**Predicting iron absorption from an effervescent iron supplement in obese patients before
and after Roux-en-Y gastric bypass: a preliminary study**

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65 **Running title:** Prediction of iron absorption pre- and post-RYGB

Introduction

Obesity as well as the surgical treatment of obesity, namely bariatric surgery, is associated with nutritional deficiencies [1-3]. In persons with obesity, the low-grade chronic inflammation associated with excessive fat accumulation inhibits iron absorption [1]. The anatomical changes associated with the most performed bariatric procedure, namely Roux-en-Y Gastric Bypass (RYGB), adversely affect iron absorption resulting in an increased risk for iron deficiency [4]. Currently, bariatric surgery patients are recommended to use oral iron supplements without any assurance of successful treatment [5]. The absence of a successful outcome results in a switch to parenteral iron administration [6-8]. Identifying patients, who may or may not benefit from oral iron supplementation, would allow us to avoid the unnecessary use of parenteral iron formulations for patients responsive to iron supplements or to immediately switch to parenteral iron formulations for patients non-responsive to iron supplements. Therefore, a screen-and-treat approach using biomarkers is needed, which allows to predict which patients would benefit from oral iron supplementation. Hepcidin is a promising biomarker to implement in a screen-and-treat approach. Cepeda-Lopez *et al.* recently demonstrated that serum hepcidin concentrations predict iron absorption in obese patients [9]. Hepcidin has a central role in iron metabolism as it is a negative regulator of the iron metabolism. It inhibits intestinal iron absorption, iron recycling by macrophages and iron mobilization from hepatic stores by internalization and degradation of ferroportin [10]. Therefore, hepcidin could serve as a biomarker to identify patients that will benefit from oral iron supplementation, though at present no data are available pre- and post-RYGB. The main objective of this preliminary study was to evaluate whether hepcidin could potentially be identified as a biomarker predicting the extent of oral iron absorption. Furthermore, we compared the disposition of an effervescent tablet containing 695 mg iron gluconate (80 mg Fe²⁺) before and after surgery.

Material and methods

Selection of patients

Obese patients that were planned to undergo RYGB surgery in the University Hospitals Leuven, Belgium and had a low iron status (ferritin < 30 µg/L and/or transferrin saturation (TSAT) < 20%) were eligible for the study. Patients with a history of bariatric surgery or female patients that were pregnant

or lactating were not allowed to enrol as were patients with a positive *Helicobacter pylori* screening as this infection can reduce iron absorption [11]. All patients underwent a laparoscopic RYGB with an alimentary limb of 120 cm and a small gastric pouch, performed by the same surgeon according to the same procedure.

Study design and procedure

A single-dose pharmacokinetic study with an effervescent tablet containing 695 mg of iron gluconate (further referred to as ‘iron’), equivalent to 80 mg Fe²⁺ (Losferron®, Hermes Pharma Ges.m.b.H., Wolfsburg, Austria) was performed in all patients before and six to eight months after RYGB (on average 6.4 months (SD 0.6); further referred to as ‘post-RYGB’).

Following an overnight fast of at least 10 hours, subjects came to the Clinical Pharmacology Unit of the University Hospitals Leuven, Belgium. Weight and height of the subjects were measured with calibrated equipment (Seca GmbH & Co, Hamburg, Germany). The weight was measured to the nearest 0.1 kg, with the subjects having an emptied bladder and wearing indoor clothing with empty pockets and without shoes. Body mass index (BMI) was calculated by dividing the weight (kg) by the square of height (m²). Dual Energy X-ray Absorptiometry (DXA) was performed (Hologic Discovery, Marlborough, MA, USA) to measure the amount of body fat. After the insertion of an intravenous catheter, subjects ingested 695 mg of iron gluconate, which was dissolved in 150 mL of water and completely effervesced with minimal carbonation. Before oral administration, blood samples were collected for the determination of the serum concentration of iron, hemoglobin, mean corpuscular volume (MCV), TSAT, transferrin, ferritin, C-reactive protein (CRP) and hepcidin. After oral administration of iron, blood samples were collected at 15, 30, 60, 90 minutes and 2; 2.5; 3; 3.5; 4; 5; 6; 7; 8; 9; 10 and 24 hours to determine the serum iron concentration. A standardized meal (containing 0.8 mg of iron, determined based on the Belgian Food Composition Database - NUBEL) and a standardized snack (containing 0.1 mg of iron) were administered 4 hours and 8 hours, respectively, after dosing of the effervescent tablet. Participants had to consume the entire meal. The use of water was allowed, except for one hour before and four hours after supplement administration. During four hours after the administration, the patients had to remain semi-supine in bed. After the 10-h blood sample, the subjects were discharged and they had to return the next morning fasted for the 24-h blood sampling. The

included patients were asked to stop multivitamin and iron supplements, proton pump inhibitors, H₂-receptor antagonists and antacids during the week preceding the study, as their use can influence the absorption of the iron supplement. Before surgery, nobody was taking an iron containing supplement. At the postoperative assessment, four patients were taking iron containing multivitamin and mineral supplements (14.0-70.0 mg Fe²⁺), four patients used a specific iron supplement (80-105 mg Fe²⁺) and one patient required parenteral iron administration containing ferric carboxymaltose.

Ethical approval

This study was performed in accordance with the ethical standards of the institutional and/or national research committee on human experimentation (UZ Leuven, ML8433) and in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained from all individual participants included in the study. The EudraCT number is 2012-001244-22.

Iron status and haematological analysis

Blood samples for the determination of serum concentration of iron, hemoglobin, MCV, transferrin, ferritin and CRP were immediately sent to the Clinical Laboratory of the University Hospitals Leuven for analysis using the tests shown in **Table 1**. Additionally, TSAT was calculated using the following formula: $TSAT(\%) = [Iron] (\mu g/dL) / ([Transferrin] (g/L) \times 1.4)$. Hepcidin concentrations were determined using the hepcidin-25 (human) Enzyme-Linked Immunosorbent Assay (ELISA) kit for human serum (Peninsula Laboratories International, San Carlos, USA).

Sample size calculations

Ruz *et al.* demonstrated a decrease in oral iron absorption of 40% after RYGB. Therefore, we pre-defined a 40% decrease in oral iron absorption post-RYGB as the difference we wanted to be able to detect. Based on this decrease and the variability observed, we calculated that a sample size of 13 patients was needed to detect such decrease with $\alpha = 0.05$ and a power of 80% [6, 8].

Data analysis

The oral exposure of iron gluconate was estimated by changes in serum iron concentration. For that purpose, serum iron concentrations after administration of the iron supplement were adjusted for the basal iron concentration using the following formula:

$$\frac{\text{serum iron concentration after administration } (\mu\text{g/dL})}{\text{serum iron concentration before administration } (\mu\text{g/dL})}$$

Subsequently, the concentration-versus-time curves were plotted and $\text{AUC}_{0-24\text{h}}$ was determined using the linear trapezoidal rule [12]. Based on the concentration-versus-time curves, the peak plasma concentration (C_{max}) and the time of peak plasma concentration (T_{max}) were determined. For visualisation of the mean changes in serum iron concentration, serum iron concentrations at the different time points after administration of both supplements were adjusted for the basal iron concentration by subtracting each iron concentration with the basal iron concentration.

Wilcoxon signed-rank tests were performed to compare the characteristics and status markers of the participants before and post-RYGB as well as to compare the pharmacokinetic results before and post-RYGB. Furthermore, Mann-Whitney U tests were performed to compare the postoperative status markers of the participants who received supplementation compared to the participants who did not received supplementation. A multivariate regression analysis was performed to investigate the effect of hepcidin concentrations, iron and hematologic indices; personal (e.g. age) and anthropometric characteristics on iron absorption. Subsequently, Receiver Operating Characteristic (ROC) curves were used to propose cut-off values for hepcidin concentrations above which obese and RYGB patients are possibly unlikely to benefit from oral iron supplementation [13]. All results are presented as mean $\pm$ SD. Statistical significance was set at $P<0.05$. The data were analysed with Graphpad Prism 7.0 (Graphpad, San Diego, USA).

Results

In total, we screened 122 medical records of eligible patients and withheld 28 patients that had a low iron status (23.0%). Thirteen non-smoking patients (3 men, 10 female) agreed to participate in the study (46.4%). The mean age at the time of surgery was 45.2 ± 12.5 years. The mean age of the participating females was 42.1 ± 12.0 , while the mean age of the participating men was 55.3 ± 9.3 . Of the participating females, two subjects were post-menopausal. In the group of eligible patients, no differences in clinical characteristics between participants and non-participants were observed, apart from the BMI. The anthropometric, body-composition, inflammatory, iron and hematologic indexes of the patients before and post-RYGB are shown in **Table 2**. As expected, weight, BMI and fat mass percentage all

significantly decreased post-RYGB (Weight: 26.5% ↓, BMI: 26.5% ↓ and fat mass percentage: 19% ↓). Regarding the status markers, hemoglobin concentration was significantly decreased post-surgery (7.3% ↓) ($P=0.006$). No significant difference in MCV was observed. The iron status markers transferrin, TSAT, ferritin, hepcidin and CRP did not differ statistically pre- and post-surgery. No significant differences were found in the postoperative iron status markers of the patients who received supplementation compared to the patients who did not received supplementation.

The mean serum iron changes after administration of the oral iron supplement are shown in **Figure 1** and the pharmacokinetic parameters are given in **Table 3**. No significant differences of the pharmacokinetic parameters were observed between pre- and post-RYGB. The AUC_{0-24h} , the C_{max} and the T_{max} was similar pre-RYGB and post-RYGB.

Correlations between serum hepcidin concentrations and TSAT and iron AUC_{0-24h} are shown in **Figure 2**. Post-RYGB, iron AUC_{0-24h} showed a significant negative correlation with hepcidin concentrations and showed a clear trend for a similar correlation between AUC_{0-24h} and TSAT. Pre-RYGB, there was a clear trend for similar negative correlations. Taking pre-and post-RYGB data together, these negative correlations were confirmed for both hepcidin concentrations and TSAT. In **Table 4**, the results of the multivariate regression are presented. Pre-surgery, the R^2 for the AUC_{0-24h} was 0.924 although none significant ($P=0.32$). The model included hepcidin, age, weight, BMI, fat percentage, hemoglobin, MCV, transferrin, TSAT and ferritin. However, none of the predictors were significant. Post-surgery, the R^2 for the AUC_{0-24h} reached one and was significant ($P = 0.012$). The model included the same predictors, where all variables except hemoglobin and TSAT, were significant predictors.

The $AUC^{ROC}=0.87$ (95%CI 0.71; 1.00) showed an optimal sensitivity/specificity cut-off at hepcidin concentrations of 26.8 ng/mL (sensitivity of 0.89 and specificity of 0.77).

Discussion and conclusion

This preliminary study demonstrates that hepcidin concentrations and TSAT values can potentially indicate to which extent oral iron supplementation will be successful in obese patients both pre- and post-RYGB. In addition, we show that on average the oral disposition of iron from an effervescent tablet is unaffected by RYGB. Nonetheless, we fully acknowledge that iron disposition is extremely variable

both before and post-RYGB, mandating careful monitoring of the iron status after initiation of oral supplementation.

In our patients, iron status markers were not improved post-RYGB as no significant differences were found in ferritin, TSAT and hepcidin between pre- and post-RYGB. Although, a clear trend in the level of inflammation was observed by a decrease in CRP. Therefore, we may conclude that iron status remained poor post-RYGB as we included patients who suffered from iron deficiency pre-operatively. This was to avoid interference of the well-known fact that iron deficiency facilitates the oral absorption of iron [14].

From a theoretical point of view, different factors may negatively affect iron absorption in patients with obesity and after bariatric surgery. Therefore, there is an increased risk of developing iron deficiency. In obese patients, the presence of low-grade chronic inflammation may negatively affect iron absorption by increasing hepcidin expression as mentioned earlier [15]. Hepcidin will bind to the transport protein ferroportin, which is responsible for the transfer of iron (Fe^{2+}) from the enterocyte into the plasma, and will trigger internalization and degradation of ferroportin. Therefore, the low-grade chronic inflammation will attenuate iron absorption in obese patients [16]. After bariatric surgery, a reduced secretion of hydrochloric acid and a reduced surface area for absorption may negatively affect iron absorption, resulting in the persisting or worsening of iron deficiency after surgery [15].

However, our preliminary data does not show a decrease in the disposition of iron from an effervescent tablet after RYGB, which is in contrast with the theory of the postoperative anatomical changes and with previous studies [6-8]. The absence of a postoperative decrease in iron absorption may be explained by three mechanisms. First, there is a postoperative reduction in the level of low-grade chronic inflammation due to a reduction in adipose tissue. The latter will reduce the expression of pro-inflammatory proteins that are known to inhibit the absorption of iron through upregulating the expression of hepcidin. Therefore, the reduction in the low-grade chronic inflammation may partially counteract the reduced absorption after surgery. Second, an effervescent tablet formulation was used that eliminates the need for tablet disintegration and drug dissolution in the stomach. Previously, Miller *et al.* recommended switching tablets into a liquid drug formulation as the reduced secretion of hydrochloric acid may affect drug solubility [17]. Our preliminary data support the use of liquid

formulations containing iron in comparison to tablet formulations as it may partially counteract the reduced absorption after surgery. In addition to the formulation, the type of salt may also influence iron absorption. Previously, Rhode *et al.* performed iron absorption tests with iron gluconate in patients after gastric bypass and showed normal absorption, which was defined as more than 100% change in serum iron concentration over 3 hours after administration [18]. On the contrary, other studies with other iron salts have shown an impaired absorption of iron after RYGB [6-8]. Therefore, the use of an iron gluconate may have partially counteracted the reduced absorption after surgery. Nonetheless, it is important to state that other elements including study design and supplement formulation might as well explain the observed differences between different studies due to a lack of standardised methodologies. In our patients, both serum hepcidin concentration and TSAT continued to show a negative correlation with iron AUC_{0-24h} in both obese and RYGB patients. This could indicate that low hepcidin concentrations and low TSAT values are potentially predictive for oral iron absorption in post-RYGB-patients. Before RYGB, the same trend was observed, but no statistical significance was reached. The lack of statistical significance could be explained by the small sample size and high variability both in iron absorption and hepcidin concentrations before RYGB.

To investigate the effect of different variables on iron absorption, a multivariate regression was conducted before and after surgery. After surgery, based on our results, it seems relevant to include other predictors such as hepcidin, age, weight, BMI, fat percentage, hemoglobin, MCV, transferrin, TSAT and ferritin. However, a multivariate regression model with a small sample size requires attention. In general, this type of statistical models needs a larger sample size than currently included in our preliminary study. In a simple linear regression model, statisticians have observed that at least 80-100 patients are needed to estimate the intercept in the model. Furthermore, this does not take into consideration the number of patients needed for each dependent variable in the model (which requires about 15 subjects per variable at a minimum)[19]. Therefore, caution is needed to avoid any form of overinterpretation of the data and potential misleading conclusions.

Nevertheless, the potential predictive value of hepcidin concentration and TSAT for oral iron absorption has already been confirmed in overweight and obese women [9]. This strengthens our hypothesis that the determination of hepcidin concentration and TSAT should be used to identify which obese patients

will benefit from the initiation of oral iron supplementation both before and post-RYGB. Based on our preliminary data, a serum hepcidin concentration cut-off of 26.8 ng/mL would enable to distinguish potential responders from non-responders. In other words, obese patients before and post-RYGB suffering from iron deficiency, who have hepcidin levels below 26.8 ng/mL could potentially benefit from oral iron supplementation, whereas hepcidin serum concentrations above 26.8 ng/mL indicate that oral supplementation could fail and thus that parenteral formulations of iron should be considered. However, further studies with a larger sample size are warranted to further substantiate this claim and to validate these proposed cut-off value for obese and bariatric patients.

Our preliminary study has strengths and weaknesses. The most important strength is the fact that we fully characterized the iron status of our patients as well as their ability to absorb a 695 mg effervescent iron gluconate supplement. Therefore, we were able to directly assess the correlation between absorption and status markers and allowed us to propose hepcidin and TSAT as possible predictive markers for absorption. A limitation of our preliminary study is the fact that we have a small sample size. Nevertheless, this study provides background data to calculate an accurate sample size to confirm our hypothesis. Furthermore, the majority of subjects were premenopausal females, who are at higher risk to develop iron deficiency. Another limitation is that only patients planned for RYGB were included. This surgery is not representative for all bariatric surgeries, but is the most performed so far [20]. Additionally, we need to take into account that a significant difference in BMI between participants and non-participants was observed. The different limitations affect the external validity of the results.

In conclusion, iron AUC_{0-24h} showed a strong negative correlation with the hepcidin concentration and TSAT of obese patients, in particular post-RYGB. Together, our preliminary data clearly support the use of oral effervescent iron supplements even post-RYGB, but only when hepcidin levels and/or TSAT are low. When hepcidin concentrations are high oral absorption of iron is unlikely to restore adequate iron levels.

Abstract

Background & aims Oral iron absorption is hampered in obese and bariatric patients, especially after Roux-en-Y gastric bypass (RYGB). As a result, iron deficiency, which is common in both patient

groups, can be difficult to treat by oral supplements, often necessitating a switch to parenteral administration. The aim of this study was to find possible predictors of the extent of absorption of an effervescent iron gluconate oral supplement, which enables to pre-emptively identify those patients in which oral supplementation is likely to fail.

Methods The pharmacokinetic properties of 695 mg effervescent iron gluconate (80 mg Fe^{2+}) were assessed in 13 obese patients (female=10; mean age $\pm$ SD: 45.2 $\pm$ 12.5years) pre- and six months post-RYGB by measuring serum iron concentrations during 24 hours and by calculating the adjusted for baseline $\text{AUC}_{0-24\text{h}}$, C_{max} and T_{max} . A multivariate regression analysis was performed to investigate the effect of hepcidin concentrations, iron and hematologic indices; personal and anthropometric characteristics on iron absorption. Subsequently, Receiver Operating Characteristic (ROC) curves were used to propose the cut-off value for hepcidin concentrations above which obese patients are unlikely to benefit from oral iron supplementation. Data are expressed as mean $\pm$ SD.

Results Low iron status persisted after surgery as there was no significant difference observed in TSAT (17.3 $\pm$ 5.2 vs. 20.2 $\pm$ 6.6%), ferritin (91.8 $\pm$ 68.6 vs. 136.2 $\pm$ 176.9 $\mu\text{g/L}$) and hepcidin concentration (32.0 $\pm$ 30.1 vs. 28.3 $\pm$ 21.3 ng/mL) after RYGB. The absorption of effervescent iron gluconate was similar pre- and post-RYGB [$\text{AUC}_{0-24\text{h,pre-RYGB}}$: 28.6 $\pm$ 10.8 $\mu\text{g/dL}\cdot\text{h}$; $\text{AUC}_{0-24\text{h,post-RYGB}}$: 27.5 $\pm$ 9.11 $\mu\text{g/dL}\cdot\text{h}$ ($P=0.84$)]. Post-RYGB, iron $\text{AUC}_{0-24\text{h}}$ showed a strong negative correlation with both hepcidin concentrations and TSAT ($R=-0.51$; $P=0.08$ and $R=-0.81$; $P=0.001$), respectively. Pre-RYGB, there was a clear trend for the same negative correlations for hepcidin concentrations and TSAT ($R=-0.47$; $P=0.11$; $R=-0.41$; $P=0.16$), respectively. Taking pre-and post-RYGB data together, the negative correlations were confirmed for hepcidin concentrations and TSAT ($R=-0.54$; $P=0.004$; $R=-0.60$; $P=0.001$), , respectively. The $\text{AUC}^{\text{ROC}}=0.87$ (95%CI 0.71; 1.00) showed an optimal sensitivity/specificity cut-off at hepcidin concentrations of 26.8 ng/mL.

Conclusions

The iron $\text{AUC}_{0-24\text{h}}$ showed a negative correlation with the hepcidin concentration and TSAT of obese patients, in particular post-RYGB. Therefore, our data support the use of hepcidin concentration and TSAT to distinguish potential responders from non-responders for iron supplementation particularly

post-RYGB. Additionally, this study showed that the pharmacokinetic properties of iron gluconate from an effervescent tablet were unaffected by RYGB-surgery.

Keywords: iron deficiency, obesity, bariatric surgery, Roux-en-Y gastric bypass, oral iron absorption, hepcidin

Highlight

- Careful monitoring of the iron status is necessary in obese and bariatric patients.
- Necessary to distinguish responders from non-responders for iron supplementation.
- Hepcidin and TSAT are predictors for iron absorption pre- and post-RYGB
- Pharmacokinetic properties of an effervescent iron tablet are unaffected by RYGB

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Conflict of interest statement

The authors declare no conflicts of interest.

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Figures

Figure 1 Mean ($\pm$ Standard Deviation) serum iron change during 24 hours after oral administration of iron gluconate before and after Roux-en-Y Gastric Bypass.

Figure 2 Spearman correlation between serum concentration of hepcidin and AUC_{0-24h} before (A) and after (B) RYGB and overall (C); and between TSAT and AUC_{0-24h}, before (D) and after RYGB (E) and overall (F).

Tables

Table 1 Laboratory methods and reference values used by the Clinical Laboratory of the University Hospitals Leuven, Belgium.

Parameter	Testing Method	Reference Value
Iron (µg/dL)	Ferrozin colorimetric analysis (Hitachi/Roche 8000c702)	50-170 µg/dL
Hemoglobin (g/dL)	Sodium lauryl sulphate colorimetric analysis (Sysmex XE 5000)	♂: 14.0-18.0 g/dL ♀: 12.0-16.0 g/dL
MCV (fL)	Electrical impedance (Sysmex XE 5000)	76.0-96.0 fL
Ferritin (µg/L)	Electrochemiluminescence immunoassay (Hitachi/Roche E-analyzer)	13.0-150.0 µg/dL
Transferrin (g/L)	Immunoturbidimetry (Hitachi/Roche Cobas 8000c702)	2.00-3.60 g/L
CRP (mg/L)	Immunoturbidimetry (Hitachi/Roche Cobas 8000c702)	≤ 5.0 mg/L

MCV: Mean Corpuscular Volume; CRP: C-Reactive Protein.

Table 2 Characteristics of the enrolled patients before and after Roux-en-Y Gastric Bypass.

Parameter^a	Before RYGB (n=13)	Post-RYGB (n=13)	<i>P</i>^b
Weight (kg)	111.8±12.7	82.2±9.1	<0.001
Body mass index (kg/m²)	39.9±3.1	29.3±2.1	<0.001
Fat mass (%)	42.4±6.3	34.3±6.9	<0.001
Hemoglobin (g/dL)	13.5±0.8	12.5±1.1	0.006
MCV (fL)	89.5±2.9	91.0±4.2	0.11
Transferrin (g/L)	2.7±0.3	2.5±0.4	0.09
TSAT (%)	17.3±5.2	20.2±6.6	0.2515
Serum ferritin (µg/L)	91.8±68.6	136.2±176.9	0.69
Hepcidin (ng/mL)	32.0±30.1	28.3±21.3	0.64
CRP (mg/L)	4.4±4.6	3.0±3.7	0.06

MCV: Mean Corpuscular Volume; TSAT: Transferrin Saturation; CRP: C-Reactive Protein.

^aData are expressed as mean±SD; ^b*P*-values were derived from Wilcoxon signed rank test.

Table 3 Pharmacokinetic results after oral administration of Losferron[®], based on the adjusted concentrations, before and after Roux-en-Y Gastric Bypass

Parameter ^a	Before RYGB	Post-RYGB	<i>P</i> ^b
AUC _{0-24h} (µg/dL*h)	28.6±10.8	27.5±9.11	0.84
C _{max} (µg/dL)	1.9±1.1	1.6±0.6	0.45
T _{max} (h)	2.9±2.6	2.7±1.7	0.92

AUC: Area Under the Curve; C_{max}: Maximum (or peak) serum concentration; T_{max}: Time at which C_{max} is reached. ^aData are expressed as mean±SD; ^b*P*-values were derived from Wilcoxon signed rank test.

Table 4 Multivariate model for the Area Under the Curve_{0-24h} before and after Roux-en-Y Gastric Bypass

Before RYGB				
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>P-value</i>
Stepwise model 1				
Constant	32.109	4.435		<0.001
Hepcidin (ng/mL)	-.111	.103	-.309	0.305
Stepwise model 2				
Constant	-523.444	196.267		0.117
Hepcidin (ng/mL)	.125	.239	.348	0.655
Age (years)	.238	.297	.275	0.506
Weight (kg)	.143	.554	.168	0.821
Body mass index (kg/m ²)	1.762	2.911	.503	0.601
Fat (%)	-2.153	.929	-1.260	0.146
Hemoglobin (g/dL)	16.993	8.692	1.280	0.190
MCV (fL)	5.035	1.859	1.353	0.114
Transferrin (g/L)	-29.372	14.447	-.912	0.179
TSAT (%)	-.819	.770	-.394	0.399
Ferritin (μg/L)	-.493	.189	-3.131	0.121
After RYGB				
	<i>B</i>	<i>SE B</i>	<i>β</i>	<i>P-value</i>
Stepwise model 1				
Constant	34.814	3.738		<0.001
Hepcidin (ng/mL)	-.250	.112	-.578*	0.049
Stepwise model 2				
Constant	-58.848	6.682		0.072
Hepcidin (ng/mL)	-.263	.005	-.609	0.012
Age (years)	.163	.009	.228	0.036
Weight (kg)	-.860	.011	-.880	0.008
Body mass index (kg/m ²)	2.092	.063	.493	0.019
Fat (%)	.355	.016	.277	0.028
Hemoglobin (g/dL)	.987	.154	.106	0.098

MCV (fL)	-4.475	.036	-.216	0.049
Transferrin (g/L)	38.674	.709	1.678	0.012
TSAT (%)	.281	.023	.154	0.053
Ferritin (µg/L)	.129	.002	.814	0.012

MCV: Mean Corpuscular Volume; TSAT: Transferrin Saturation; *P*-values were derived from multivariate regression.