Eur J Nutr
DOI 10.1007/s00394-015-1048-1

ORIGINAL CONTRIBUTION

Increased risk of iron deficiency and reduced iron absorption but no difference in zinc, vitamin A or B-vitamin status in obese women in India

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Received: 22 January 2015 / Accepted: 16 September 2015

Abstract
Purpose Two objectives were investigated: (1) to assess the risk of micronutrient deficiencies in relation to weight status in Indian women with a focus on iron but also including zinc, vitamin A and B vitamins and (2) to compare fractional iron absorption between obese (OB) and normal weight (NW) women.
Methods Part 1 was a cross-sectional study including 146 healthy, middle-class women from Bangalore, India, with a BMI between 19 and 40 kg/m^2. Anthropometrics and blood pressure were measured, and a fasting blood sample was obtained for the analysis of vitamin and mineral status, hepcidin, blood lipids and glucose. In part 2, 16 OB and 13 NW women consumed a standardized test meal labeled with the stable iron isotope ^{57}Fe. Incorporation of the iron isotope into erythrocytes was measured 14 days later. In addition, iron status, hepcidin and inflammatory markers were determined.
Results In part 1, compared to NW women, overweight/OB subjects had significantly higher C-reactive protein, serum ferritin, soluble transferrin receptor (sTfR) and hepcidin concentrations ($p < 0.05$). The odds ratio for having high sTfR concentrations (i.e., low iron status) with increasing BMI was 1.09 (95 % CI 1.02–1.17). None of the other micronutrients investigated showed any differences between weight status groups. In part 2, fractional iron absorption was significantly lower in the OB group compared to the NW group even after controlling for differences in iron status (10.0 ± 6.5 vs. 16.7 ± 4.6 %; $p = 0.038$).
Conclusions OB women in Bangalore have an increased risk of low iron status and absorb less dietary iron; however, their risk of other micronutrient deficiencies was similar to NW women. Our results clearly demonstrate the importance of considering the double burden of malnutrition in the planning of prevention strategies especially in transition countries with emerging obesity epidemics.

Keywords Obesity · Iron deficiency · Iron absorption · Micronutrients · Hepcidin · Double burden

Introduction

Many developing countries undergoing rapid urbanization and economic development are experiencing the 'double burden' of over- and undernutrition. Obesity and diet-related chronic disease, specifically the metabolic syndrome, are emerging as important health problems, yet vulnerable population groups remain micronutrient deficient. Potential interactions within this 'double burden' could have important public health consequences [1–3]. There is intriguing evidence that the combination of obesity and possibly the metabolic syndrome with micronutrient deficiencies may be even more detrimental to health than either condition alone [4, 5].

To date, considering the micronutrients investigated here, the most pronounced evidence for interactions between obesity and micronutrient deficiency exists for iron. Higher rates of iron deficiency have consistently been found in overweight (OW) and obese (OB) subjects

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Published online: 10 October 2015

in different studies [6–14]. This is believed to be a result of reduced absorption of dietary iron due to chronic low-grade inflammation in OW and obesity [15], which leads to increased secretion of hepcidin from the liver and increased expression in adipose tissue [16, 17]. Even though increased hepcidin concentrations have repeatedly been shown in OW and OB subjects [18, 19], and weight loss was associated with both a decrease in hepcidin concentrations and an improvement in iron status [20], the direct effect of hepcidin on iron absorption in the context of obesity or weight loss remains unknown. Besides its clear effect on cognitive function [21], iron deficiency has also been related to impaired physical health, reduced physical activity, increased fatigue and reduced work performance [22–24]. Taken together, obesity-related iron deficiency may exacerbate a vicious cycle of increased weight gain due to decreased physical activity which in turn could lead to even more pronounced iron deficiency.

Other micronutrient deficiencies are also associated with obesity. Vitamin A or serum retinol (SR) has been found to be reduced in several OW and OB populations [25–27]. Even though the underlying mechanism is poorly understood, systemic inflammation is suspected to play a role, as recovery from various infections was associated with correction of low serum retinol concentrations in children [28, 29]. On the other hand, it was also suggested that vitamin A may regulate adiposity through several different mechanisms [30–32], again opening the question of cause and effect.

Similarly, considerable cross-sectional evidence exists showing lower zinc status with increasing adiposity [33–35]. Whether obesity causes zinc deficiency or vice versa cannot be judged from the existing cross-sectional studies. Some data suggest that zinc deficiency may be a risk factor for obesity or increased body fat accumulation [35, 36]; however, other studies demonstrated that short-term weight loss resulted in an improvement in zinc status [37, 38].

The association between vitamins of the B complex and obesity is even less understood and controversial to some extent. Even though cross-sectional evidence supports lower status of B complex vitamins in OB subjects [39–43], the precise mechanism(s) is unknown.

Taking into account the existing literature and what remains unknown, the first aim of the present study was to assess iron, zinc, vitamin A, B2, and B12 as well as folic acid status in relation to body mass index (BMI), body composition and the metabolic syndrome in middle-class Indian women. The second aim was to better explain the increased risk of iron deficiency in obesity based on measures of inflammation and hepcidin. Finally, the third aim was to determine fractional iron absorption from a standardized test meal in a group of OB and NW Indian women.

Subjects and methods

Subjects

Subjects for the first part of this study were 150 women of reproductive age with a weight status over a large BMI range between 19 and 40 kg/m^2. Women were recruited from the campus of St. John's Medical School and Nursing College and St. John's Hospital in Bangalore, India, between September 2011 and February 2012. In order to be included, subjects had to be between 18 and 35 years old, apparently healthy, not pregnant or lactating and willing to comply with the study procedures (the age limitation was set to 35 years in order to limit the risk of obesity-related comorbidities). Pregnancy or giving birth in the 6 months prior to the study was a further exclusion criterion. Significant medical conditions other than obesity that might influence micronutrient status (cancer, HIV/AIDS, inflammatory bowel disease or hemochromatosis) were considered as exclusion criteria, while signs of the metabolic syndrome (dyslipidemia, high fasting glucose or high blood pressure) were not. Based on data from previous studies comparing iron status of NW and OW/OB subjects [18, 19, 44], a sample size of 50 subjects per group was deemed to be sufficient to detect a difference of 25 % in iron status between groups (power 80 %). The aim was therefore to recruit a total sample of 150 women, 50 in each of the three BMI groups.

Subjects for the second part of the study were 16 OB (BMI > 30 kg/m^2) and 13 NW (BMI 19–23 kg/m^2) subjects, otherwise meeting the same inclusion criteria as described above. Based on a 38 % difference in iron absorption in OB subjects before and after weight loss in a study in adolescents using the serum iron appearance method [45], we used an expected 35 % lower fractional iron absorption in the OB compared to the NW women for our sample size calculation. Using a power of 90 %, the calculation indicated that 13 subjects were needed in each group. To account for potential attrition, we aimed at including 16 subjects in each group.

Informed written consent was obtained from each subject before starting the examinations. Ethical approval was obtained from the institutional ethical review board at St. John's Medical College Hospital.

Study design

In part 1 of the cross-sectional study, each subject presented for one or two visits at St. John's Research Institute depending on scheduling availability for the BodPod measurement. For anthropometric measurements and blood sampling, subjects were asked to present in the morning after an overnight fast (minimum 8 h) at the St. John's Research

Institute. Weight was measured to the nearest 0.1 kg using a digital balance (Salter 9016, Tonbridge, UK) calibrated with standard weights and height was measured to the nearest 0.1 cm using a portable stadiometer (built in-house and calibrated against a standard length measure). Waist circumference was measured to the nearest 0.1 cm using a non-stretchable measuring tape midway between the lowest rib and the top of the iliac crest. At the time of measurement, subjects were asked to gently breathe out [46]. Next, after sitting and resting for 15 min, blood pressure was measured twice using an automated device (OMRON, Model HEM-711DLXCAN, Health Care Canada) with the cubital fossa supported at heart level. During the 15-min resting period, a questionnaire on general health and lifestyle was completed. Following the blood pressure measurement, a venous blood sample of ca. 20 ml was taken: 10 ml into an uncoated tube and 10 ml into an EDTA-coated tube. Immediately following or on a separate day, body volume was measured by BodPod (COSMED, Rome, Italy) with the subjects wearing a swimsuit to rule out air being trapped in clothing. The BodPod was calibrated daily according to the manufacturer's instructions. Using the BodPod software suite, body composition was calculated from body density, body weight and body volume and percent fat mass (% FM) from assumed constant densities of fat-free mass (FFM) and fat mass (FM) [47].

For part 2 of the study, subjects were asked to present in the morning after an overnight fast at St. John's Research Institute. After weight and height were determined as described above and a general health questionnaire was completed, subjects were given a standardized test meal (see below) containing 5 mg ^{57}Fe as iron sulfate together with 2 dl deionized water. After completion of the test meal, subjects were instructed not to eat or drink anything during the following 3 h and not to engage in strenuous activities.

Test meal preparation

The standardized test meal consisted of tomato rice (rice, tomatoes, cooking oil, salt, turmeric, chili) which was prepared the day before test meal administration, stored in the fridge and re-heated in a microwave prior to consumption. The isotope solution was added directly to the test meal before consumption. The natural iron content of the test meal was 1.3 mg/meal.

Preparation of isotopes

The preparation of the isotopic label was similar to the method described by Walczyk et al. and Kastenmayer et al. [48, 49]. The label of [^{57}Fe]-$FeSO_4$ was prepared from isotopically enriched elemental iron (^{57}Fe at 97.83 % enrichment: Chemgas, Boulogne, France) by dissolution in 3 mol H_2SO_4/L solution. The solution was further diluted with water to yield 1 mol H_2SO_4/L. Solution of $^{57}FeSO_4$ so obtained was stored in polytetrafluoroethylene container that was flushed with argon to keep the iron in the 2^+ oxidation state.

Laboratory analysis

Biochemical measurements were taken for hemoglobin (Hb), total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, glucose, ferritin (SF), soluble transferrin receptor (sTfR), C-reactive protein (CRP), hepcidin, serum retinol, vitamin B_2, vitamin B_{12}, RBC folate and serum zinc. Hb concentrations were measured using an ABX Pentra 60 C^+ hematology analyzer (Horiba group, France). Lipid profiles, glucose, sTfR and CRP were measured using immuno turbidometry (Hitachi-902, Roche diagnostics, Risch, Switzerland). Ferritin, vitamin B12 and RBC folate were measured by electro-chemiluminescence (Elecsys 2010, Roche diagnostics, Risch, Switzerland). Hepcidin was analyzed by competitive immunoassay (Peninsula Laboratories, USA). Vitamin B2 was measured by erythrocyte glutathione reductase activation coefficient (EGRAC) method using UV–Vis 1800° spectrophotometer (Shimadzu Corporation, Japan). Serum retinol was measured by HPLC (Shimadzu Corporation, Japan), while serum zinc was measured using flame atomic absorption spectrometry (FAAS), (iCE 3500 series, Thermo Scientific, Bremen, Germany). To assess fractional iron absorption, whole blood samples were ashed and then subjected to acid digestion using a HNO_3/H_2O_2/HCl mixture, followed by separation of the iron from the sample matrix by anion exchange chromatography (200–400 mesh Ag 1-X8, Bio-Rad, California, USA) and a solvent/solvent extraction step into diethyl ether. Iron isotopic ratios were measured by negative thermal ionization mass spectrometry with a multicollector system for simultaneous ion beam detection (Triton, Thermo Scientific, Bremen, Germany) [48, 49]. The amount of circulating label was calculated as the product of the shift in the iron isotopic ratio and the amount of circulating iron in the blood [48]. Circulating iron was calculated from blood volume and hemoglobin concentration, and blood volume was calculated based on weight and height measurements [50]. Iron incorporation into red blood cells was assumed to be 80 % of the absorbed iron. All the biochemical analyses were carried out at the Micronutrient Laboratory, Division of Nutrition, St John's Research Institute, Bangalore.

Data analysis

BMI was calculated as weight (kg) divided by height $(m)^2$. For the classification of subjects by weight status, three

groups were defined based on BMI: NW (BMI 19–22.9 kg/m^2), OW (23–29.9 kg/m^2) and OB (≥30 kg/m^2). The cut-off for OW was chosen as 23 kg/m^2 a value previously suggested as a public health action point in Asian populations by a WHO expert consultation [51]. The harmonizing definition for the metabolic syndrome issued in a joint statement by the International Diabetes Federation Task Force on Epidemiology and Prevention, the National Heart, Lung, and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society and the International Association for the Study of Obesity was used [52]: At least three of the following five criteria must be present for the diagnosis of metabolic syndrome: waist circumference >80 cm for women (in South Asian populations), elevated triglycerides (>150 mg/dl), reduced HDL cholesterol (<50 mg/dl for women), elevated blood pressure (systolic BP > 130 or diastolic BP > 85 mm HG) and elevated fasting glucose (>100 mg/dl, or previously diagnosed type 2 diabetes). Anemia was defined based on the WHO recommendations as Hb < 12 g/dl [53].

Data were analyzed using SPSS Statistics version 19 (IBM Company, Armonk, NY, USA) and Excel (Microsoft Office 2010, Microsoft Corporation, Redmond, WA, USA). All data were checked for normal distribution visually before analysis. Non-normally distributed data were log-transformed. If log-transformed values were normally distributed, they were used for statistical testing; if they were still not normally distributed, nonparametric tests were used (CRP and hepcidin). Normally distributed values were reported as arithmetic mean ± SD, non-normally distributed values with normally distributed log-transformed values as geometric mean (95 % CI) and non-normally distributed values with non-normal log values as median (min–max). Independent samples *t* test and one-way ANOVA (with post hoc Bonferroni correction) were used for the comparison between groups in normally distributed data and those normally distributed after log transformation. For all other data, Kruskal–Wallis test with multiple Mann–Whitney tests for individual comparisons was used (also with post hoc Bonferroni correction). Logistic regression was used to determine odds ratios (ORs) for having higher sTfR concentrations depending on BMI or BMI group in study part 1. Spearman correlations and linear regression models were further used to investigate relationships between the different continuous parameters. In study part 2, Pearson correlations were used to investigate associations between iron absorption, hepcidin, iron status and inflammatory markers. ANCOVA was used to investigate the combined impact of those parameters on iron absorption. For all analyses, the level of significance was set at $p < 0.05$.

Table 1 Basic anthropometric characteristics (mean ± SD) of normal weight, overweight and obese women participating in a cross-sectional study in Bangalore, India

	NW	OW	OB
N	52	71	23
Age (year)	25.1 ± 4.4^{a}	27.9 ± 4.7^{b}	$26.3 \pm 4.6^{a,b}$
Weight (kg)	47.2 ± 6.2^{a}	63.0 ± 6.9^{b}	79.8 ± 8.8^{c}
Height (m)	$1.54 \pm 0.05^{a,b}$	1.53 ± 0.06^{a}	1.56 ± 0.05^{b}
BMI (kg/m^2)	19.8 ± 2.2^{a}	27.0 ± 1.7^{b}	32.6 ± 2.4^{c}
Waist circumference (cm)	66.4 ± 7.2^{a}	83.7 ± 7.4^{b}	93.2 ± 6.4^{c}
% body fat	30.6 ± 6.6^{a}	40.6 ± 4.0^{b}	47.3 ± 3.0^{c}
% lean tissue	69.4 ± 6.6^{a}	59.3 ± 4.0^{b}	54.0 ± 6.5^{c}

NW normal weight (BMI < 23 kg/km^2), *OW* overweight (BMI 23–29.9 kg/m^2), *OB* obese (BMI ≥ 30 kg/m^2), *BMI* body mass index

Means not sharing a common superscript letter are significantly different from each other (one-way ANOVA with post hoc Bonferroni test; $p < 0.05$)

Results

Cross-sectional assessment of micronutrient status (study part 1)

From a total of 150 subjects recruited, two were excluded because it was not possible to draw blood and two because of missing anthropometric data. Data from 146 women were used for analysis. Each subject was assigned to one of three groups based on their BMI: 52 were NW (BMI 19–22.9 kg/m^2), 71 OW (BMI 23–29.9 kg/m^2) and 23 OB (BMI ≥ 30 kg/m^2). Additionally, subjects were grouped based on the presence or absence of the metabolic syndrome; 19 were diagnosed with the metabolic syndrome, 8 were OB, and 11 were OW.

Basic anthropometric characteristics presented by BMI group are displayed in Table 1. Except for height, which was only significantly different between the OW and the OB group, all parameters differed significantly between all groups ($p < 0.01$). Comparison between the group with the metabolic syndrome and the group without showed significant differences in all anthropometric parameters except for height ($p < 0.01$, data not shown). When comparing subjects with the metabolic syndrome with OW and OB subjects without metabolic syndrome, OW and OB with metabolic syndrome had significantly higher weight ($p = 0.011$), BMI ($p = 0.012$) and WC ($p = 0.001$). However, % body fat or % lean tissue were similar between OW and OB with and without metabolic syndrome ($p > 0.05$).

Table 2 shows lipid profile, fasting glucose, the inflammatory marker CRP and blood pressure by BMI group. Except for total cholesterol and fasting glucose

Table 2 Metabolic parameters (lipid profile, fasting glucose, CRP) and blood pressure as well as vitamin and mineral status in 146 normal weight, overweight and obese women participating in a cross-sectional study in Bangalore, India

	NW	OW	OB
N	52	71	23
N with MS (%)	0 (0)	11 (15.5)	8 (34.8)
Total cholesterol (mg/dl)[1]	153.0 ± 30.1[a]	165.7 ± 33.2[a]	159.1 ± 19.5[a]
LDL cholesterol (mg/dl)[1]	93.8 ± 23.7[a]	108.5 ± 26.3[b]	105.0 ± 51.2[a,b]
HDL cholesterol (mg/dl)[1]	46.3 ± 10.5[a]	40.7 ± 9.6[b]	38.0 ± 7.1[b]
Triglycerides (mg/dl)[2]	63.9 (57.3, 71.3)[a]	88.1 (77.9, 99.7)[b]	94.9 (78.5, 114.7)[b]
Glucose (mg/dl)[2]	78.6 (76.6, 80.6)[a]	79.6 (75.5, 83.9)[a]	80.7 (75.0, 86.8)[a]
CRP (mg/dl)[3]	0.05 (0–0.86)[a]	0.20 (0–1.53)[b]	0.41 (0–1.61)[b]
Systolic blood pressure (mmHg)[1]	107.9 ± 7.5[a]	111.9 ± 10.5[a,b]	114.8 ± 8.7[b]
Diastolic blood pressure (mmHg)[1]	71.0 ± 7.5[a]	74.9 ± 7.4[b]	79.3 ± 7.3[c]
SF (ng/ml)[2]	15.0 (11.7, 19.2)[a]	22.0 (17.1, 28.4)[a,b]	29.3 (20.9, 41.0)[b]
sTfR (mg/dl)[2]	2.01 (1.68, 2.30)[a]	2.68 (2.31, 3.10)[b]	2.49 (1.93, 3.15)[a,b]
Hb (g/dl)[1]	12.2 ± 1.5[a]	12.5 ± 1.7[a]	12.8 ± 1.1[a]
Hepcidin (ng/ml)[3]	0.73 (0–53.9)[a]	4.43 (0.02–20.46)[b]	6.48 (0.46–26.08)[b]
Serum retinol (µmol/l)[1]	0.46 ± 0.10[a]	0.50 ± 0.11[a]	0.51 ± 0.11[a]
EGRAC[1]	1.11 ± 0.06[a]	1.10 ± 0.06[a]	1.11 ± 0.06[a]
Vitamin B12 (pg/ml)[2]	281.8 (52.9, 314.0)[a]	302.9 (273.0, 336.1)[a]	252.0 (210.4, 301.8)[a]
Folate (ng/ml)[1]	476.0 ± 180.3[a]	491.1 ± 162.3[a]	431.2 ± 86.6[a]
Serum zinc (µg/dl)[1]	114.1 ± 42.9[a]	117.1 ± 30.7[a]	116.1 ± 25.7[a]

NW normal weight (BMI < 23 kg/km^2), *OW* overweight (BMI 23–29.9 kg/m^2), *OB* obese (BMI ≥ 30 kg/m^2), *MS* metabolic syndrome (at least three of the following: high waist circumference, high triglycerides, low HDL cholesterol, high glucose, high blood pressure), *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *CRP* C-reactive protein, *SF* serum ferritin, *sTfR* soluble transferrin receptor, *Hb* hemoglobin, *EGRAC* erythrocyte glutathione reductase activation (to define vitamin B2 deficiency)

Means and medians not sharing a common superscript letter are significantly different from each other (one-way ANOVA with post hoc Bonferroni correction and, for CRP and hepcidin, Kruskal–Wallis test with multiple Mann–Whitney tests for individual comparisons (including Bonferroni correction), always between NW, OW and OB; $p < 0.05$)

[1] Arithmetic mean ± SD

[2] Geometric mean (95 % CI)

[3] Median (min–max)

concentrations, all parameters were significantly different between NW and OW and/or OB subjects. Generally, differences between NW and OW or OB were more pronounced than those between OW and OB. The inflammatory marker CRP increased significantly from the NW to the OW and the OB group [0.05 (0–0.86) mg/dl, 0.20 (0–1.53) mg/dl, and 0.41 (0–1.61) mg/dl, respectively, $p < 0.05$], but was not significantly different between OW and OB. By far, the highest concentration, however, was found in the group with the metabolic syndrome [0.67 (0.01–1.61) mg/dl, $p < 0.001$ compared to OW/OB non-metabolic syndrome subjects].

Biochemical parameters related to iron status (SF, sTfR, Hb, hepcidin) as well as serum retinol, EGRAC, vitamin B12, folate and serum zinc are also shown in Table 2. In parallel with increased inflammation, SF and hepcidin increased significantly from the NW to the OW and/or OB groups ($p < 0.05$). Furthermore, a significant increase in sTfR was observed from the NW to the OW group ($p = 0.035$) with no further increase in the OB group. None of the other vitamins or minerals were found to be different between the BMI groups. The OW/OB subjects with the metabolic syndrome showed significantly higher concentrations of SF [41.5 (27.5, 62.6) ng/ml vs. 20.0 (15.8, 25.3) ng/ml, $p = 0.005$), Hb (13.3 ± 1.1 g/dl vs. 12.4 ± 1.7 g/dl, $p = 0.032$) and CRP [0.67 (0.01–1.61) mg/dl vs. 0.20 (0.0–1.53) mg/dl, $p < 0.001$] compared to the OW/OB subjects without metabolic syndrome. None of the other parameters showed significant differences between subjects with or without the metabolic syndrome.

Bivariate spearman correlations of BMI and % BF with SF, sTfR, CRP and hepcidin are shown in Table 3 and Fig. 1. Strong associations between indicators of adiposity and inflammation but also iron status and hepcidin can be observed. Furthermore, in a linear regression model, BMI was a predictor of hepcidin ($p = 0.008$), independent of sTfR ($p = 0.171$, model $R^2 = 0.058$). To define the risk of having lower iron status with increasing BMI, subjects

Table 3 Correlation matrix (Spearman correlations) of BMI and % body fat with iron status (SF and sTfR), hepcidin and inflammation (CRP) status in 146 normal weight, overweight and obese subjects participating in a cross-sectional study in Bangalore, India

		BMI	% BF	SF	sTfR	CRP	Hepcidin
BMI	*r*	1.0	0.885	0.309	0.203	0.596	0.442
	p		<0.001	<0.001	0.014	<0.001	<0.001
% BF	*r*		1.0	0.168	0.186	0.561	0.314
	p			0.046	0.026	<0.001	<0.001
SF	*r*			1.0	-0.190	0.294	0.753
	p				0.022	<0.001	<0.001
sTfR	*r*				1.0	0.071	0.029
	p					0.395	0.730
CRP	*r*					1.0	0.336
	p						<0.001
Hepcidin	*r*						1.0
	p						

BMI body mass index, *% BF* % body fat, *SF* serum ferritin, *sTfR* soluble transferrin receptor, *CRP* C-reactive protein

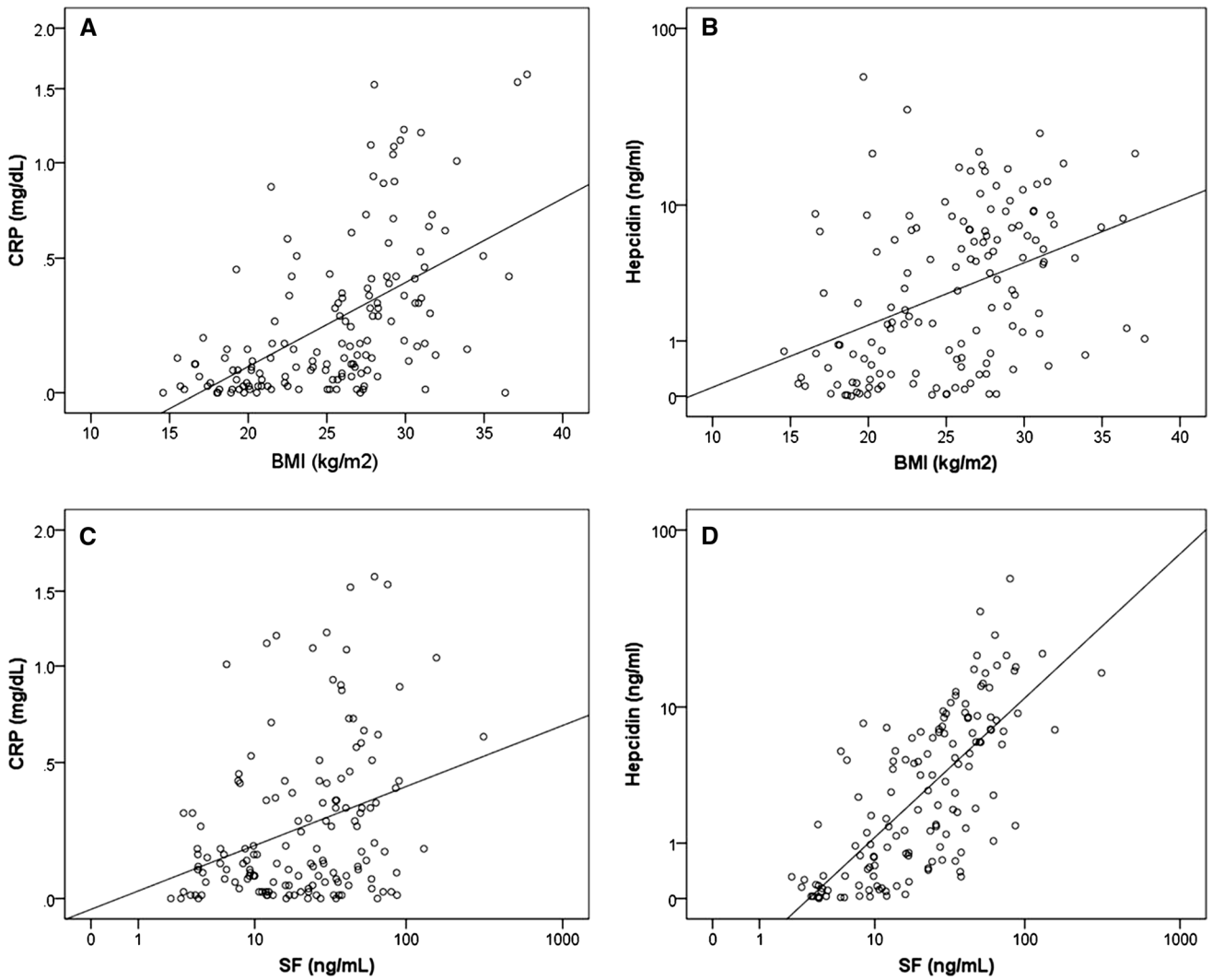

Fig. 1 Scatterplots showing the associations of BMI and CRP **a** as well as hepcidin **b** and of serum ferritin (SF) and CRP **c** as well as hepcidin **d** in 146 Indian women. Logarithmic scales are used for non-normally distributed data and a *linear regression line* is shown

Table 4 Characteristics of the obese and normal weight subjects participating in the iron absorption study conducted in Bangalore, India

	OB	NW
n	16	13
Weight (kg)[1]	84.5 ± 12.8[a]	51.9 ± 6.5[b]
Height (m)[1]	1.58 ± 0.04[a]	1.54 ± 0.06[a]
BMI (kg/m^2)[2]	33.7 (31.6, 36.0)[a]	21.7 (20.4, 23.1)[b]
Hb[1]	13.6 ± 1.0[a]	13.1 ± 1.2[a]
SF (ng/ml)[2]	46.7 (31.1, 70.2)[a]	17.3 (9.37, 31.8)[b]
sTfR (mg/dl)[2]	1.60 (1.11, 2.21)[a]	5.16 (3.79, 6.91)[b]
CRP (mg/dl)[3]	0.38 (0–1.63)[a]	0.07 (0–0.28)[b]
Hepcidin (ng/ml)[3]	2.93 (0.53–8.72)[a]	1.26 (0.12–8.29)[a]

OB obese, *NW* normal weight, *BMI* body mass index, *SF* serum ferritin, *sTfR* soluble transferrin receptor, *Hb* hemoglobin, *CRP* C-reactive protein

Means and medians not sharing a common superscript letter are significantly different from each other (independent samples *t* test on original data for weight, height and Hb, independent samples *t* test on log-transformed data for BMI, SF and sTfR, and Mann–Whitney *U* test for hepcidin and CRP)

[1] Arithmetic mean ± SD

[2] Geometric mean (95 % CI)

[3] Median (min–max)

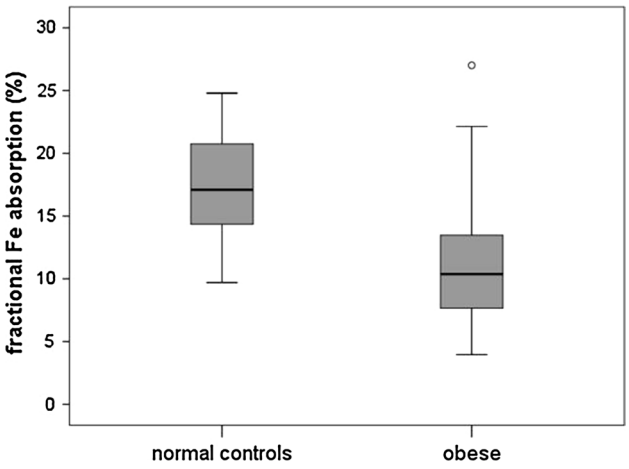

Fig. 2 Fractional iron absorption from a test meal labeled with ^{57}Fe in a group of normal weight and a group of obese subjects. The *box plots* show the median, with the *box* indicating the first to third quartile and the *whiskers* indicating the lowest and highest data points. *Outliers* are marked with *circles* and indicate data points >1.5 IQR above the third quartile

were divided in two groups (upper and lower 50 %, mean sTfR = 1.50 ± 0.58 and 4.0 ± 1.81 ng/ml) based on sTfR concentrations. Using these categories, logistic regression revealed a slightly but significantly increased risk of having high sTfR concentrations (i.e., a lower iron status) with increasing BMI (OR 1.09, 95 % CI 1.02–1.17). The effect was even more pronounced using categorical variables for weight status [NW (BMI < 23 kg/m^2) and OW (BMI ≥ 23 kg/m^2)]: The OR for having a higher sTfR in the OW group was 2.66 (95 % CI 1.318–5.382).

Bivariate spearman correlations were also conducted between BMI, % BF, WC and serum retinol, EGRAC, vitamin B12, folate and serum zinc, but no significant associations were seen except for the correlation of BMI and WC with serum retinol (BMI $r = 0.193$, $p = 0.022$; WC $r = 0.254$, $p = 0.002$).

Iron absorption study (study part 2)

General characteristics of the 16 OB and 13 NW subjects are described in Table 4. Based on the inclusion criteria, it was not surprising that weight and BMI were significantly different, while height was similar between the groups. Further differences were seen in regard to iron status. SF was significantly greater in the OB, while sTfR was higher in the NW group. CRP and hepcidin were also higher in the OB group, although only CRP reached statistical significance.

Fractional iron absorption was determined 14 days after ingestion of the stable iron isotopes (^{57}Fe). As depicted in Fig. 2, the geometric mean (±SD) fractional iron absorption was 10.0 % ± 6.5 in the OB and 16.7 % ± 4.6 in the NW control group. Comparing the two groups using independent samples *t* test revealed a significant difference in iron absorption between OB and NW subjects ($p = 0.007$).

Since iron status of the NW control group was significantly lower compared to the OB group (compare Table 4) and iron status is known to be a significant predictor of iron absorption, an analysis of covariance was conducted to compare iron absorption between the two groups using sTfR as a covariate. Using this analysis, the difference in iron absorption between the two groups still remained significant ($p = 0.038$). Furthermore, hepcidin concentrations were significantly negatively correlated with fractional iron absorption in the study subjects ($r = -0.384$, $p = 0.048$) and were also associated with sTfR ($r = -0.451$, $p = 0.018$).

Discussion

By investigating the associations between micronutrient deficiencies and OW/obesity, we have for the first time shown that the double burden of iron deficiency and obesity exists in a sample of middle-class women living in Bangalore, India. At the same time, we have not been able to demonstrate associations between body weight and any of the other micronutrients investigated. However, most importantly, with the second part of the study we have

demonstrated that iron absorption is indeed reduced in OB subjects compared to NW controls, even after controlling for iron status.

India is a country rapidly undergoing dietary and lifestyle changes. Based on data from the Indian National Family Health Survey 2005–2006 (NFHS 3), the national prevalence of OW (including obesity) was 9.7 % in men and 12.6 % in women. With considerable differences between geographic regions, the prevalence was 15.9 and 23.5 % in urban areas and 5.6 and 7.4 % in rural areas. At the same time, NFHS 3 reported 24.7 % of men and 56.2 % of women were affected by anemia [54]. Other indicators of micronutrient status were not assessed in the NFHS, but already the available data (on anemia and anthropometrics) clearly indicate the growing importance of excess adiposity and malnutrition in India.

Serum ferritin (SF) is an accurate measure of body iron stores in relatively healthy populations. However, SF is also an acute phase protein that is increased with inflammation even in the presence of low body iron stores. Thus, SF is an inaccurate marker of body iron stores in persons with systemic inflammation [53]. Obesity has been widely recognized as a state of low-grade systemic inflammation [15]. Consequently, we have shown that SF is positively correlated with CRP, a marker of systemic inflammation, indicating that SF is not a good indicator of iron stores in our OB population. Therefore, for further evaluation of iron status, sTfR was used. sTfR was not associated with CRP in our population and was thus assumed to be a reliable marker of iron status.

In the present study (part 1), in both discrete comparisons of BMI groups and the continuous analysis, a higher BMI was associated with a higher sTfR concentration and thus reduced tissue iron. This is in good agreement with previous studies showing an increased risk of iron deficiency in obesity [6–14].

Low iron status observed in obesity is thought to be due to an up-regulation of the iron regulatory peptide hormone hepcidin. Generally, hepcidin expression is down-regulated in iron deficiency and hypoxia and up-regulated in inflammatory states. Increased circulating hepcidin concentrations diminish dietary iron absorption and promote iron sequestration [55]. In obesity, an inflammatory state, macrophages infiltrating adipose tissue secrete interleukin 6 (IL6), which can then promote hepcidin production [15, 56]. Consequently, hepcidin concentrations have repeatedly been shown to be increased in obesity independent of iron stores [18, 19]. Similarly, in our data, we have shown a significant positive association between BMI and hepcidin independent of sTfR. Further, hepcidin was strongly associated with the inflammatory marker CRP. CRP is mainly secreted from hepatocytes, where its expression is up-regulated predominantly by IL6 [57]. Thus, we can conclude that the observed increase in hepcidin seen in the OW and OB subjects was likely triggered by low-grade systemic inflammation. The fact that sTfR was higher in OW/OB subjects and that hepcidin was overexpressed indicates that the low-grade systemic inflammation supersedes the down-regulation mechanism due to low iron status [58]. Furthermore, those data confirm the fear of public health experts worldwide that the increasing epidemic of obesity, especially in transition countries such as India, may undermine the efforts to control iron deficiency.

Generally, in iron deficiency, hepcidin is down-regulated and dietary iron absorption increased. However, in inflammatory states including obesity, hepcidin is up-regulated, potentially reducing dietary iron absorption [59]. It has previously been shown that iron absorption is indeed reduced with increasing BMI in Thai women [5], although only in a relatively low BMI range up to 27 kg/m^2. Consistent with those data, we have shown in our current work that iron absorption was significantly reduced in OB subjects compared to NW controls, even after controlling for differences in iron status. However, from our data it is not possible to determine the role of hepcidin in this process as, against expectations, hepcidin was not significantly different between the two groups. We would have expected hepcidin to be up-regulated in the OB subjects compared to the NW for two reasons: They had increased systemic inflammation (CRP) and better iron status. Nevertheless, hepcidin was slightly but not significantly higher in the OB group. The hepcidin concentration observed in the NW group included in the absorption analysis (part 2) (2.35 ng/ml) was higher compared to the NW group included in part 1 (0.73 ng/ml) despite lower iron status. This may to some extent be explained by somewhat higher inflammation in the NW group in part 2.

Even though some evidence from smaller studies exists linking obesity with increased zinc deficiency [33–35], not all studies agree [34, 60] and large epidemiological studies are lacking. The lack of data can be explained by the challenges in assessing zinc status and the lack of a reliable biomarker. In our sample of NW, OW and OB subjects, using the currently recommended biomarker for zinc status, serum zinc, we have not been able to detect differences in zinc status between the groups despite standardizing our methodology to fasting morning blood samples. This can most probably be explained by the low sensitivity of serum zinc as a marker for low zinc status as our subjects were middle-class women with a generally good health status. In order to clarify the impact of obesity on zinc status or vice versa, larger epidemiological studies assessing serum zinc would be desirable.

Several metabolites of vitamin A play a role in body weight regulation. Our data indicate no difference in vitamin A status between NW, OW and OB women, but we have shown a significant positive association between BMI as well as WC and serum retinol. On the other hand, in

animal studies, oral or intravenous administration of vitamin A can precipitate a reduction in body weight [61–63]. Despite some mechanistic evidence [31], it is unclear whether low vitamin A status promotes obesity given the existing studies were conducted in preclinical animal models and specific evidence linking vitamin A deficiency to body fat accumulation in humans is lacking. Our findings of a positive correlation between serum retinol and BMI as well as WC would rather point in the opposite direction.

B vitamins are essential cofactors for enzymes important in cell function and energy production [64, 65]. A deficiency in one or several B vitamins may therefore be important for body energy imbalance and thus in the development of obesity. In the past, different studies have shown low serum concentrations of several B vitamins in obesity, including vitamin B12 and folic acid, but findings have not been consistent [31]. We were not able to confirm associations in our study between weight status, waist circumference or body fat content and B vitamins (B2, B12 and folic acid).

The metabolic syndrome is a clustering of risk factors for the development of cardiovascular disease [66]. The prevalence of the metabolic syndrome in our study population was 15.5 % in the OW subjects and increased to 34.8 % in the OB group. Low-grade systemic inflammation has long been accepted as a factor closely linked with the metabolic syndrome and was found to be more prevalent in subjects diagnosed with the syndrome. As inflammation is thought to be the causal link between obesity and iron deficiency and may also be important in the deficiency of other micronutrients, subjects with the metabolic syndrome may be expected to be at an increased risk of the double burden of micronutrient deficiency. In line with the expectations, CRP was significantly higher in the metabolic syndrome group compared to OW/OB subjects without metabolic syndrome and so was SF. On the other hand, patients with the metabolic syndrome have been diagnosed with the dysmetabolic iron overload syndrome (DIOS), a syndrome characterized by elevated SF concentrations, high-normal transferrin saturation and liver iron deposition [67]. Based on our results, we cannot exclude the possibility that at least some of our OW/OB subjects with the metabolic syndrome suffered from DIOS but can also not confirm it as we did not determine all necessary parameters (e.g., transferrin saturation, liver transaminases and ideally hepatic iron content from biopsies). However, the elevated Hb concentration found in our OW/OB subjects with the metabolic syndrome may point toward higher iron stores in this group. For example, a meta-analysis found higher Hb concentrations in OB subjects and argued this might be related to tissue hypoxia secondary to obstructive sleep apnea and other respiratory conditions [68]. With regard to sTfR or the status of other micronutrients, no differences were found between subjects with and without the metabolic syndrome. However, as the number of subjects with the metabolic syndrome included in this study was relatively small (19 subjects), we cannot rule out the possibility that a lack of power was the reason for not seeing an association.

This study is not without limitations. The fact that we have not been able to recruit equal numbers of subjects in all groups in part 1, especially the low number of OB subjects, is a limitation of the current work. This may have contributed to our inability to detect associations between micronutrient status and obesity for all nutrients but iron, as small differences may not have been detected. Furthermore, we have unfortunately not been able to assess dietary intake (including supplement consumption) of our subjects which would have helped rule out differences in intake being responsible for differences in micronutrient status. However, previous studies have reported that suboptimal dietary iron intake is not associated with low iron status observed in OB populations [14, 18].

Another important limitation is certainly the difference in iron status between OB and NW subjects in part 2. As we did not screen the subjects for iron status, this was unfortunately only detected after test meal consumption. Nevertheless, by controlling the statistical analysis for iron status we are confident that the detected difference between OB and NW subjects is a true difference. One further potential limitation of our study is that we have used a single meal design for assessing iron absorption. Single meal iron absorption studies have been shown to overestimate the effect of dietary inhibitors and enhancers on absorption compared to daily diets [69, 70]. It has not been investigated to date whether the influence of subject factors such as inflammation or iron status also varies between single meal and multiple meal studies, but we do not expect this to be the case. Thus, even though we may not be able to judge the absolute iron absorption from normal meals based on this single meal study, we are confident that the differences seen between groups would remain proportional. Overall, we are convinced that the advantages of the study, combining a cross-sectional analysis in a larger number of subjects and with a large number of parameters analyzed with the exact determination of iron absorption using a stable isotope, outweigh the mentioned disadvantages.

In conclusion, we have demonstrated that OW and OB middle-class women in Bangalore have an increased risk of developing iron deficiency compared to their NW counterparts but are not at an elevated risk of a deficiency of any of the other micronutrients analyzed. The increased risk of iron deficiency seems to be mediated by increased inflammation which triggers hepcidin secretion and reduces iron absorption in OW and OB subjects. Using a stable iron isotope, we have further demonstrated that iron absorption was impaired in OB compared to NW subjects we have, however, not been able to demonstrate the hypothesized

role of hepcidin in this respect. Thus, the increasing obesity epidemic observed in India and other transition countries may undermine current efforts to control iron deficiency anemia in those areas. Therefore, public health approaches designed to combat iron deficiency need to consider the impact of obesity on the effectiveness of the selected iron repletion strategy. In populations with a high prevalence of obesity, a careful evaluation should be made as to how best to implement a fortification or supplementation program, and the amount of iron given may have to be adapted. The results of this study clearly show the importance of considering both over- and undernutrition when planning prevention or intervention strategies especially in transition countries with emerging obesity epidemics.

Acknowledgments We would like to thank Shanti Johnson, Vani Amalrajan, Blaze Correia, Bernice Edwards and Smitha Bath for their great work during recruitment and data collection and Christophe Zeder for his support with the isotope analyses. This study was supported by the Indian Council of Medical Research and a fellowship from the Swiss National Science Foundation.

Authors' contributions IHA, PT and AVK designed the study; IHA and PT conducted the research; IHA performed statistical analyses; BB performed essential analyses; IAH had primary responsibility for final content. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest Isabelle Herter-Aeberli, Prashanth Thankachan, Beena Bose and Anura V. Kurpad have no conflicts of interest.

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