In overweight and obese women, dietary iron absorption is reduced and the enhancement of iron absorption by ascorbic acid is one-half that in normal-weight women

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ABSTRACT

Background: Iron deficiency is common in overweight and obese individuals. This deficiency may be due to adiposity-related inflammation that increases serum hepcidin and decreases dietary iron absorption. Because hepcidin reduces iron efflux from the basolateral enterocyte, it is uncertain whether luminal enhancers of dietary iron absorption such as ascorbic acid can be effective in overweight and obese individuals.

Objective: In this study, we compared iron absorption from a meal with ascorbic acid (+AA) and a meal without ascorbic acid (−AA) in women in a normal-weight group (NW) with those in overweight and obese groups combined (OW/OB).

Design: Healthy, nonanemic women [n = 62; BMI (in kg/m²): 18.5–39.9] consumed a stable-isotope–labeled wheat-based test meal −AA and a wheat-based test meal +AA (31.4 mg ascorbic acid). We measured iron absorption and body composition with the use of dual-energy X-ray absorptiometry, blood volume with the use of a carbon monoxide (CO)–rebreathing method, iron status, inflammation, and serum hepcidin.

Results: Inflammatory biomarkers (all P < 0.05) and hepcidin (P = 0.08) were lower in the NW than in the OW/OB. Geometric mean (95% CI) iron absorptions in the NW and OW/OB were 19.0% (15.2%, 23.5%) and 12.9% (9.7%, 16.9%) (P = 0.049), respectively, from −AA meals and 29.5% (23.3%, 38.2%) and 16.6% (12.8%, 21.7%) (P = 0.004), respectively, from +AA meals. Median percentage increases in iron absorption for −AA to +AA meals were 56% in the NW (P < 0.001) and 28% in the OW/OB (P = 0.006). Serum ferritin [R² = 0.22; β = −0.17 (95% CI: −0.25, −0.09)], transferrin receptor [R² = 0.23; β = 2.79 (95% CI: 1.47, 4.11)], and hepcidin [R² = 0.13; β = −0.85 (95% CI: −1.41, −0.28)] were significant predictors of iron absorption.

Conclusions: In overweight and obese women, iron absorption is two-thirds that in normal-weight women, and the enhancing effect of ascorbic acid on iron absorption is one-half of that in normal-weight women. Recommending higher intakes of ascorbic acid (or other luminal enhancers of iron absorption) in obese individuals to improve iron status may have a limited effect. This trial was registered at clinicaltrials.gov as NCT01884506.


Keywords: inflammation, iron absorption, obesity, overweight, stable isotopes

INTRODUCTION

Iron depletion is common in obese subjects (1–14). The hypoferremia of obesity may be caused by higher circulating hepcidin that is stimulated by subclinical inflammation; serum hepcidin may originate from both adipose tissue and the liver (15, 16). Hepcidin, which is the master regulator of iron metabolism, is a 25–amino acid protein that is produced mainly in the liver in response to iron stores and inflammation (17). Hepcidin interacts with ferroportin, which is the cellular iron exporter expressed on intestinal cells and macrophages, and regulates the iron flow from cells into the circulation (18). Thus, in states of high hepcidin concentrations, intestinal iron absorption (through enterocytes) and the recycling of iron from senescent red cells (through macrophages) are reduced, thereby depriving developing erythroblasts of an iron supply. An array of pro-inflammatory cytokines and adipokines are increased in the obese state including leptin, TNF-α, IL-6, and others (19). Leptin and IL-6 directly mediate the hypoferremia of inflammation by inducing hepcidin synthesis (20). Consequently, obese subjects have increased hepcidin expression (9, 21–25), which may lead to reduced iron absorption and, eventually, to iron deficiency (26). We have previously shown associations between iron absorption and body weight in the normal-weight to overweight range [BMI (in kg/m²) =27] (27) and also relations between overweight, hepcidin, and iron status (22). In additional, weight loss has been associated with reductions in hepcidin concentrations that may improve functional iron status (28). However, the direct influence of overweight and obesity on iron metabolism remains unclear.

The extent to which nonheme iron is absorbed from the diet is influenced by the composition of the diet (29). Ascorbic acid...
(AA)\textsuperscript{5} is a potent enhancer of nonheme iron absorption (30–33), likely through a luminal reduction of dietary ferric iron (Fe\textsuperscript{3+}) to more soluble ferrous iron (Fe\textsuperscript{2+}) (29, 34, 35). However, because hepcidin reduces iron efflux at the basolateral membrane of the enterocyte, it is unclear whether luminal enhancers of iron absorption would be effective in overweight and obese subjects. Data on the effects of AA on iron absorption in obesity are needed to be able to provide dietary recommendations to improve iron bioavailability in obese subjects. Thus, the purpose of the current study was to evaluate the influence of body weight and body composition on iron absorption from a standardized test meal with ascorbic acid (+AA) and a standardized test meal without ascorbic acid (−AA).

METHODS

Subjects

We recruited 64 women for the study as follows: 24 normal-weight women (BMI: 18.5–24.9), 20 overweight women (BMI: 25–29.9), and 20 obese women (BMI: 30–39.9). No men were recruited because iron absorption is generally higher in women because of lower iron stores and higher losses during menstruation. In a screening visit, we informed interested volunteers about the study procedure, and written informed consent was obtained from all women after they were given a full oral and written description of the aims and procedures of the study and associated risks. The study criteria were assessed with the use of a questionnaire, weight and height were measured to determine BMI, and a pregnancy test was carried out. Inclusion criteria for the study were as follows: 1) female, 2) age 18–45 y, 3) premenopausal (no absence of a menstrual cycle in the past 12 mo), 4) BMI from 18.5 to 39.9, 5) no chronic illness and no significant medical conditions that could influence iron or inflammation status other than obesity, 6) nonsmoking (or at least ≤1 cigarette/wk and not smoking 3 d before the first measurement and during the study), and 7) nonpregnant and not planning a pregnancy. The protocol was approved by the ethics committees of the Swiss Federal Institute of Technology and the Ethics Committee of the Canton of Zurich (clinicaltrials.gov; NCT01884506).

Sample-size calculation

The sample-size calculation was based on the expected differences between normal-weight and obese subjects in iron absorption. On the basis of the differences in hepcidin concentrations observed in previous studies (22, 24), a difference in iron absorption of 30–40% was expected. The use of an SD for the log of the difference of fractional iron absorption of 0.25 (this was a mean value calculated from a large series of iron-absorption studies that were carried out at the Human Nutrition Laboratory, Swiss Federal Institute of Technology), an expected difference in absorption of 35%, a power of 80%, and an α-level of 0.05 resulted in a required sample size of 22 subjects/group. With an expected 10% noncompletion rate taken into account, we aimed for a sample size of 25 subjects/group.

Study design

Subjects reported fasting to the Human Nutrition Laboratory (no food for 12 h and no drinks after midnight) on 2 consecutive mornings. At the first visit (day 1), anthropometric measurements were conducted, after which subjects consumed a first labeled test meal that was consumed under standardized conditions and close supervision. The next day (day 2), subjects consumed the second labeled test meal under the same conditions. The order of the meals (−AA and +AA) was randomly assigned in all subjects. The test meal −AA consisted of a standardized meal labeled with 5 mg \textsuperscript{55}Fe as ferrous sulfate (FeSO\textsubscript{4}) and served as the reference meal, and the test meal +AA consisted of the same meal but labeled with 5 mg \textsuperscript{59}Fe as FeSO\textsubscript{4} as well as AA at a molar ratio of AA:iron of 2:1 (31.4 mg) as an iron-absorption enhancer. The ratio of AA and iron used has been suggested to effectively enhance the iron absorption from meals with low to medium amounts of iron-absorption inhibitors (36). The isotopes (and AA) were added to the test meal just before consumption. The additional iron provided by bread, butter, and honey amounted to 1.4 mg/meal.

The standardized test meal consisted of white bread rolls with butter and honey and was bought in a single large batch. The bread and butter were frozen and thawed the evening before the test-meal administration. A stock solution of food-grade l-AA was prepared so that 1 mL stock solution corresponded to the 2:1 molar ratio of AA to added iron. One milliliter of the AA stock solution was pipetted onto the bread rolls with the isolate solution. After ingestion of the test meal, subjects were asked not to eat or drink for 3 h to minimize the impact of additional foodstuffs on iron absorption. Fourteen days after the second test meal was consumed (day 16), a fasting blood sample was obtained for an analysis of hemoglobin and erythrocyte isotopic composition as well as for iron status, inflammation, and hepcidin determination. Within 2 wk before and after the administration of the test meal, all subjects were scheduled to undergo dual-energy X-ray absorptiometry for the determination of body compositions at University Zurich Irchel and a blood volume measurement with the use of the carbon monoxide (CO)–rebreathing method at the University Clinic Balgrist in Zurich. To minimize fluctuations in blood volume, all measurements were, if possible, performed between 7 and 14 d after the beginning of the last menstrual cycle.

Anthropometric and body-composition determinations

Body weight (kg) was measured to the nearest 0.1 kg on a digital scale, and height (m) was measured to the nearest 1.0 cm with the use of a stadiometer according to standardized procedures (37). Total body and segmental (arms, legs, trunk, and gluteofemoral and abdominal region) fat and lean mass were determined with the use of dual-energy X-ray absorptiometry (Lunar iDXA; GE Healthcare) at the University of Zurich, Irchel. Visceral and subcutaneous adipose tissues at the level of the abdomen were estimated with the use of the Lunar iDXA CoreScan function (GE Healthcare).

\textsuperscript{5}Abbreviations used: AA, ascorbic acid; −AA, without ascorbic acid; +AA, with ascorbic acid; AGP, α-1 glycoprotein; BMP-HJV, bone morphogenetic protein-hemojuvelin; CO, carbon monoxide; CRP, C-reactive protein; mRNA, messenger RNA; NW, normal-weight group; OW/OB, overweight and obese groups combined; SF, serum ferritin; TIR, transferrin receptor; TIBC, total iron-binding capacity.
Blood volume determination

The optimized CO-rebreathing method (38) is a routinely applicable, minimally invasive, reliable method for the assessment of blood volume (39). This method has been described in detail elsewhere (38, 40, 41). Briefly, baseline venous samples were obtained (2 mL in an tube containing EDTA) 15 min after the adoption of a seated position for hemoglobin and hematocrit determination. All blood samples were immediately analyzed with the use of a spectrophotometer for the blood gas determination (ABL 700 Serie; Radiometer A/S). Furthermore, capillary blood samples from the earlobe were obtained (35 μL in preheparinized glass capillary tubes) in triplicate at baseline and at 6, 7, and 8 min after the start of the rebreathing procedure. The percentage of carboxyhemoglobin saturation was measured with the use of a blood gas analyzer. The mean value of measurements at 6 and 8 min was taken as the plateau value after CO rebreathing with the sample at minute 7 as a backup. Total hemoglobin mass was calculated as described previously (42) with the use of a slightly different correction for the loss of CO to myoglobin (0.3%/min of administered CO) (38). A bolus of chemically pure CO of 0.8 mL/kg body mass was administered during the first inspiration from a closed spirometric system (Blood tec GbR) and was rebreathed for 1 min and 50 s together with a small amount of oxygen (4 L). To verify that no gas was leaking during the CO-rebreathing procedure, the entire apparatus as well as the mouth piece and nose clip were checked with the use of a portable CO-gas analyzer (Dräger PAC 7000; Dräger Safety) with a parts-per-million sensitivity to monitor local CO concentrations. The analyzer was also used to calculate end-tidal CO concentrations before the CO-rebreathing and after the onset of the rebreathing procedure with the subject wearing a nose-clip and blowing into a mouthpiece until the maximal value of CO observed was recorded. The amount of CO remaining in the spirometer after rebreathing was also measured with the portable CO gas analyzer. The determined variables were first used to calculate total hemoglobin mass, and from this calculation, blood, plasma and red blood cell volumes were derived with the use of equations published previously (38, 40, 42).

Preparation of isotopically labeled iron

Isotopic labels $^{57}$Fe-FeSO$_4$ and $^{56}$Fe-FeSO$_4$ were prepared from isotopically enriched elemental iron by dissolution in diluted sulfuric acid. The solutions were stored in polytetrafluoroethylene containers and flushed with argon to keep the iron in the +2 oxidation state. The preparation of isotopic labels was done according to the method described by Walczyk et al. (43).

Laboratory analysis

Hemoglobin concentrations were assessed with the use of a Coulter counter (Beckman Coulter) with 3-level quality-control material (Liquichek; Bio-Rad) on the day of blood collection. Serum iron and total iron-binding capacity (TIBC) were determined by colorimetry as described previously (44, 45). Serum transferrin receptor (TfR), serum ferritin (SF), and high-sensitive C-reactive protein (CRP) and α-1 glycoprotein (AGP) were assessed with the use of an automated chemiluminescent immunoassay system (Immulite; Diagnostic Products Corp.). IL-6 was measured with the use of a Quantikine ELISA kit (R&D Systems), and hepcidin concentrations were determined with the use of a commercial ELISA kit (Bachem). Iron-deficiency anemia was defined as a hemoglobin concentration $<12$ g/dL and SF concentration $<15 \mu$g/L or TIR concentration $>8.5 \text{ mg/L}$, and iron deficiency without anemia was defined as a hemoglobin concentration $>12 \text{ g/dL}$ and SF concentration $<15 \mu$g/L or TIR concentration $>8.5 \text{ mg/L}$ (46).

For the isotope analysis, whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography and a subsequent solvent-solvent extraction step into diethylether. The isotopic analysis of $^{58}$Fe and $^{57}$Fe were performed with the use of inductively coupled plasma mass spectrometry with a high-resolution double focusing mass spectrometer (Neptune; Thermo-Finnigan) equipped with a multicollector system for simultaneous ion-beam detection (43). The calculation of the amount of isotopic label present in the blood of the subject was based on the shift of the isotopic ratios in the blood after red cell incorporation of the absorbed isotopic label. When the circulating amount of isotopic label was known, the amount of label absorbed from the test meal and, thus, the fractional iron absorption could be calculated (43). The amount of natural iron circulating in the blood was calculated on the basis of the blood volume determined by the CO-rebreathing method and the hemoglobin concentration in the blood (38). The estimation of fractional iron absorption was based on the assumption of an 80% incorporation of absorbed iron into the erythrocytes. The observed shift in iron isotope ratios was converted to fractional iron absorption with the use of standard algorithms (43).

Statistical analysis

All statistical analyses were conducted with the use of IBM SPSS Version 20 software (IBM Co.). Data were checked for normal distribution (as assessed with the use of Kolmogorov-Smirnov and Levene’s normality testing) and for the presence of outliers (±3 SDs from the mean). Nonnormally distributed data were logarithmically transformed for statistical analyses. Two subjects (one overweight subject and one obese subject) did not complete the study measurements and, therefore, were excluded from all analyses. The mean ± SD (for normally distributed data), geometric mean (95% CI) (for data with normal distribution after log transformation), or median (IQR) (for nonnormally distributed data even after log transformation) values for each variable were determined. Nonparametric tests were applied for data that remained nonnormally distributed after logarithmic transformation. Comparisons between all BMI groups (NW, overweight, and obese) were conducted with the use of an ANOVA with post hoc Bonferroni correction and the Kruskal-Wallis test followed by the Mann-Whitney U test as appropriate. Independent sample t tests and nonparametric Mann-Whitney U tests were used to compare variables between NW and OW/OB. Paired Student’s t tests or Wilcoxon’s signed rank tests were used to test differences in iron absorption from the 2 test meals (−AA and +AA) within BMI groups. A linear mixed-effect model analysis was used to investigate the effect of AA and body fat on iron absorption. The BMI group, meal type (−AA and +AA), and interaction between the 2 variables were defined as fixed factors.
The subject identifier was considered as the random factor, the variance component was considered as the covariance type, and the maximum likelihood was considered as the estimation. A 1-factor ANCOVA was conducted to determine the difference between BMI groups (NW, overweight, and obese) on iron absorption with confounders (age and TfR) controlled for. Because overweight and obese subjects were shown to have similar fractional iron-absorption rates, these groups were combined for some of the analyses. Bivariate Pearson or Spearman correlations and multiple linear regression models, including correction for confounding variables, were used to study associations between continuous variables. Differences were considered significant at \( P < 0.05 \).

RESULTS

Anthropometric characteristics, body composition, inflammation, and iron-status indexes by BMI groups are summarized in Table 1. Sixty-two subjects completed the study (NW: \( n = 24 \); overweight: \( n = 19 \); and obese: \( n = 19 \)). The age range was 20–44 y, and women in the OW/OB had a significantly higher median age (27 y; range: 23–31 y) than that of women in the NW (23 y; range: 22–26 y). Only 4 subjects presented with iron-deficiency anemia (NW: \( n = 2 \); overweight: \( n = 1 \); and obese: \( n = 1 \)). Hemoglobin, TIR, and SF were in the normal range and comparable between the 3 BMI groups, whereas serum iron (\( P = 0.025 \)) and TIBC (\( P = 0.04 \)) were significantly lower in the obese group than in the NW. Figure 1 shows differences in inflammation and serum hepcidin between BMI groups. As shown in Table 1, fractional iron absorption from the –AA meal was 19.0% in NW compared with 12.2% in the overweight group, and 13.6% in the obese group. With the +AA meal, iron absorption increased to 29.5% in the NW compared with 14.9% in the overweight group and 18.4% in the obese group. In a linear mixed-effect model analysis, both the meal type (\( P < 0.001 \)) and the interaction of the meal type and BMI group (\( P = 0.008 \)) significantly predicted iron absorption, but the BMI group did not (\( P = 0.08 \) data not shown). After age and TIR were controlled for, there was a significant difference between the 3 BMI groups (NW, overweight, and obese) in iron absorption from the test meal –AA (\( P = 0.02 \)). Because iron absorption was very similar in overweight and obese groups, we decided to combine them for some additional analyses. The geometric mean (95% CI) iron absorption from the –AA meal in women in the OW/OB was 12.9% (9.7%, 16.9%) (compared with the NW, \( P = 0.049 \)), and the geometric mean (95% CI) was 16.6% (12.8%, 21.7%) from the +AA meal (compared with the NW, \( P = 0.004 \)) (Figure 2). Thus, iron absorption in the –AA meal in the OW/OB was 68% of that in the NW (\( P = 0.049 \)). The median percentage of change in iron absorption for the comparison of –AA to +AA was 56% in the NW (\( P < 0.001 \)) and 28% in the OW/OB (\( P = 0.006 \)), which represented a significantly higher increase with +AA in women in the NW (\( P = 0.004 \)).

Table 1

Baseline characteristics of the study population of apparently healthy women in NW, overweight, obese, and OW/OB groups participating in the iron-absorption study.  

<table>
<thead>
<tr>
<th></th>
<th>NW</th>
<th>Overweight</th>
<th>Obese</th>
<th>OW/OB</th>
</tr>
</thead>
<tbody>
<tr>
<td>( n )</td>
<td>24</td>
<td>19</td>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>Age, y</td>
<td>( 23 ) ( (22–26) ) ( ^{bc} )</td>
<td>( 26 ) ( (22–29) )</td>
<td>( 27 ) ( (23–33) )</td>
<td>( 27 ) ( (23–31) )</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61.8</td>
<td>73.7 ( ^{a} )</td>
<td>89.0 ( ^{a} )</td>
<td>83.0 ( ^{a} )</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>21.9</td>
<td>27.3 ( ^{a} )</td>
<td>32.8 ( ^{a} )</td>
<td>30.1 ( ^{a} )</td>
</tr>
<tr>
<td>Total body fat, %</td>
<td>29.8</td>
<td>40.1 ( ^{b} )</td>
<td>45.8</td>
<td>43.0 ( ^{b} )</td>
</tr>
<tr>
<td>Blood volume, mL/kg</td>
<td>74.9</td>
<td>64.3 ( ^{c} )</td>
<td>59.4 ( ^{c} )</td>
<td>61.8 ( ^{c} )</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>13.5</td>
<td>13.7 ( ^{a} )</td>
<td>13.7 ( ^{a} )</td>
<td>13.7 ( ^{a} )</td>
</tr>
<tr>
<td>Serum iron, ( \mu )g/mL</td>
<td>1.06 ( ^{b} )</td>
<td>1.01 ( ^{b} )</td>
<td>0.76 ( ^{b} )</td>
<td>0.89 ( ^{b} )</td>
</tr>
<tr>
<td>TIBC, ( \mu )g/mL</td>
<td>3.68 ( ^{a} )</td>
<td>3.62 ( ^{a} )</td>
<td>3.17 ( ^{a} )</td>
<td>3.39 ( ^{a} )</td>
</tr>
<tr>
<td>TIR, mg/L</td>
<td>6.61 ( (5.86, 7.45) )</td>
<td>6.69 ( (5.82, 7.68) )</td>
<td>7.00 ( (5.90, 8.30) )</td>
<td>6.84 ( (6.16, 7.59) )</td>
</tr>
<tr>
<td>Serum ferritin, ( \mu )g/L</td>
<td>50.6 ( (40.2, 63.4) )</td>
<td>59.9 ( (41.3, 86.8) )</td>
<td>62.8 ( (45.4, 86.9) )</td>
<td>61.35 ( (48.5, 77.6) )</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>1.05 ( (0.60, 1.85) )</td>
<td>2.23 ( (1.23, 4.02) )</td>
<td>3.22 ( (1.77, 5.86) )</td>
<td>2.68 ( (1.78, 4.01) )</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.69 ( (0.53, 0.91) )</td>
<td>0.75 ( (0.57, 1.00) )</td>
<td>1.25 ( (1.03, 1.53) )</td>
<td>0.97 ( (0.81, 1.17) )</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>0.79 ( (0.23) )</td>
<td>0.90 ( ^{b} )</td>
<td>1.00 ( ^{b} )</td>
<td>0.95 ( ^{b} )</td>
</tr>
<tr>
<td>Hepcidin, ng/mL</td>
<td>9.20</td>
<td>11.6 ( ^{b} )</td>
<td>12.4 ( ^{b} )</td>
<td>11.92 ( ^{b} )</td>
</tr>
<tr>
<td>Iron absorption, %</td>
<td>19.0 ( (15.2, 23.5) )</td>
<td>12.2 ( (9.1, 17.0) )</td>
<td>13.6 ( (8.8, 21.0) )</td>
<td>12.9 ( (9.7, 16.9) )</td>
</tr>
</tbody>
</table>

\(^{1}\)BMI ranges were as follows: NW, 18.5–24.9; overweight, 25–29.9; and obese, 30–39.9. Differences between NW, overweight, and obese groups were assessed with the use of 1-factor ANOVA with post hoc Bonferroni correction and the Kruskal-Wallis test followed by an independent-samples Mann-Whitney \( U \) test corrected for multiple comparisons. Differences between NW and OW/OB groups were assessed with the use of an independent samples \( t \) test. Significantly different from overweight, \( P < 0.05 \); \(^{a}\)significantly different from obese, \( P < 0.05 \); \(^{b}\)significantly different from OW/OB, \( P < 0.05 \); and \(^{c}\)significantly different from the meal –AA in the same BMI group, \( P < 0.05 \). AGP, \( \alpha \)-1 glycoprotein; CRP, C-reactive protein; NW, normal weight; OW/OB, overweight and obese combined; TIR, transferrin receptor; TIBC, total iron-binding capacity; –AA, meal without ascorbic acid (control meal); +AA, meal with ascorbic acid at a molar ratio of ascorbic acid:iron of 2:1.

\(^{2}\)Median; IQR in parentheses (all such values).

\(^{3}\)Mean \( \pm \) SD (all such values).

\(^{4}\)Geometric mean; 95% CI in parentheses (all such values).
BMI was negatively correlated with serum iron ($r = -0.37$, $P = 0.003$) and TIBC ($r = -0.34$, $P = 0.007$) and positively correlated with all 3 of the following inflammatory markers: CRP ($r = 0.39$, $P = 0.002$), IL-6 ($r = 0.38$, $P = 0.003$), and AGP ($r = 0.43$, $P = 0.001$) (data not shown). There was no correlation between BMI and SF, TfR, or hepcidin. The percentage of body fat was negatively correlated with serum iron ($r = -0.38$, $P = 0.002$) and TIBC ($r = -0.33$, $P = 0.009$) and positively correlated with SF ($r = 0.26$, $P = 0.04$), all 3 inflammatory markers [CRP ($r = 0.42$, $P = 0.001$), IL-6 ($r = 0.34$, $P = 0.006$), and AGP ($r = 0.47$, $P < 0.001$)], and hepcidin ($r = 0.27$, $P = 0.03$) but not with hemoglobin and TfR (Table 2). CRP was positively correlated with AGP ($r = 0.26$, $P = 0.039$), IL-6 ($r = 0.36$, $P = 0.004$), and ferritin ($r = 0.32$, $P = 0.01$) (data not shown). Moreover, serum hepcidin was significantly correlated with hemoglobin ($r = 0.41$, $P = 0.001$), TIBC ($r = -0.37$, $P = 0.003$), SF ($r = 0.59$, $P < 0.001$), TfR ($r = -0.29$, $P = 0.02$), and AGP ($r = 0.29$, $P = 0.02$) but not with serum iron, CRP, and IL-6 (Table 2). In multiple linear regression analyses, (log)SF ($\beta = 0.59$, $P < 0.001$) and (log)hemoglobin ($\beta = 0.27$, $P = 0.006$) were significant predictors of (log)serum hepcidin ($r^2 = 0.58$). When (log)AGP ($\beta = 0.09$, $P = 0.31$), (log)TIBC ($\beta = -0.11$, $P = 0.29$), and (log)TfR ($\beta = -0.03$, $P = 0.75$) were added to the regression model, they appeared not to be independent predictors of the hepcidin concentration and did not change the predictive power of the model ($r^2 = 0.58$).

Iron absorption (−AA) was correlated significantly with hemoglobin ($r = -0.27$, $P = 0.03$), SF ($r = -0.58$, $P < 0.001$), TfR ($r = 0.35$, $P = 0.006$), CRP ($r = -0.26$, $P = 0.04$), and hepcidin ($r = -0.36$, $P = 0.004$) but not with body fat, serum iron, IL-6, and AGP (Table 2). There was also no significant correlation between iron absorption and age (−AA: $r = 0.125$, $P = 0.334$; +AA: $r = -0.044$, $P = 0.733$) (data not shown). 

**Figure 1** CRP (A), IL-6 (B), AGP (C), and serum hepcidin (D) concentrations in 3 BMI groups. Error bars are means ± SDs (for serum hepcidin and AGP) or geometric means (95% CIs) (for IL-6 and CRP). Comparisons between all BMI groups were conducted with the use of a 1-factor ANOVA with post hoc Bonferroni correction ($n = 62$). AGP, alpha 1 glycoprotein; CRP, C-reactive protein; NW, normal-weight group; OB, obese group; OW, overweight group.
predictors of iron absorption \( R^2 = 0.30, \beta = -0.58 \) (95% CI: 
-1.14, -0.03). However, when either CRP or SF was included in the model, hepcidin was no longer an independent predictor of iron absorption (data not shown), SF \( R^2 = 0.22, \beta = -0.17 \) (95% CI: 
-0.25, -0.09), TfR \( R^2 = 0.23, \beta = 2.79 \) (95% CI: 
1.47, 4.11), and hepcidin \( R^2 = 0.13, \beta = -0.85 \) (95% CI: 
-1.41, -0.28) were significant predictors of iron absorption.

**DISCUSSION**

In this study, we showed that dietary iron absorption was lower in women in the OW/OB than in the NW, and the enhancing effect of AA on iron absorption in the OW/OB was only half of that in the NW. There is expanding evidence that obesity and iron deficiency are linked (15, 47). It appears that the hypoferremia of obesity is not associated with dietary factors (2, 9, 22, 48), and it has been proposed that inflammation via hepcidin may be an important factor that links obesity with iron dysregulation (27). Our findings of lower serum iron and transferrin saturation are consistent with previous studies that described hypoferremia in obese subjects (6, 7). Moreover, our results showed that TfR was a better predictor of iron absorption than SF or hepcidin was in populations with different BMI. The results are also consistent with the hypothesis that increasing hepcidin concentrations, along with subclinical inflammation, limit dietary iron absorption in subjects with excessive body fat. To our knowledge, this is the first study to assess iron absorption with the use of stable isotopes over a broad range of BMI that has measured serum hepcidin concentrations and several markers of inflammation.

Our results of the association of excess weight with reduced iron absorption are in line with previous studies: In Thai women, both adiposity and inflammation (assessed with the use of CRP) were negatively correlated with iron absorption, independent of iron status, but hepcidin was not measured (27); similarly, in a recent cross-sectional study in Chilean women, obese women had lower fractional iron absorption than did overweight and normal-weight women \( P < 0.05 \), but inflammatory biomarkers and serum hepcidin concentrations were not assessed (49).

Numerous studies have reported that serum hepcidin was significantly elevated in obese compared with lean individuals, but none of the studies directly measured iron absorption (24, 28, 50, 51). In our study, there was an increasing trend in hepcidin concentrations throughout the 3 BMI groups, and the hepcidin concentration was a negative predictor of iron absorption. One important result of the current study was that only a small portion of the variability of iron absorption was associated with and, thus, potentially explained by hepcidin, with \( R^2 \) values that ranged from 0.13 to 0.30 in simple and multivariate analyses. When we included SF together with serum hepcidin, hepcidin was no longer a predictor of iron absorption. However, when TfR was included in the model, both serum hepcidin and TfR were predictors of iron absorption. These results show that, when taking into account the separate effects of age and TfR on iron absorption, hepcidin still remained a significant predictor. Thus, hepcidin might play an important role in the regulation of iron absorption regardless of iron status. We previously reported that, in 6–14-y-old children, overweight was associated with increased serum hepcidin than in a normal-weight group, and overweight children had poorer

**FIGURE 2** Geometric means (95% CIs) of fractional iron absorption from a wheat-based test meal –AA and a wheat-based test meal +AA (31.4 mg). Iron absorption in women in the NW was 19.0% (15.2%, 23.5%) from the meal –AA and 29.5% (23.3%, 38.2%) from the meal +AA \( P < 0.001 \). Iron absorption in the OW/OB was 12.9% (9.7%, 16.9%) from the meal –AA and 16.6% (12.8%, 21.7%) from the meal +AA \( P = 0.006 \). Paired \( t \) tests were used to assess differences in log iron absorption between meals within BMI groups. Independent \( t \) tests were used to assess differences in log iron absorption from each meal between the NW and OW/OB. NW, normal-weight group; OW/OB, overweight and obese groups combined; –AA, without ascorbic acid; +AA, with ascorbic acid.

**TABLE 2** Correlations between body fat, hepcidin, and iron absorption (–AA) with iron and inflammation markers in a group of women with BMI (in kg/m²) between 18.5 and 39.9 \( (n = 62) \)

<table>
<thead>
<tr>
<th></th>
<th>Body fat, %</th>
<th>Hepcidin, ng/mL</th>
<th>Iron absorption in –AA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( r )</td>
<td>( P )</td>
<td>( r )</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>—</td>
<td>0.27</td>
<td>0.03</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>0.17</td>
<td>0.18</td>
<td>0.41</td>
</tr>
<tr>
<td>Serum iron, µg/mL</td>
<td>0.38</td>
<td>0.002</td>
<td>0.22</td>
</tr>
<tr>
<td>TIBC, µg/mL</td>
<td>0.33</td>
<td>0.009</td>
<td>-0.37</td>
</tr>
<tr>
<td>TfR, mg/L</td>
<td>-0.06</td>
<td>0.67</td>
<td>-0.29</td>
</tr>
<tr>
<td>Serum ferritin, µg/L</td>
<td>0.26</td>
<td>0.04</td>
<td>0.59</td>
</tr>
<tr>
<td>Hepcidin, ng/mL</td>
<td>0.27</td>
<td>0.03</td>
<td>—</td>
</tr>
<tr>
<td>CRP, mg/mL</td>
<td>0.42</td>
<td>0.001</td>
<td>0.16</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.34</td>
<td>0.006</td>
<td>-0.01</td>
</tr>
<tr>
<td>AGP, g/L</td>
<td>0.47</td>
<td>&lt;0.001</td>
<td>0.29</td>
</tr>
</tbody>
</table>

\( ^1 \)Iron-absorption values from the control meal without ascorbic acid were used (5 mg \(^5^7\)Fe as FeSO₄). –AA, meal without ascorbic acid; AGP, α-1 glycoprotein; CRP, C-reactive protein; TIBC, total iron-binding capacity; TfR, transferrin receptor.
iron status, despite similar dietary iron intakes (22). In that study, although serum hepcidin was positively correlated with BMI and body iron, there was no relation between hepcidin and CRP, IL-6, or leptin (22). Similarly, another study showed that elevated serum hepcidin in obese women was not correlated with IL-6 or CRP (24). Another study showed that overweight impaired the efficacy of iron supplementation in iron-deficient South African children. In that study, iron-deficient children with higher BMI-for-age z scores had 2-fold higher risk of remaining iron deficient after iron supplementation (52).

The mechanism of the upregulation of hepcidin by adiposity and its influence on iron status are not fully understood. Hepcidin expression in the liver is regulated by a combination of factors including iron stores, erythropoiesis, and inflammation, which control transcription (53). Bekri et al. (21) reported that hepcidin was produced by visceral and subcutaneous adipose tissue in vitro, and hepcidin expression was positively correlated with BMI and IL-6, thereby suggesting that the liver is not the only source of increased hepcidin in obesity. However, Tussing-Humphreys et al. (54) showed that there was no net release of hepcidin from the abdominal subcutaneous adipose tissue depot in vivo in obese and lean women. Moreover, the authors reported that liver hepcidin messenger RNA (mRNA) expression was positively correlated with serum hepcidin concentrations, whereas adipose mRNA expression was not (24). Another study reported that leptin stimulated hepcidin mRNA production via the Janus kinase-2 signal transducer and activator of transcription-3 signaling pathway in human hepatoma cells (55), similar to the effect of IL-6 (17, 20). However, it has also been shown that adipose tissue may influence iron homeostasis in obese patients by the expression of major iron-regulatory proteins, and the bone morphogenetic protein-hemojuvelin (BMP-HJV) signaling pathway could be involved in the regulation of hepcidin expression in this tissue (16). Thus, the multimodal regulation of the increased hepcidin expression in obese subjects may explain the lack of correlation between hepcidin concentrations and the inflammatory markers CRP and IL-6 in our study and in other studies. The other major factor that affects hepcidin secretion by the liver is iron status (53). It is difficult to define iron status in obesity because the associated inflammation increases SF. Although, in our study, there were no differences in TfR with weight status, both serum iron and transferrin saturation were lower in obese subjects.

If the obese subjects had slightly lower iron status, this would have been a trigger to lower serum hepcidin and increase iron absorption, but this signal was likely overridden by the inflammatory pathways described previously.

The enhancing effects of AA on nonheme iron absorption can be used to overcome the effect of food components that inhibit iron absorption, such as phytic acid (29), and to enhance iron

| TABLE 3 |

<table>
<thead>
<tr>
<th>Iron absorption</th>
<th>β (95% CI)</th>
<th>P</th>
<th>β (95% CI)</th>
<th>P</th>
<th>β (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td></td>
<td>r²</td>
<td></td>
<td>r²</td>
<td></td>
</tr>
<tr>
<td>−AA</td>
<td>0.13</td>
<td>−0.85</td>
<td>(−1.41, −0.28)</td>
<td>0.004</td>
<td>0.22</td>
<td>−0.17</td>
</tr>
<tr>
<td>+AA</td>
<td>0.18</td>
<td>−1.41</td>
<td>(−2.18, −0.63)</td>
<td>0.001</td>
<td>0.26</td>
<td>−0.26</td>
</tr>
</tbody>
</table>

r² is the proportion of variance explained by the model. β represents the change in iron absorption associated with a change in hepcidin concentration of 1 ng/mL. For −AA: 5 mg 57Fe as FeSO₄. For +AA: 5mg 59Fe as FeSO₄ as well as 31.4 mg ascorbic acid at a molar ratio of ascorbic acid:iron of 2:1. TfR, transferrin receptor; −AA, without ascorbic acid; +AA, meal with ascorbic acid.
absorption in iron-depleted subjects (33). In this study, we showed, for the first time to our knowledge, that the enhancing effect of AA on nonheme iron absorption in overweight and obese individuals is blunted. This effect is likely due to the different sites of action on the enterocyte of AA and serum hepcidin in dietary iron absorption. Increased hepcidin reduces iron flux into the circulation at the basolateral membrane of the enterocyte (18). In contrast, AA improves the transport of iron into enterocytes through their apical membrane (via the divalent metal transporter 1) by reducing Fe$^{3+}$ to Fe$^{2+}$ (56). Thus, an explanation of our findings could be that, although AA allows more iron to enter the enterocyte, increased hepcidin reduces its efflux into the circulation, and when the enterocytes shed from the luminal surface, the iron is lost. These findings argue against recommending increased intakes of AA in obese individuals to improve iron status.

Strengths of the current study were as follows: 1) the assessment of fractional iron absorption over a wide BMI range; 2) the direct measurement of blood volume that allowed for a more-precise calculation of iron absorption with the use of stable isotopes, which was particularly valuable in obese subjects in whom the estimation of blood volume was challenging; and 3) the measurement of most factors known to influence iron absorption, including hepcidin, iron status, IL-6, leptin, and CRP. However, there were also some limitations to the study. Because we only used oral isotopic labels, we were not able to directly determine the iron incorporation into erythrocytes, but we assumed that it was the same for all subjects (80%). In a study in subjects with afebrile malaria, it was previously shown that, although iron absorption was reduced significantly before malaria treatment, incorporation was not affected (57). On the basis of this and other earlier studies in different subjects, we concluded that iron incorporations seems to be relatively constant (58, 59). We only studied women in Switzerland in this trial. Whether the results would have been the same in men and in other ethnicities remains to be shown.

In conclusion, the widespread increase in overweight and obesity may limit current dietary strategies to improve iron absorption in iron-deficient women, especially in certain low- and middle-income countries where both conditions are prevalent. Additional research is needed to better understand the underlying mechanisms that reduce iron absorption in overweight individuals to develop specific dietary recommendations to improve iron status in this population group.

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The authors’ responsibilities were as follows—ACC-L: wrote the manuscript draft; ACC-L and IH-A: conducted the research, data collection, and analysis and performed the statistical analysis; AM-B, MBZ, and IH-A: provided critical revision of the manuscript; and all authors: contributed to the study design and the writing and editing of the manuscript and read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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