



Clinical Research

# The effect of central obesity on inflammation, hepcidin, and iron metabolism in young women

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## Abstract

**Background/objectives** In overweight and obesity (OW/OB), greater total body fat predicts higher serum hepcidin (SHep) which can impair iron homeostasis and increase risk for iron deficiency (ID). However, the effect of body fat distribution on SHep and iron homeostasis is unclear. In central obesity, interleukin (IL)-6 released from visceral adipose tissue into portal blood could strongly stimulate hepatic hepcidin synthesis. Thus, our hypothesis was that higher amounts of android fat, rather than gynoid fat, would predict impaired iron metabolism in OW/OB.

**Subjects/methods** In this cross-sectional study, we enrolled 117 otherwise-healthy women into two groups: normal weight; BMI < 25 ( $n = 36$ ) and OW/OB; BMI  $\geq 25$  ( $n = 81$ ); we then subdivided the OW/OB using DEXA into tertiles based on the ratio of android fat/total body fat (AF/TBF). We measured inflammation and iron status, and assessed iron absorption in two ways: by measuring erythrocyte isotope incorporation from a labeled test meal containing 6 mg <sup>57</sup>Fe (representing dietary iron); and by measuring change in serum iron ( $\Delta$ SeFe) after a 100 mg oral iron challenge (representing supplemental iron).

**Results** Greater AF/TBF correlated with higher CRP, AGP, SHep, and TIBC, and lower transferrin saturation and SeFe/SHep ratio (for all,  $p < 0.05$ ). Greater AF/TBF correlated with lower supplemental iron absorption ( $\Delta$ SeFe) ( $p = 0.08$ ) but not lower dietary iron absorption. In multiple regressions, AF/TBF positively predicted CRP ( $p < 0.001$ ) and SHep ( $p < 0.05$ ); a model including AF/TBF and serum ferritin as covariates explained 65% of the variance in SHep. AF/TBF negatively predicted TSAT ( $p < 0.05$ ) and iron absorption ( $\Delta$ SeFe) ( $p = 0.07$ ). In contrast, the ratio of gynoid fat/total body fat was not significantly associated with these variables.

**Conclusion** Body fat distribution affects iron metabolism: women with greater central adiposity have higher SHep, greater impairments in iron homeostasis, and reduced iron absorption from a supplemental iron dose.

## Introduction

Obesity alters iron metabolism: in adults and children, obesity is linked to hypoferremia, impaired iron absorption

and lower iron stores despite adequate dietary iron intake [1–6]. These impairments are likely due to high-circulating interleukin (IL)-6 concentrations in obesity [2] that increase hepatic hepcidin expression through the JAK-STAT signaling pathway in the liver [7]. Higher serum hepcidin (SHep) reduces ferroportin-mediated export of iron from macrophages and duodenal enterocytes, resulting in hypoferremia and decreased dietary iron absorption [8]. Concentrations of serum IL-6 and SHep are sharply higher in overweight and obese (OW/OB) individuals compared with normal-weight individuals [1, 9–11]. Weight loss in iron-deficient OW/OB women decreases systemic inflammation and SHep [12], and improves dietary iron absorption [2].

Although increased BMI and total body fat (TBF) predict impaired iron homeostasis, the effect of body fat distribution on iron metabolism is unclear. Compared with peripheral fat, visceral adipose tissue (VAT) is more heavily infiltrated by

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macrophages producing inflammatory cytokines, including IL-6 [13]. VAT secretes IL-6 into the portal circulation that drains directly to the liver [14]. It is possible that high concentrations of IL-6 in portal blood in central obesity strongly and directly stimulate hepatic hepcidin synthesis. Independent of BMI and TBF, increased VAT is a stronger predictor of systemic inflammation [15] and higher risk of cardiometabolic disorders than fat depots in other anatomic regions [15, 16]. In countries affected by the double burden of malnutrition, up to 30% of young women are affected by both central obesity and iron deficiency (ID) [17].

Therefore, our study aim was to assess the relationships between body fat distribution, inflammation, SHep, and iron homeostasis in young women. Our hypotheses were: (1) greater android fat (AF) would be a positive predictor of inflammation and SHep, and a negative predictor of iron absorption, serum iron, and transferrin saturation; and (2) greater gynoid fat would not predict these variables. We assessed body fat distribution by using dual-energy X-ray absorptiometry (DEXA) and iron absorption by administering stable iron isotopes in a test meal (at a dietary level of iron) as well as change in serum iron after an oral iron challenge (at a supplementation level of iron).

## Methods

### Subjects

Subjects were recruited from the students and staff of the American University of Beirut or through physicians at the American University of Beirut Medical Center, Lebanon. We recruited young women because they are a group at high risk of ID. We screened 220 women; 99 women did not meet the inclusion criteria, mainly due to chronic diseases or medications, and were excluded (Fig. 1, study design). At screening, we administered a questionnaire and we measured weight and height. Inclusion criteria for the study were as follows: (1) female, (2) age 18–55 years, (3) BMI from 18.5 to 50.0 kg/m<sup>2</sup>, (4) no chronic illness and no significant medical conditions that could influence iron or inflammatory status other than obesity, (5) nonsmoking, and (6) nonpregnant and not planning a pregnancy, (7) no iron supplement intake within 2 weeks before study start, (8) no regular use of medication (except oral contraceptives), (9) no blood donation or surgery within the last 4 months. We obtained written informed consent from all women. The ethics committees of the ETH Zurich, Switzerland and the Ethics Committee of the American University of Beirut, Lebanon approved the protocol and it was registered at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT 03642223).

### Sample size calculation

To detect a 40% difference in iron absorption between normal-weight and OW/OB subjects, with an SD for the log of the difference of fractional iron absorption (FIA) of 0.25 (calculated from a large series of iron absorption studies at the ETH Zurich), a power of 80%, and an  $\alpha$ -level of 0.05, we required the sample size that was 25 subjects per group. Anticipating a 20% noncompletion rate, we aimed for a sample size of 30 subjects in the normal-weight group and 30 subjects in each tertile of AF/TBF in the OW/OB group.

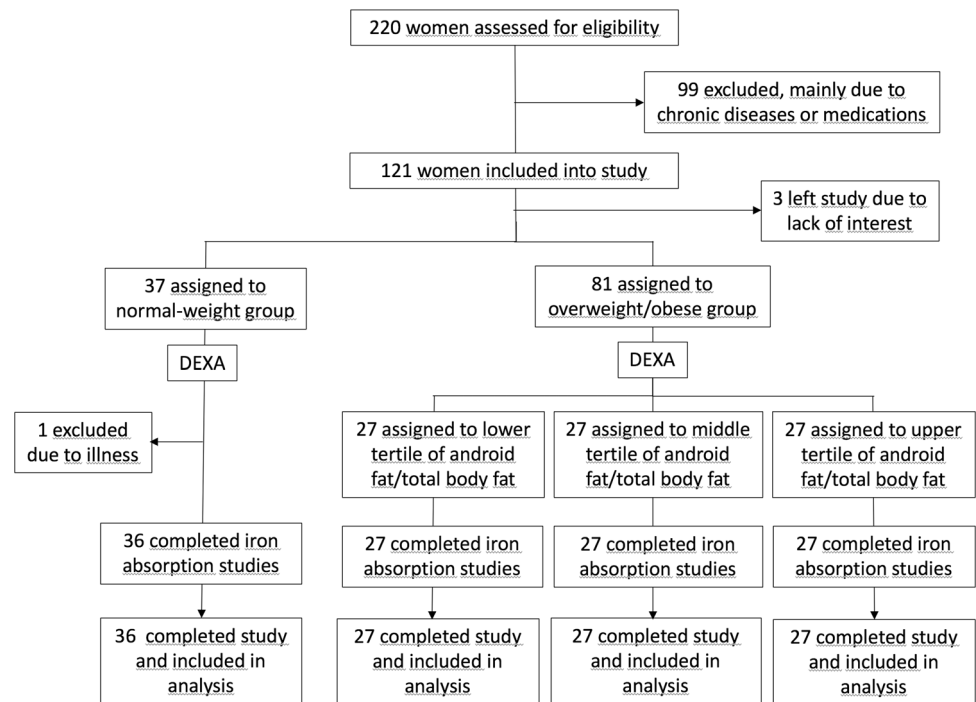
### Study design

We enrolled normal-weight women (BMI: 18.5–24.9 kg/m<sup>2</sup>) and overweight or obese (OW/OB) women (BMI: 25.0–50.0 kg/m<sup>2</sup>). DEXA measurement (Hologic Inc., Bedford, MA, USA) was done at the American University of Beirut Medical Center. The OW/OB group was then subdivided into tertiles of the ratio of AF/TBF based on the DEXA measurement. Subjects reported fasting at 08.00 a. m. (no food for 12 h and no drinks after midnight) and consumed an iron-isotope labeled standardized test meal that was consumed under standardized conditions and close supervision (Fig. 1). We labeled the test meals with 6 mg <sup>57</sup>Fe as ferrous sulfate (FeSO<sub>4</sub>) added directly into the test meals immediately before consumption. The test meal consisted of a white-flour bread roll ( $\approx$ 70 g) purchased in a single-large batch, cut in half, with butter (10 g) and honey ( $\approx$ 25 g) spread on top. The bread was frozen and thawed the evening before the test-meal administration. The subjects were given 300 ml of double deionized water with the meal. After ingestion of the test meal, we asked subjects not to eat or drink for 3 h. Fourteen days after the test meal was consumed, we obtained a fasting venipuncture blood sample for an analysis of hemoglobin (Hb) and erythrocyte isotopic composition as well as for iron status, inflammation, and hepcidin determination. On day 15, immediately after the blood sample was taken, subjects consumed a solution containing 75 g of glucose and 100 mg of iron as sodium ferrous citrate (Sanferol, Eisai, Japan). Two hours after the ingestion of the syrup, we obtained a final venipuncture blood sample for measurement of TIBC and serum iron.

### Anthropometric and body-composition determinations

We measured body weight (kg) to the nearest 0.1 kg on a digital scale, and height (m) to the nearest 1.0 cm with the use of a stadiometer, according to standardized procedures [18]. From the DEXA measurements, TBF, AF, gynoid fat, segmental (arms, legs, trunk, and gluteofemoral and

Fig. 1 Study flow-chart.



abdominal region) fat and lean mass and visceral and subcutaneous adipose tissues at the level of the abdomen were calculated with the use of the Hologic Inc. software. The android and gynoid fat to TBF ratios (AF/TBF and GF/TBF) were calculated by dividing the mass of android and gynoid fat (g) by TBF (g).

### Preparation of isotopically labeled iron

We prepared isotopic-labeled  $^{57}\text{Fe}$ - $\text{FeSO}_4$  from isotopically enriched elemental iron by dissolution in diluted sulfuric acid [19]. The solutions were stored in polytetrafluoroethylene containers and flushed with argon to keep the iron in the +2 oxidation state.

### Laboratory analysis

We measured Hb by using a Coulter Counter (Beckman Coulter, Miami, FL, USA) with 3-level quality-control material on the day of blood collection. We measured serum iron (SeFe) and total iron-binding capacity (TIBC) by using colorimetry, and used these to calculate transferrin saturation (TSAT). We measured serum transferrin receptor (sTfR), serum ferritin (SF), and high-sensitive C-reactive protein (CRP) and  $\alpha$ -1 glycoprotein (AGP) by using a multiplex immunoassay [20], and SHep by using immunoassay (DRG Instruments GmbH, Marburg, Germany). Body iron stores (BIS) were calculated from the sTfR/SF ratio [21]. We defined ID as SF < 15  $\mu\text{g/L}$  and/or sTfR > 8.3 mg/L [22], anemia as Hb < 12 g/dL, and ID anemia as

ID and anemia [23]. Change in serum iron ( $\Delta\text{SeFe}$ ) after the 100 mg oral iron dose was calculated as the difference between serum iron measured immediately before and 2 h after consuming the dose.

For the isotope analyses, whole blood was mineralized by microwave digestion, and iron was separated by anion-exchange chromatography and a subsequent solvent-solvent extraction step into diethyl ether. The isotopic analysis of  $^{57}\text{Fe}$  was 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 [24]. 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 FIA could be calculated [24]. The amount of natural iron circulating in the blood was calculated on the basis of the blood volume determined by using a previously validated algorithm [25] and Hb. We estimated FIA assuming an 80% incorporation of absorbed iron into the erythrocytes. The observed shift in iron isotope ratios was converted to FIA by using standard algorithms [24].

### Statistical analysis

Statistical analyses were conducted with the use of SPSS (IBM SPSS statistics, Version 22). Data were checked for

normal distribution by using Shapiro–Wilk test and for the presence of outliers (defined as  $\pm 3$  SDs from the mean). Nonnormally distributed data were logarithmically transformed for statistical analyses. Data was expressed as mean  $\pm$  SD (for normally distributed data) or median (IQR) (for nonnormally distributed data). Comparisons between groups (normal weight, tertiles of AF/TBF) were done using a 1-factor ANOVA with post hoc Bonferroni correction. Group was defined as fixed effect. BIS was added as a covariate to the model on SHep, TIBC was added as a covariate to the model on  $\Delta$ SeFe and SeFe was added as a covariate to the model on TSAT. Spearman correlations were used to study associations between continuous variables. We conducted linear multiple regression analysis on SHep, CRP, TSAT, and  $\Delta$ SeFe. If the dependent variable was not normally distributed, we used log-transformed data. We corrected SF for inflammation [26] and FIA was adjusted to a serum ferritin level of 15  $\mu$ g/L [27]. Differences were considered significant at  $P < 0.05$ .

## Results

We began recruiting on September 10, 2017 and completed the study on October 18, 2018. We screened 220 women and 99 were excluded because they did not meet the inclusion criteria, mainly due to chronic diseases and medications (Fig. 1). We enrolled 121 women but 3 left the study due to lack of interest before the DEXA measurement. We performed DEXA measurements on 118 women (37 in the normal-weight group and 81 in the OW/OB group). One woman in the normal-weight group was excluded because she developed a febrile illness. Of the 81 women assigned to OW/OB group, all completed the study. Therefore, final analyses were performed on data from 117 women: 36 in the normal-weight group and 81 in the OW/OB group (Fig. 1).

Age and anthropometric characteristics of the subjects, by group (normal weight, and in the OW/OB, by tertiles of the AF/TBF), are shown in Table 1. There were significant between-group differences in all anthropometric measures

**Table 1** Age and anthropometric measurements in otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ), by tertile of the ratio of android fat/total body fat (AF/TBF).

	Normal-weight	Overweight/obese tertiles of android fat/total fat ratio			<i>p</i> value
		Lower tertile (peripheral adiposity)	Middle tertile	Upper tertile (central adiposity)	
<i>n</i>	36	27	27	27	
Age, years <sup>1</sup>	32 (24–42)	29 (23–40)	38 (30–41)	36 (33–43)	0.053
Body weight, kg	61 (56–64) <sup>b</sup>	73 (65–82)	84 (75–93)	89 (79–99)	<0.05
BMI, kg/m <sup>2</sup>	23.1 (21.6–24.2) <sup>a,c,f</sup>	28.4 (26.0–31.6) <sup>a,c</sup>	33.7 (30.5–36.3)	35.0 (32.5–36.9)	<0.001
WC, cm	76 (69–81) <sup>a,c,f</sup>	89 (82–95) <sup>a,c</sup>	101 (91–109)	104 (95–110)	<0.001
Total fat, kg	23.5 (20.5–27.8) <sup>a,c,f</sup>	35.2 (30.2–40.0)	40.6 (35.4–47.0)	43.3 (37.5–49.2)	<0.001
VAT, g <sup>2</sup>	302 $\pm$ 95 <sup>a,c,f</sup>	495 $\pm$ 151 <sup>a,d</sup>	699 $\pm$ 192	853 $\pm$ 241	<0.001
Android fat, kg	1.4 (1.1–1.7) <sup>a,c,f</sup>	2.2 (2.0–2.7) <sup>a,e</sup>	3.2 (2.7–3.6)	3.9 (3.2–4.4)	<0.001
AF/TBF	0.058 (0.051–0.064) <sup>a,c,f</sup>	0.066 (0.068–0.070) <sup>a,c</sup>	0.078 (0.076–0.079) <sup>a</sup>	0.086 (0.084–0.092)	<0.001
Gynoid fat, kg	5.0 (4.0–5.4) <sup>a,c,f</sup>	6.5 (5.8–7.5)	6.8 (6.4–8.6)	7.6 (6.7–8.4)	<0.001
GF/TBF	0.199 (0.189–0.211) <sup>a,c</sup>	0.185 (0.174–0.204) <sup>b,e</sup>	0.176 (0.166–0.189)	0.176 (0.165–0.187)	<0.001

BMI ranges as follows: normal weight, 18.5–24.9 kg/m<sup>2</sup>; overweight and obese,  $\geq 25$  kg/m<sup>2</sup>. The overweight and obese participants were separated into tertiles according to the AF/TBF, with central adiposity defined as the upper tertile for AF/TBF; peripheral adiposity defined as the lower tertile for AF/TBF. Differences between the four groups were assessed with the use of 1-factor ANOVA with post hoc Bonferroni correction

WC waist circumference, VAT visceral adipose tissue, GF/TBF gynoid fat/total fat

<sup>1</sup>All such data as medians (IQR)

<sup>2</sup>All such data as means  $\pm$  SD

<sup>a</sup>Different from upper tertile,  $p < 0.001$

<sup>b</sup>Different from upper tertile,  $p < 0.05$

<sup>c</sup>Different from middle tertile,  $p < 0.001$

<sup>d</sup>Different from middle tertile,  $p < 0.01$

<sup>e</sup>Different from middle tertile,  $p < 0.05$

<sup>f</sup>Different from lower tertile,  $p < 0.001$

**Table 2** Iron and inflammation status and iron absorption parameters in otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ), by tertile of the ratio of android fat/total body fat (AF/TBF).

	Normal-weight	Overweight/obese tertiles of android fat/total fat ratio			<i>p</i> value
		Lower tertile (peripheral adiposity)	Middle tertile	Upper tertile (central adiposity)	
Hemoglobin, g/dl <sup>1</sup>	13.2 (12.4–13.6)	13.3 (12.6–13.6)	12.6 (11.7–13.3)	13.1 (12.4–13.8)	0.220
Serum ferritin, µg/L	17.7 (10.2–36.0)	17.1 (12.3–30.0)	12.0 (5.4–33.2)	20.1 (7.7–34.6)	0.757
TfR, mg/L	5.4 (4.7–7.1)	6.0 (5.0–7.3)	6.3 (5.1–7.7)	5.7 (4.9–7.8)	0.582
BIS, mg/kg BW <sup>2</sup>	2.42 ± 3.86	2.59 ± 3.27	1.30 ± 5.03	2.06 ± 4.94	0.687
SHep, ng/ml	4.48 (1.93–6.90) <sup>a,c</sup>	5.42 (3.89–8.43)	6.11 (1.38–10.21)	7.85 (3.94–14.1)	<0.001
CRP, mg/L	0.49 (0.28–2.68) <sup>a,d</sup>	1.84 (0.94–2.82)	2.54 (1.29–5.91)	3.35 (1.95–8.82)	<0.001
AGP, g/L	0.65 (0.49–0.97) <sup>a,c,e</sup>	0.91 (0.72–1.17)	1.1 (0.89–1.62)	1.28 (1.07–1.52)	<0.001
Serum iron, mcg/dl	79.5 (56.5–96.5)	66 (52–90)	64.0 (49.0–79.5)	71.0 (58.5–96.0)	0.477
ΔSerum iron	47.0 (20.0–81.0)	33.0 (9.5–64.0)	43.0 (25.0–106.5)	33.0 (21.5–66.5)	0.080
Serum iron/SHep	23.3 (11.0–37.1) <sup>b</sup>	13.2 (7.4–22.6)	13.7 (7.3–30.5)	10.9 (5.3–19.0)	0.034
ΔSerum iron/SHep	10.5 (3.4–34.7)	6.1 (1.0–14.8)	14.2 (3.2–78.1)	3.2 (1.8–13.6)	0.071
TIBC, mcg/dl	469.4 ± 84.2 <sup>b</sup>	505.6 ± 68.2	505.7 ± 69.5	518.7 ± 74.8	<0.05
TSAT, %	16.8 (12.1–21.5) <sup>b</sup>	13.7 (10.4–17.1)	14.1 (9.1–15.6)	13.6 (11.0–21.5)	<0.05
FIA, %	12.0 (8.9–20.0)	17.6 (9.4–23.8)	13.9 (9.3–21.8)	20.9 (10.0–27.0)	0.400
FIA/SHep	3.7 (2.0–6.5)	2.8 (1.6–6.0)	4.4 (2.1–7.8)	2.4 (1.4–4.7)	0.320

BMI ranges as follows: normal weight, 18.5–24.9 kg/m<sup>2</sup>; overweight and obese, ≥25 kg/m<sup>2</sup>. The overweight and obese participants were separated into tertiles according to the AF/TBF; central adiposity defined as the upper tertile for AF/TBF; peripheral adiposity defined as the lower tertile for AF/TBF. Differences between the four groups were assessed with the use of 1-factor ANOVA with post hoc Bonferroni correction. Serum ferritin was corrected for inflammation [26]. BIS were calculated using the corrected serum ferritin values. FIA was adjusted for a serum ferritin level of 15 µg/L using the inflammation corrected serum ferritin [27]. BIS was added as a covariate to the model on SHep. TIBC was added as a covariate to the model on ΔSerum iron. Serum iron was added as a covariate to the model on TSAT

TfR transferrin receptor, BIS body iron stores, SHep serum hepcidin, CRP C-reactive protein, AGP α-1 glycoprotein, FIA fractional iron absorption, ΔSerum iron increase in serum iron 2 h after the ingestion of 100 mg iron as sodium ferrous citrate, TSAT transferrin saturation, TIBC total iron-binding capacity

<sup>1</sup>All such data as medians (IQR)

<sup>2</sup>All such data as means ± SD

<sup>a</sup>Different from upper tertile,  $p < 0.001$

<sup>b</sup>Different from upper tertile,  $p < 0.05$

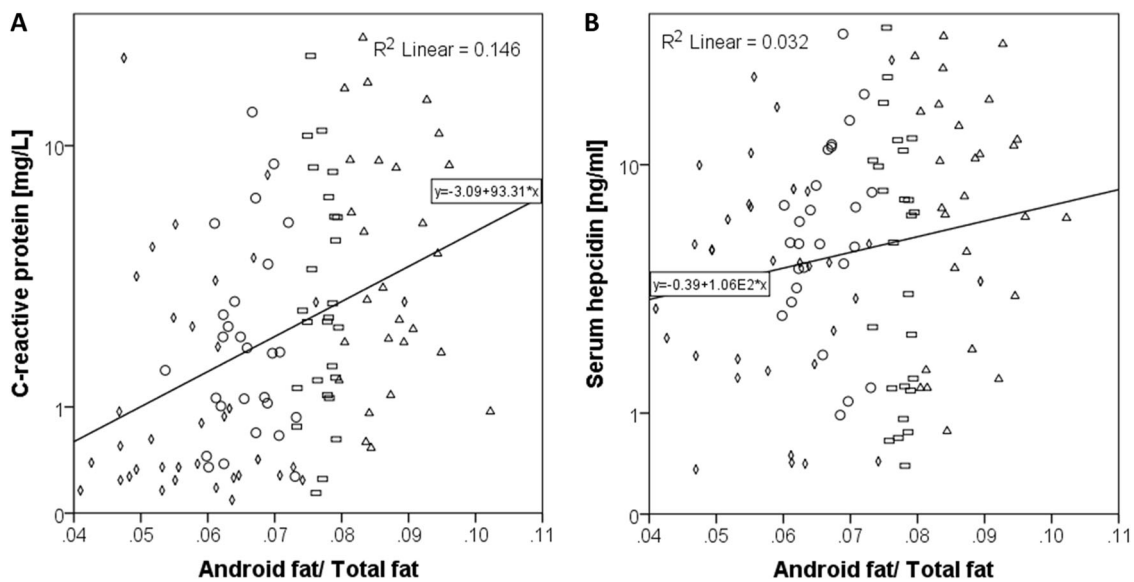
<sup>c</sup>Different from middle tertile,  $p < 0.001$

<sup>d</sup>Different from middle tertile,  $p < 0.01$

<sup>e</sup>Different from lower tertile,  $p < 0.05$

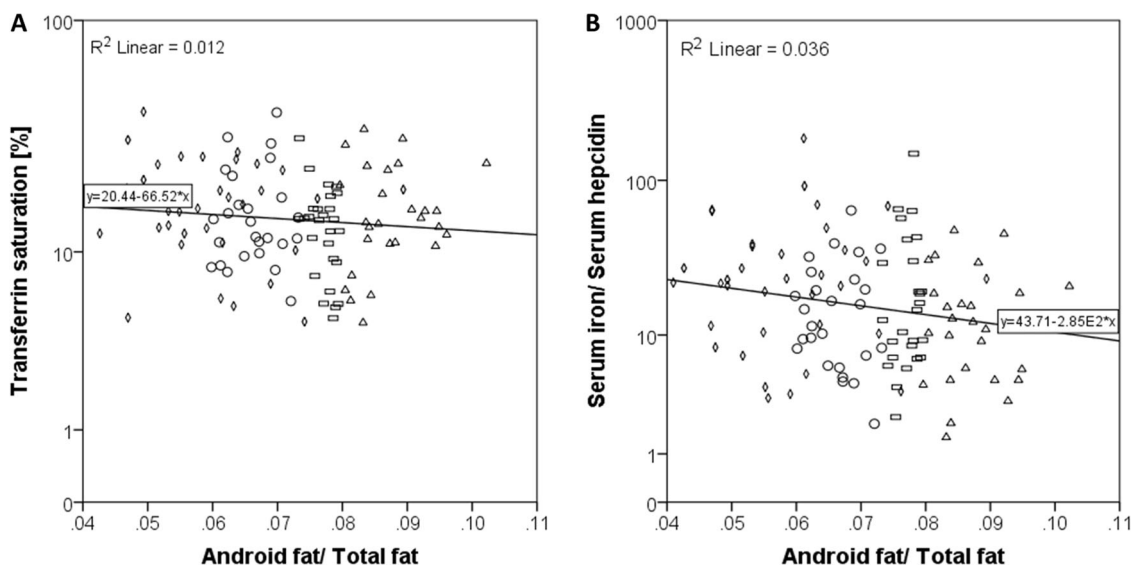
(for all  $p < 0.001$ ). Table 2 shows, by group (normal weight, and in the OW/OB, by tertiles of the AF/TBF), inflammation and iron biomarkers, as well as change in serum iron (ΔSeFe) during the oral iron challenge and FIA from the labeled test meal. There were significant between-group differences in CRP, AGP, SHep, SeFe/SHep, TSAT, and TIBC (for all,  $p < 0.05$ ), and a borderline significant difference in ΔSeFe/SHep ( $p = 0.07$ ) and ΔSeFe after the oral iron dose ( $p = 0.08$ ). There were no significant between-group differences in FIA from the labeled test meal. Anemia and ID prevalence in the normal-weight group were 16.7% and 8.3%, respectively, and in the lower, middle, and upper tertiles of AF/TBF in the OW/OB group, were 14.8%, 33.3%, 18.5% and 7.4%, 18.5%, 14.8%, respectively.

Figure 2a, b shows the correlations between AF/TBF and CRP, and AF/TBF and SHep. There was a significant positive correlation between AF/TBF and CRP (Fig. 2a) ( $r_s = 0.419$ ,  $p < 0.001$ ) and between AF/TBF and SHep (Fig. 2b) ( $r_s = 0.181$ ,  $p = 0.05$ ). The positive correlation between AF/TBF and SHep was stronger, when adjusting for BIS (Table 3). Figure 3a, b shows the correlations between AF/TBF and iron status parameters. There were negative associations between AF/TBF and TSAT (Fig. 3a) and between AF/TBF and ΔSeFe after oral iron challenge, but these relationships were significant only when adjusting for SeFe and TIBC, respectively (Table 3). There was a negative correlation between AF/TBF and the SeFe/SHep ratio ( $r_s = -0.187$ ,  $p < 0.05$ ) (Fig. 3b), and a



**Fig. 2** In otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ), correlations between android fat/total fat ratio, inflammation and serum hepcidin. **a** Android fat/total fat ratio and C-reactive protein. **b** Android fat/total fat ratio and

Serum hepcidin. Both figures are on a log<sub>10</sub>-scale. Diamonds: normal-weight; circles: lower tertile AF/TBF; squares: middle tertile AF/TBF; triangles: upper tertile AF/TBF.



**Fig. 3** In otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ), correlations between android fat/total fat ratio and iron status parameters. **a** Android fat/total fat ratio and transferrin saturation. **b** Android fat/total fat ratio and serum

iron/serum hepcidin ratio. Both figures are on a log<sub>10</sub>-scale. Diamonds: normal-weight; circles: lower tertile AF/TBF; squares: middle tertile AF/TBF; triangles: upper tertile AF/TBF.

negative correlation between SHep and  $\Delta\text{SeFe}$  ( $r_s = -0.572$ ,  $p < 0.001$ ) (Fig. 4).

As shown in Table 3, we performed separate hierarchical regression analyses with CRP, SHep, TSAT, and  $\Delta\text{SeFe}$  as dependent variables. For CRP, including AF/TBF and gynoid fat/total fat ratio (GF/TBF) as covariates, the model explained 18.4% of the variance in CRP; AF/TBF was a significant positive predictor of CRP ( $p < 0.001$ ), GF/TBF was not. For SHep, including AF/TBF, GF/TBF, and SF as

covariates, the model explained 64.5% of the variance in SHep; AF/TBF ( $p < 0.05$ ) and SF ( $p < 0.001$ ) were significant positive predictors of SHep, while GF/TBF was not. In the regression analyses on TSAT (Table 3), including AF/TBF, GF/TBF, SF, and age as covariates, the model explained 37.4% of the variance in TSAT; SF was a positive predictor ( $p < 0.001$ ), AF/TBF ( $p < 0.05$ ) was a negative predictor of TSAT. In the regression analyses on  $\Delta\text{SeFe}$ , the model including AF/TBF, GF/TBF, age, TIBC, and AGP

**Table 3** Predictors of C-reactive protein, serum hepcidin, transferrin saturation and  $\Delta$ serum iron after oral iron challenge, in otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ).

	<i>B</i>	Standard error of <i>B</i>	Standardized $\beta$
Model I dependent variable: CRP $R^2 = 0.184$			
Android fat/total fat ratio	2.628	0.630	0.384***
Gynoid fat/total fat ratio	-1.092	1.061	-0.095
Model II dependent variable: SHep $R^2 = 0.645$			
Android fat/total fat ratio	0.670	0.337	0.122*
Gynoid fat/total fat ratio	0.886	0.582	0.096
Serum ferritin	0.914	0.066	0.805***
Model III dependent variable: TSAT $R^2 = 0.374$			
Android fat/total fat ratio	-0.447	0.219	-0.173*
Gynoid fat/total fat ratio	0.128	0.364	0.029
Serum ferritin	0.326	0.042	0.609***
Age	0.025	0.140	0.014
Model IV dependent variable: $\Delta$ serum iron $R^2 = 0.093$			
Android fat/total fat ratio	-129.661	69.928	-0.228 <sup>+</sup>
Gynoid fat/total fat ratio	-8.705	96.127	-0.009
Age	33.335	38.087	0.088
TIBC	207.076	65.965	0.300**
AGP	23.253	26.803	0.098

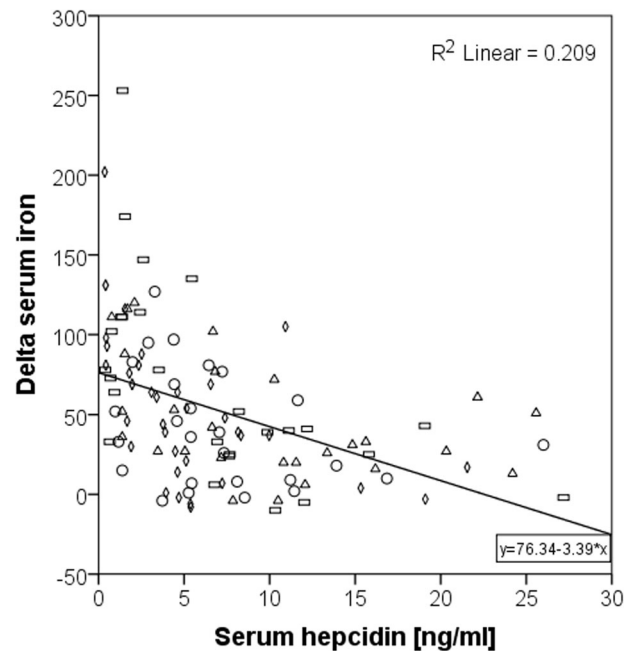
Regression analysis with CRP (Model I), SHep (Model II), TSAT (Model III), and  $\Delta$ serum iron (Model IV) as dependent variable and android fat/total fat ratio, gynoid fat/total fat ratio, serum ferritin, age, TIBC and AGP as independent variables. For models I–III the dependent variable was log transformed (Log10).  $R^2$  indicates the proportion of variance explained by the model. The *b* values (*B*) indicate the individual contribution of each predictor to the model. A positive value indicates a positive relationship between the predictor and the outcome, whereas a negative coefficient represents a negative relationship. CRP C-reactive protein, SHep serum hepcidin, TSAT transferrin saturation, TIBC total iron-binding capacity, AGP  $\alpha$ -1 glycoprotein.

\*\*\* $p < 0.001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; <sup>+</sup> $p = 0.066$

explained 9.3% of the variance; TIBC ( $p < 0.01$ ) was a positive predictor of  $\Delta$ SeFe ( $p < 0.01$ ) and AF/TBF was a negative predictor ( $p = 0.07$ ) of  $\Delta$ SeFe.

## Discussion

To our knowledge, this is the first study to assess the effects of body fat distribution on SHep and iron metabolism. Our



**Fig. 4** In otherwise-healthy normal-weight women ( $n = 36$ ) and overweight/obese women ( $n = 81$ ), correlation between  $\Delta$ serum iron and serum hepcidin. Diamonds: normal-weight; circles: lower tertile AF/TBF; squares: middle tertile AF/TBF; triangles: upper tertile AF/TBF. AF/TBF android fat/total fat ratio.

main findings are, in young women: (1) AF/TBF was a significant positive predictor of inflammation (CRP) and SHep (2); AF/TBF was a negative predictor of iron status (TSAT) and supplemental iron absorption ( $\Delta$ SeFe) (3); the GF/TBF ratio was not a significant predictor of any of these variables; and (4) neither AF/TBF or the gynoid fat/TBF ratio predicted iron absorption from the labeled test meal.

Adipose tissue produces a variety of pro-inflammatory factors, including IL-6; it is estimated that one third of total circulating IL-6 is released from adipose tissue [14]. Increasing waist circumference predicts plasma IL-6 concentrations [28]; and VAT is more heavily infiltrated by macrophages producing IL-6 than peripheral fat [13, 29–32]. In contrast to peripheral adipose tissue, IL-6 released by VAT into the portal circulation drains directly to the liver, and in obese adults, mean plasma IL-6 is  $\approx 50\%$  higher in the portal vein than in the peripheral circulation [14]. High concentrations of IL-6 in the portal circulation can stimulate hepatocytes to increase CRP synthesis and release [14, 33, 34]. This is consistent with our findings that android but not gynoid fat positively predicted CRP (Table 3). Cytokines may also directly decrease red cell lifespan [35, 36] and suppress erythropoiesis [37, 38], and this effect could have contributed to the slightly lower Hb and the high prevalence of anemia in the middle and upper tertiles of AF/TBF.

It is possible that high concentrations of IL-6 in portal blood released from VAT could also strongly stimulate hepatic hepcidin synthesis [32, 33]. This hypothesis is supported by our findings that, controlling for body iron stores, AF positively predicted SHep, but gynoid fat did not (Table 3). Although SHep was higher in OB/OW women with greater central adiposity, a greater difference in SHep may have been partially masked by the opposing effects of obesity-related inflammation (increasing hepcidin expression) and ID/anemia (decreasing hepcidin expression): net SHep depends on the relative strength of these opposing stimuli [39]. Women with greater central adiposity had generally poorer iron status (Table 2). This may have partially offset the effects of higher VAT-derived inflammation on SHep. This explanation is consistent with our data showing the serum iron/SHep ratio was lower in central adiposity, suggesting relatively greater hypoferrremia compared with SHep, likely due to poor iron status.

High concentrations of circulating hepcidin reduce ferroportin-mediated export of iron from reticuloendothelial macrophages and duodenal enterocytes, resulting in iron sequestration, hypoferrremia, and decreased dietary iron absorption [8, 40]. In our subjects, there was evidence of SHep-induced iron sequestration [41] in central adiposity: TSAT, SeFe, and the SeFe/SHep ratio were lower, while TIBC was higher with increasing central adiposity (Table 2). These findings are consistent with a recent study in OW/OB women that reported total circulating mass of hepcidin were higher, while total mass of serum iron was lower, compared with normal-weight women [25].

Previous stable iron isotope studies have reported that iron absorption from labeled test meals is reduced in women with OW/OB [9] and that absorption is negatively correlated with SHep [42]. Weight loss in OW/OB women reduces SHep and improves iron absorption [2]. In our study, we assessed the effect of central adiposity on iron absorption in two ways: (a) we measured the increase in serum iron 2 h after the administration of 100 mg iron as ferrous citrate, as a measure of absorption from supplemental doses of iron [19]; (b) as a proxy for dietary iron, we provided an iron-isotope labeled standardized test meal containing 6 mg of iron and measured erythrocyte incorporation of iron 14 days later [43].  $\Delta\text{SeFe}$  after the oral iron challenge was lowest in those women with greater central adiposity (Table 2), and in the regression analysis, AF/TBF was a negative predictor of  $\Delta\text{SeFe}$  after oral iron; GF/TBF was not a predictor (Table 3). These data suggest iron absorption from a supplemental dose (100 mg) was impaired in women with greater central adiposity. In contrast, there were no significant between-group differences in iron absorption from the lower dose (6 mg) of iron, assessed by stable isotope techniques (Table 2). This may have been due to the difference in iron dose and/or the difference in

the measurement of absorption ( $\Delta\text{SeFe}$  vs. erythrocyte isotope incorporation at 14 days). However, accurate determination of blood volume, which underpins the calculation of iron absorption using stable iron isotopes, is challenging in overweight/obese subjects [2, 25] and this may have biased our findings. In addition, poorer iron status and greater anemia in the central adiposity group may have enhanced absorption through direct effects on the enterocyte rather than through hepcidin [39, 44, 45].

The strengths of our study are: (1) we used DEXA to precisely measure regional body fat content [46]; (2) our subjects were young women, a risk group for ID and anemia, who were otherwise healthy and free of potential confounding comorbidities; (3) we studied women with a wide range of total and android body fat; and (4) we assessed iron absorption using two methods, stable iron isotopes in a test meal and an oral iron challenge. Limitations of our study include: (1) its cross-sectional design precludes assessment of directionality of effects; (2) we did not measure serum IL-6; and (3) estimation of blood volume in obese subjects is challenging; although we used an algorithm developed in overweight/obese subjects using the  $\text{CO}_2$ -rebreathing method [25]; we are uncertain if an overestimation of blood volume, particularly in the very obese, may have biased our estimates of FIA using stable isotopes.

In conclusion, our data suggest that, in OW/OB women, distribution of body fat may be a primary determinant of disordered iron homeostasis. A plausible explanation for these findings is adipocytokines released by VAT into the portal system have stimulatory effects of hepatic hepcidin synthesis. Our findings suggest that OW/OB women with central adiposity may be at higher risk for ID and anemia than normal-weight women. Thus, clinicians should emphasize weight loss and ample dietary iron for iron-deficient women with a central distribution of body fat. On the population level, our findings suggest the current increase in obesity, particularly central obesity, in young women in many countries [17] may impair programs to reduce ID and anemia in this target group [47].

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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## References

1. Cepeda-Lopez AC, Aeberli I, Zimmermann MB. Does obesity increase risk for iron deficiency? A review of the literature and the potential mechanisms. *Int J Vitam Nutr Res.* 2010;80:263–70.

2. Cepeda-Lopez AC, Allende-Labastida J, Melse-Boonstra A, Osendarp SJ, Herter-Aeberli I, Moretti D, et al. The effects of fat loss after bariatric surgery on inflammation, serum hepcidin, and iron absorption: a prospective 6-mo iron stable isotope study. *Am J Clin Nutr.* 2016;104:1030–8.
3. Yanoff LB, Menzie CM, Denkinger B, Sebring NG, McHugh T, Remaley AT, et al. Inflammation and iron deficiency in the hypoferremia of obesity. *Int J Obes.* 2007;31:1412–9.
4. Baumgartner J, Smuts CM, Aeberli I, Malan L, Tjalsma H, Zimmermann MB. Overweight impairs efficacy of iron supplementation in iron-deficient South African children: a randomized controlled intervention. *Int J Obes.* 2013;37:24–30.
5. Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Guzman G, Holterman AX, et al. Elevated systemic hepcidin and iron depletion in obese premenopausal females. *Obesity.* 2010;18:1449–56.
6. Tussing-Humphreys LM, Liang H, Nemeth E, Freels S, Braunschweig CA. Excess adiposity, inflammation, and iron-deficiency in female adolescents. *J Am Diet Assoc.* 2009;109:297–302.
7. Wrighting DM, Andrews NC. Interleukin-6 induces hepcidin expression through STAT3. *Blood.* 2006;108:3204–9.
8. Sangkhav V, Nemeth E. Regulation of the iron homeostatic hormone hepcidin. *Adv Nutr.* 2017;8:126–36.
9. Cepeda-Lopez AC, Melse-Boonstra A, Zimmermann MB, Herter-Aeberli I. 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. *Am J Clin Nutr.* 2015;102:1389–97.
10. Aeberli I, Hurrell RF, Zimmermann MB. Overweight children have higher circulating hepcidin concentrations and lower iron status but have dietary iron intakes and bioavailability comparable with normal weight children. *Int J Obes.* 2009;33:1111–7.
11. del Giudice EM, Santoro N, Amato A, Brienza C, Calabro P, Wiegierneck ET, et al. Hepcidin in obese children as a potential mediator of the association between obesity and iron deficiency. *J Clin Endocr Metab.* 2009;94:5102–7.
12. Tussing-Humphreys LM, Nemeth E, Fantuzzi G, Freels S, Holterman AX, Galvani C, et al. Decreased serum hepcidin and improved functional iron status 6 months after restrictive bariatric surgery. *Obesity.* 2010;18:2010–6.
13. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- $\alpha$ : direct role in obesity-linked insulin resistance. *Science.* 1993;259:87–91.
14. Fontana L, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes.* 2007;56:1010–3.
15. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest.* 2006;116:1793–801.
16. Lim S, Meigs JB. Ectopic fat and cardiometabolic and vascular risk. *Int J Cardiol.* 2013;169:166–76.
17. Gartner A, El Ati J, Traissac P, Bour A, Berger J, Landais E, et al. A double burden of overall or central adiposity and anemia or iron deficiency is prevalent but with little socioeconomic patterning among Moroccan and Tunisian urban women. *J Nutr.* 2014;144:87–97.
18. Gibson RS. Principles of nutritional assessment. 2nd ed. New York: Oxford University Press; 2005.
19. Andersson M, Theis W, Zimmermann MB, Foman JT, Jakel M, Duchateau GS, et al. Random serial sampling to evaluate efficacy of iron fortification: a randomized controlled trial of margarine fortification with ferric pyrophosphate or sodium iron edetate. *Am J Clin Nutr.* 2010;92:1094–104.
20. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. *J Nutr.* 2004;134:3127–32.
21. Cook JD, Flowers CH, Skikne BS. The quantitative assessment of body iron. *Blood.* 2003;101:3359–64.
22. WHO/CDC. Assessing the iron status of populations including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level Geneva, WHO/CDC, 2004; [https://www.who.int/nutrition/publications/micronutrients/anaemia\\_iron\\_deficiency/9789241596107/en/](https://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596107/en/).
23. WHO. Iron deficiency anemia, assessment, prevention and control: a guide for programme managers. Geneva: WHO; 2001.
24. Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine the iron absorption by Peruvian school children from a breakfast meal. *Fresen J Anal Chem.* 1997;359:445–9.
25. Cepeda-Lopez AC, Zimmermann MB, Wussler S, Melse-Boonstra A, Naef N, Mueller SM, et al. Greater blood volume and Hb mass in obese women quantified by the carbon monoxide-rebreathing method affects interpretation of iron biomarkers and iron requirements. *Int J Obes.* 2018;43:999–1008.
26. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr.* 2010;92:546–55.
27. Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. *Am J Clin Nutr.* 1991;54:717–22.
28. Wannamethee SG, Tchernova J, Whincup P, Lowe GD, Kelly A, Rumley A, et al. Plasma leptin: associations with metabolic, inflammatory and haemostatic risk factors for cardiovascular disease. *Atherosclerosis.* 2007;191:418–26.
29. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab.* 1998;83:847–50.
30. Stepan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature.* 2001;409:307–12.
31. Shimomura I, Funahashi T, Takahashi M, Maeda K, Kotani K, Nakamura T, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. *Nat Med.* 1996;2:800–3.
32. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. *Science.* 2005;307:426–30.
33. Rocha VZ, Libby P. Obesity, inflammation, and atherosclerosis. *Nat Rev Cardiol.* 2009;6:399–409.
34. Vgontzas AN, Bixler EO, Papanicolaou DA, Chrousos GP. Chronic systemic inflammation in overweight and obese adults. *JAMA.* 2000;283:2235.
35. Nemeth E, Ganz T. Anemia of inflammation. *Hematol Oncol Clin North Am.* 2014;28:671–81, vi.
36. Freireich EJ, Ross JF, Bayles TB, Emerson CP, Finch SC. Radioactive iron metabolism and erythrocyte survival studies of the mechanism of the anemia associated with rheumatoid arthritis. *J Clin Invest.* 1957;36:1043–58.
37. Libregts SF, Gutierrez L, de Bruin AM, Wensveen FM, Papadopoulos P, van Ijcken W, et al. Chronic IFN- $\gamma$  production in mice induces anemia by reducing erythrocyte life span and inhibiting erythropoiesis through an IRF-1/PU.1 axis. *Blood.* 2011;118:2578–88.
38. Johnson RA, Waddelow TA, Caro J, Oliff A, Roodman GD. Chronic exposure to tumor necrosis factor in vivo preferentially inhibits erythropoiesis in nude mice. *Blood.* 1989;74:130–8.

39. Stoffel NU, Lazrak M, Bellitir S, El Mir N, El Hamdouchi A, Barkat A, et al. The opposing effects of acute inflammation and iron deficiency anemia on serum hepcidin and iron absorption in young women. *Haematologica*. 2019;104:1143–9.
40. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferrremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest*. 2004;113:1271–6.
41. Michels K, Nemeth E, Ganz T, Mehrad B. Hepcidin and host defense against infectious diseases. *Plos Pathog*. 2015;11:e1004998.
42. Zimmermann MB, Troesch B, Biebinger R, Egli I, Zeder C, Hurrell RF. Plasma hepcidin is a modest predictor of dietary iron bioavailability in humans, whereas oral iron loading, measured by stable-isotope appearance curves, increases plasma hepcidin. *Am J Clin Nutr*. 2009;90:1280–7.
43. Cercamondi CI, Egli IM, Mitchikpe E, Tossou F, Zeder C, Hounhouigan JD, et al. Total iron absorption by young women from iron-biofortified pearl millet composite meals is double that from regular millet meals but less than that from post-harvest iron-fortified millet meals. *J Nutr*. 2013;143:1376–82.
44. Galy B, Ferring-Appel D, Becker C, Gretz N, Grone HJ, Schumann K, et al. Iron regulatory proteins control a mucosal block to intestinal iron absorption. *Cell Rep*. 2013;3:844–57.
45. Taylor M, Qu AJ, Anderson ER, Matsubara T, Martin A, Gonzalez FJ, et al. Hypoxia-inducible factor-2 alpha mediates the adaptive increase of intestinal ferroportin during iron deficiency in mice. *Gastroenterology*. 2011;140:2044–55.
46. Hind K, Oldroyd B, Truscott JG. In vivo precision of the GE Lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *Eur J Clin Nutr*. 2011;65:140–2.
47. Cepeda-Lopez AC, Osendarp SJ, Melse-Boonstra A, Aeberli I, Gonzalez-Salazar F, Feskens E, et al. Sharply higher rates of iron deficiency in obese Mexican women and children are predicted by obesity-related inflammation rather than by differences in dietary iron intake. *Am J Clin Nutr*. 2011;93:975–83.