.
21. **Byrnes V**, **Barrett S**, **Ryan E**, **Kelleher T**, **O'Keane C**, **Coughlan B**, **Crowe J.** Increased duodenal DMT-1 expression and unchanged HFE mRNA levels in HFE-associated hereditary hemochromatosis and iron deficiency. *Blood Cells Mol Dis* 29: 251–260, 2002. doi:10.1006/bcmd.2002.0554.
22. **Griffiths WJ**, **Sly WS**, **Cox TM.** Intestinal iron uptake determined by divalent metal transporter is enhanced in HFE-deficient mice with hemochromatosis. *Gastroenterology* 120: 1420–1429, 2001. doi:10.1053/gast.2001.24050.
23. **Hutchinson C**, **Geissler CA**, **Powell JJ**, **Bomford A.** Proton pump inhibitors suppress absorption of dietary non-heme iron in hereditary hemochromatosis. *Gut* 56: 1291–1295, 2007. doi:10.1136/gut.2006.108613.
24. **Anderson GJ**, **Frazer DM.** Current understanding of iron homeostasis. *Am J Clin Nutr* 106: 1559s–1566s, 2017. doi:10.3945/ajcn.117.155804.
25. **Girelli D**, **Trombini P**, **Busti F**, **Campostrini N**, **Sandri M**, **Pelucchi S**, **Westerman M**, **Ganz T**, **Nemeth E**, **Piperno A**, **Camaschella C.** A time course of hepcidin response to iron challenge in patients with HFE and TFR2 hemochromatosis. *Haematologica* 96: 500–506, 2011. doi:10.3324/haematol.2010.033449.
26. **Sangwaiya A**, **Manglam V**, **Busbridge M**, **Thursz M**, **Arnold J.** Blunted increase in serum hepcidin as response to oral iron in HFE-hemochromatosis. *Euro J GastroenterolHepatol* 23: 721–724, 2011. doi:10.1097/MEG.0b013e3283484716.
27. **Kloepfer K**, **Schmid P**, **Wuillemin WA**, **Rufer A.** Reference values for oral iron absorption of bivalent iron in healthy volunteers. *Swiss Med Wkly* 145: w14063, 2015. doi:10.4414/smw.2015.14063.
28. **Kaltwasser JP**, **Werner E**, **Niechzial M.** Bioavailability and therapeutic efficacy of bivalent and trivalent iron preparations. *Arzneimittel-Forschung* 37: 122–129, 1987.
29. **Hartmann M**, **Theiss U**, **Huber R**, **Luhmann R**, **Bliesath H**, **Wurst W**, **Lucker PW.** Twenty-four-hour intragastric pH profiles and pharmacokinetics following single and repeated oral administration of the proton pump inhibitor pantoprazole in comparison to omeprazole. *Aliment Pharmacol Ther* 10: 359–366, 1996. doi:10.1111/j.0953-0673.1996.00359.x.