

## Weight status and iron deficiency among urban Malian women of reproductive age

Nadia Fanou-Fogny<sup>1,2\*</sup>, Naomi J. Saronga<sup>2</sup>, Yara Koreissi<sup>2,3</sup>, Romain A. M. Dossa<sup>1</sup>, Alida Melse-Boonstra<sup>2</sup> and Inge D. Brouwer<sup>2</sup>

<sup>1</sup>Department of Nutrition and Food Sciences, Faculty of Agronomic Sciences, University of Abomey Calavi, 01 BP 526, Cotonou, Benin

<sup>2</sup>Division of Human Nutrition, Wageningen University, PO Box 8129, 6700 EV Wageningen, The Netherlands

<sup>3</sup>Food Technology Laboratory, Regional Agronomic Research Center of Sotuba, Institute of Rural Economics, BP 258 – Rue Mohamed V, Bamako, Mali

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The present study investigated the association between weight status and Fe deficiency (ID) among urban Malian women of reproductive age. Height, weight, serum ferritin (SF), soluble transferrin receptor (sTfR) and C-reactive protein (CRP) concentrations were measured in sixty apparently healthy women aged 15–49 years old in Bamako, Mali. Prevalences of overweight and obese were 19 and 9%, respectively. SF was non-significantly different between overweight (84 µg/l) and normal-weight women (52 µg/l). The prevalence of ID (SF < 12 µg/l) was 9% in the overweight group and no true ID (sTfR > 8.3 mg/l) cases were recorded in the overweight and obese groups. The prevalence OR of ID (SF < 12 µg/l) in the overweight group was NS (OR = 0.3; *P* = 0.363). Conversely, the chronic energy deficiency group was at a significantly higher risk of ID than the normal-weight group, adjusting or not for CRP (OR = 7.7; 95% CI 1.49, 39.96; *P* = 0.015). The lack of association between overweight and ID in the present study could be due to the fact that the excess of body fat of the women might not be critical to induce chronic inflammation related to reduced Fe absorption. Future research based on a larger convenience sample should be designed to further investigate associations between overweight, obesity and ID in developing countries.

### Overweight and iron deficiency: Mali: Women of reproductive age: Urban areas: West Africa

Fe deficiency (ID) continues to be the most prevalent single micronutrient deficiency among women of reproductive age in Mali, West Africa<sup>(1)</sup>. In the meantime, obesity has become an emerging public health concern in developing countries, especially in urban communities<sup>(2,3)</sup>. In Bamako, 10% of women were overweight in 2001<sup>(4)</sup>. Recently, significant correlations between serum Fe, soluble transferrin receptors (sTfR), fat mass and BMI have been reported, and it was suggested that excess adiposity may negatively affect Fe status<sup>(5–8)</sup>. Both ID and obesity increase the risk of overall poor health and are associated with increased risk of mortality<sup>(1,9)</sup> but definitive mechanisms explaining low Fe status in the obese have not been clearly determined<sup>(10)</sup>. The association between Fe status and obesity should be explored further, as obesity and ID continue to evolve worldwide, and the combined impact of these nutritional comorbidities is unknown<sup>(11)</sup>. Because very few studies have investigated the association between ID and weight status in a developing context, exploratory research is a necessary step to bring forward consistent conclusions about relationships between ID and overweight/obesity. The present study examined the association between weight status and ID

among women of reproductive age in urban Mali, using part of the data originally collected for a larger research project named FONIO (EU/INCO no. 0015403). (Fonio is the name of a West African traditional cereal). Although the research was not powered to study the relationship between weight status and ID, results were found to be interesting to publish in a research communication – as data on developing countries are still lacking – and to generate points of discussion about the interaction between obesity and Fe status in developing countries for further research. We hypothesised that overweight women have a higher risk of ID than normal-weight women, irrespective of Fe intake.

### Subjects and methods

For the purpose of the FONIO project, 108 Malian women aged 15–49 years old were randomly selected from 108 households of Bamako, using a three-stage cluster sampling<sup>(12)</sup> and the random walk method<sup>(13)</sup>. The sampling procedure has been fully described elsewhere<sup>(14)</sup>.

For the present study, a sub-sample of sixty women was selected from the main sample of the FONIO project, based on

**Abbreviations:** CED, chronic energy deficiency; CRP, C-reactive protein; ID, iron deficiency; SF, serum ferritin; sTfR, soluble transferrin receptors.

\* **Corresponding author:** N. Fanou-Fogny, email [nadia.fanou@gmail.com](mailto:nadia.fanou@gmail.com)

their willingness to participate in anthropometry and blood sample collection. Due to the small sample size, statistical power of the results is limited.

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethical committee of the Malian National Institute of Research in Public Health (Mali). Verbal consent was witnessed and a written informed consent was obtained from all the participants before data collection. All the data were collected over a period of 3 months.

### Anthropometry

Body weight and height were measured early in the morning, from fasting subjects according to WHO standardised procedures<sup>(15)</sup>. Weight was measured with a SECA Model 761 platform spring balance scale with a 150 kg maximum range and 0.5 kg graduation (Seca 761; Seca, Hamburg, Germany). Height was measured with a body measuring tape with 2200 mm maximum range and 1 mm graduation. BMI ( $\text{kg/m}^2$ ) was calculated from anthropometric data and subjects were divided into four groups: chronic energy deficiency (CED) defined as  $\text{BMI} < 18.5 \text{ kg/m}^2$ , normal weight defined as  $18.5 \leq \text{BMI} \leq 24.9 \text{ kg/m}^2$ , overweight defined as  $25 \leq \text{BMI} < 30 \text{ kg/m}^2$  and obesity defined as  $\text{BMI} \geq 30 \text{ kg/m}^2$ <sup>(16)</sup>.

### Blood samples and biochemical measurements

Whole blood was collected by venepuncture from overnight fasting subjects in  $\text{K}_2$  EDTA tubes (2 ml) for Hb, and in normal tubes (6 ml) for sTfR, serum ferritin (SF) and C-reactive protein (CRP). Hb was measured using an automated counter (Horiba ABX Micros 60-OT16, Montpellier, France). Anaemia was defined as  $\text{Hb} < 120 \text{ g/l}$ <sup>(17)</sup>. SF was analysed by chemiluminescent immunoassay using the Beckman Access Immunoassay DXL System (Immulate/Ferritin, Beckman Coulter, Inc., Fullerton, CA, USA). sTfR was analysed by ELISA (Ramco, Stafford, TX, USA), using the quality-control materials supplied by the company. ID was defined as  $\text{SF} < 12 \text{ } \mu\text{g/l}$ . Because SF can be influenced by a high CRP level<sup>(18)</sup>, true ID was also defined using  $\text{sTfR} > 8.3 \text{ mg/l}$ <sup>(17)</sup>. High sensitive CRP was measured using latex nephelometry (BNProSpec System; Dade Behring Limited, Tokyo, Japan), normal reference value:  $\text{CRP} < 7.5 \text{ mg/l}$ <sup>(19)</sup>.

### Dietary and nutrient adequacy assessment

Energy and Fe intake was assessed by two non-consecutive 24 h dietary recalls as described in Gibson & Ferguson<sup>(13)</sup>. All days of the week were included in the 24 h recalls except weekends and special event days to reduce bias due to occasional consumption of foods with exceptionally high Fe or other (micronutrients) content. Foods were weighed with digital dietary scales with a 10 kg maximum range and 2 g graduation. Procedures for dietary assessment and food intake data processing have been fully described elsewhere<sup>(14)</sup>.

Probability of Fe adequacy was derived from the intake of the 2 recall days, assuming a bioavailability factor of 5% regarding the diet of the women. As Fe requirement distribution is not normally distributed<sup>(20)</sup>, probability of

Fe adequacy was determined by the intake of absorbed Fe from the Institute of Medicine table for adult women<sup>(21)</sup>.

### Statistical analysis

Statistics were performed using SPSS 15 for windows (SPSS, Inc., release 15.0.1.1, Chicago, IL, USA, 2007). A  $P$ -value  $< 0.05$  was considered significant. Normality of variable distribution in BMI groups was assessed using the Shapiro–Wilk test. Mean Fe intake, SF and sTfR distributions were (log) transformed for normality. One-way ANOVA test was used for means comparison; when significant, the Tukey's minimum significant difference was used as a *post hoc* test, assuming homogeneity of variances. Pearson's  $\chi^2$  test was used for comparison of proportions. Logistic regression was performed to determine the prevalence OR of ID in overweight and CED groups using the normal-weight group as reference. The model was adjusted for CRP by excluding three cases (each from the CED, normal-weight and obese groups) with simultaneously high CRP ( $> 20 \text{ mg/l}$ ) and ferritin levels ( $> 65 \text{ } \mu\text{g/l}$ ), and by including CRP as a covariate in the model.

### Results

The mean age of the women was 32.5 (SD 10) years. BMI ranged between 16.6 and 38.7 with a mean of  $23.4 \text{ kg/m}^2$  (Table 1). Prevalences of overweight and obesity were 19 and 9%, respectively. About 16% of the women suffered from CED. Mean daily Fe intake appeared to be the lowest in the obese group (13 mg) as compared to the normal-weight (19 mg) and CED groups (22 mg) but this was NS. Of the women, 20% had an adequate intake of Fe (probability of Fe adequacy = 0.2); 30 and 20% of the women in the CED and overweight groups had an adequate intake, respectively, while none of the obese women had an adequate Fe intake. However, there was no significant difference between the groups. Mean concentration of SF was  $39 \text{ } \mu\text{g/l}$ , being significantly higher in the obese group ( $84 \text{ } \mu\text{g/l}$ ) compared to the CED group ( $15 \text{ } \mu\text{g/l}$ ). However, there was no significant difference between SF of the overweight group ( $52 \text{ } \mu\text{g/l}$ ) and SF of the normal-weight group ( $36 \text{ } \mu\text{g/l}$ ). Using SF as an indicator, the prevalence of ID was 25%, with the lowest prevalence recorded in the overweight group (9%). No ID cases were recorded among obese women. Overall sTfR levels and means among groups were not higher than the normal reference value for detecting true ID ( $> 8.3 \text{ mg/l}$ ). Subsequently, overall prevalence of true ID among women was low (9%). Also, no true ID cases were recorded in overweight and obese groups. Mean CRP level was lower ( $2.4 \text{ mg/l}$ ) than the reference value for detecting present inflammation ( $> 7.5 \text{ mg/l}$ ). Overweight and obese groups showed higher CRP values as compared to normal and CED groups but this was NS. Except for the three cases which were deleted because of simultaneously very high CRP ( $> 20 \text{ mg/l}$ ) and ferritin levels ( $\text{SF} > 65 \text{ } \mu\text{g/l}$ ), five out of the fifty-seven women (9%) showed an elevated CRP level ( $> 7.5 \text{ mg/l}$ ), of which one was in the overweight group (9%), one was in the CED group (11%) and three were in the normal-weight group (9%). The proportion of women with elevated CRP level ( $> 7.5 \text{ mg/l}$ ) was non-significantly different between groups.

Because none of the obese women was Fe deficient, only overweight women were included in the regression model.

**Table 1.** Characteristics of the women by weight category and mean comparison between chronic energy deficiency (CED), normal, overweight and obese groups\*

(Mean values and standard deviations)

	All groups (n 57)		CED (n 9)		Normal weight (n 32)		Overweight (n 11)		Obese (n 5)		Mean comparison (P)
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
BMI (kg/m <sup>2</sup> )	23.4	4.9	17.6	0.7	21.9	1.98	27.2	1.2	34.9	3.4	
Energy intake (kJ/d)	8946	2432.7	9496	1814.4	9088	2297.4	8085	3536.4	8959	1096.2	0.596
Fe intake (mg/d)	18.4	7.9	22.3	8.8	18.5	7.4	17.4	9.1	13.0	2.7	0.209
PAiron	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.3	0.0	0.0	0.107
Hb (g/l)	123	15	113	22	123	12	128	16	128	6	0.123
SF (µg/l)	39.3	51.0	14.8	14.6	33.8	31.5	52.2	72.3	83.5	104.3	0.004†
sTfR (mg/l)	4.6	3.6	7.6	7.3	4.2	2.5	3.7	0.8	3.7	1.2	0.094
CRP (mg/l)	2.4	3.6	1.3	2.5	2.0	3.9	3.8	3.4	3.7	2.4	0.315
Fe status											
Anaemia (%)											
Hb < 120 g/l	35		44		38		36		0		0.371
Fe deficiency (%)											
SF < 12 µg/l	25		67		22		9		0		0.008†
sTfR > 8.5 mg/l	9		22		9		0		0		0.310
Elevated CRP (%)											
> 7.5 mg/l	9		11		9		9		0		0.906

PAiron, probability of iron adequacy; SF, serum ferritin; sTfR, serum transferrin receptors; CRP, C-reactive protein.

\* One-way ANOVA for means comparison and Pearson's  $\chi^2$  for comparison of proportions.†  $P < 0.05$ .

Also, because sTfR indicated zero prevalence among the overweight and obese women, only SF was used as an Fe biomarker in the model. The prevalence OR of ID (SF < 12 µg/l) in the overweight group was < 1 and NS (OR = 0.3; 95 % CI 0.04, 3.24;  $P=0.363$ ). Adjusting for CRP level did not influence the relationship (OR = 0.3; 95 % CI 0.03, 2.99;  $P=0.314$ ). Conversely, the CED group was at a significantly higher risk of ID than the normal-weight group (OR = 7.1; 95 % CI 1.41, 36.08;  $P=0.017$ ). The relationship remained significant after adjusting for CRP level (OR = 7.7; 95 % CI 1.49, 39.96;  $P=0.015$ ).

## Discussion and conclusion

Based on the studies suggesting that obesity may be a causal factor in the development of ID in adults, one might expect that indications of poor Fe status would be observed in overweight and obese adults, especially in settings where the double burden of malnutrition and micronutrient deficiencies exists simultaneously<sup>(8)</sup>. In the present study, we investigated the association between weight status and ID among urban Malian women of reproductive age. There was a lack of association between ID and BMI in overweight women; the prevalence OR of ID was low and NS for overweight women, using normal weight as reference; mean SF was not significantly different between overweight and normal-weight women; overweight women showed the lowest ID prevalence.

### Study limitation

Limitations of the present study included the use of SF as a unique biomarker in the regression model. SF may be elevated in inflammatory conditions even in the presence of true ID, and therefore resulted in a misleadingly high ferritin concentration<sup>(18)</sup>. Indeed, the present results showed a trend of higher CRP and

ferritin levels in the overweight and obese groups than in the normal-weight and CED groups, although this was not statistically significant. sTfR concentration has been suggested as the best indicator for detecting true ID because it is not influenced by infection or by inflammatory processes<sup>(22)</sup>. Also, as indicated by Looker *et al.*<sup>(23,24)</sup>, an individual must show an abnormal value for two or more indicators to be considered Fe deficient. This did not apply to the present study because sTfR concentration indicated zero prevalence of ID among overweight and obese groups and thus was not included in the model. Nonetheless, SF has been indicated as the most specific biochemical test that correlates with relative total body Fe stores. Looker *et al.*<sup>(23)</sup> reported that models including ferritin are more likely to detect an early stage of ID. Indeed, low SF levels reflect depleted Fe stores and hence are a precondition for ID in the absence of infection<sup>(17)</sup>. In our model, a correction has been made to reduce bias related to the use of ferritin by excluding three cases with simultaneously high CRP and ferritin levels (> 65 µg/l), and by including CRP as a covariate in the model.

The second limitation of the present study was the probability that under-reporting of dietary intake could have occurred, as indicated by the lowest energy intake of the overweight group as compared to the normal-weight and CED groups (non-significantly different though). Also, the prevalence of inadequate intake of Fe was almost 100 % in the obese group for a zero prevalence of ID. Dietary under-reporting has been shown to be particularly prevalent in obese subjects<sup>(25)</sup>. In the present study, the interviewers were carefully trained and supervised in order to minimise reporting errors, and it was not possible to detect under-reporting during food intake data collection.

### Iron deficiency in overweight/obese

The results of the present study confirmed those of Karl *et al.*<sup>(10)</sup> who reported that decreased Fe status was not associated with

increasing adiposity among overweight and overfat non-obese American women.

One hypothesis developed to explain the association between high rates of ID among overweight individuals is that overweight may be associated with lower Fe intake from a poor-quality diet<sup>(26)</sup>. The present study did not support this hypothesis; mean Fe intake was not-significantly different between weight categories. This confirmed the results of previous studies which showed that dietary Fe intakes in overweight adults were not lower than in normal-weight individuals<sup>(5,6)</sup>.

A second hypothesis is that chronic inflammation and increased leptin production in obesity increase hepcidin secretion from the liver<sup>(27)</sup> which, along with hepcidin produced by adipose tissue<sup>(28)</sup>, could reduce dietary Fe absorption<sup>(29)</sup>. Recently, higher circulating hepcidin concentrations have been associated with lower Fe status in overweight children<sup>(30)</sup>. Owing to the fact that obesity is an excess accumulation of body fat resulting in a chronic inflammation condition<sup>(31)</sup>, Karl *et al.*<sup>(10)</sup> suggested that a certain critical level of relative body fat mass may be required to support an association between body composition and poor Fe status in obese adults. This might be applicable in the present study. In our sample, none of the obese women was Fe deficient and although body fat was not assessed, the mean BMI of overweight women (27 kg/m<sup>2</sup>) suggested that the level of body fat may not be enough to induce the critical chronic inflammation level associated with obesity. Although hepcidin level was not measured in the present study, the rate of chronic inflammation observed among overweight women was 9% and the NS difference between BMI categories showed that CRP was not associated with weight status. All this together supported the assumption that the lack of association between ID and overweight in the present study might be due to the fact that the fat mass in overweight women did not meet the critical level needed to induce an inflammatory process, and thus the production of hepcidin leading to the reduction of absorbed Fe.

In addition, an inverse relationship has been found between physical activity and weight gain, and it has been suggested that physical inactivity could be another factor associated with ID in obesity<sup>(5)</sup>, but this assumption has not yet been tested in human subjects. McClung *et al.*<sup>(32)</sup> investigated the effects of physical activity on moderate Fe-deficient rats' body composition. They reported that moderate ID may cause increased body fat accretion, while physical activity attenuates that effect. Physical activity was not measured in the present study but 90% of the women in our sample are housewives, with activities such as cleaning the house, shopping at the market, cooking and getting water (from the well). This indicates that the potential increase of ID due to increased fat mass in overweight women could be attenuated by the physical activity level of the women, but this hypothesis could not be verified with our data.

#### *Iron deficiency in chronic energy deficiency*

The present study showed that individuals in the CED group are significantly at risk of ID using SF as the biomarker, although they apparently have the highest Fe intake as compared to the other groups. However, the probability of

Fe adequacy of the CED group (0.3) indicated that the prevalence of inadequate intake is 70%, which is close to the observed prevalence of ID in the group (67%). This confirms the recommendation that the estimate of Fe inadequacy prevalence should agree with the prevalence of ID<sup>(20)</sup>.

#### *Conclusion and public health significance*

The present study was the first exploratory research about association between ID and weight status in an urban West African context. From a public health perspective, the study revealed that ID, CED and obesity are all occurring at the public health level among women of reproductive age in urban Mali. However, based on our sample, there were no ID cases among obese women and no association between ID and weight status among overweight women. This could be due to the fact that in our sample the excess of body fat might not be critical to induce chronic inflammation related to reduced Fe absorption. Future research based on larger convenience samples should be designed to further investigate associations between overweight, obesity and ID in developing countries along with causal factors related to this association.

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