THE OFFICIAL JOURNAL OF THE BRITISH DIETETIC ASSOCIATION

Journal of Human Nutrition and Dietetics

Journal of Human Nutrition and Dietetics

RESEARCH PAPER

The double burden of obesity and iron deficiency on children and adolescents in Greece: the Healthy Growth Study

Y. Manios,* G. Moschonis,† G. P. Chrousos,† C. Lionis,‡ V. Mougios,§ M. Kantilafti,* V. Tzotzola,* K. P. Skenderi,* A. Petridou,§ G. Tsalis,§ A. Sakellaropoulou,§ G. Skouli§ & C. Katsarou*

*Department of Nutrition and Dietetics, Harokopio University of Athens, Athens, Greece
†First Department of Pediatrics, Athens University Medical School, 'Aghia Sophia' Children's Hospital, Athens, Greece
‡Clinic of Social and Family Medicine, School of Medicine, University of Crete, Crete, Greece
§Department of Physical Education and Sport Science, Aristotle University of Thessaloniki, Thessaloniki, Greece

Keywords
adolescents, children, diet, inflammation, iron deficiency, obesity.

Correspondence
Y. Manios, Department of Nutrition and Dietetics, Harokopio University of Athens, 70, El.Venizelou Ave, 17671 Kallithea, Athens, Greece.
Tel.: +30 210 9549156
Fax: +30 210 9514759
E-mail: manios@hua.gr

How to cite this article
Manios Y., Moschonis G., Chrousos G.P., Lionis C., Mougios V., Kantilafti M., Tzotzola V., Skenderi K.P., Petridou A., Tsalis G., Sakellaropoulou A., Skouli G. & Katsarou C. (2012) The double burden of obesity and iron deficiency on children and adolescents in Greece: the Healthy Growth Study. *J Hum Nutr Diet*. doi:10.1111/jhn.12025

Abstract

Background: Some small cohort studies have noted that obesity co-exists with lower serum iron levels. The present study aimed to examine the association between being overweight and iron deficiency (ID) in a large cohort of Greek children and adolescents.
Methods: A representative sample of 2492 primary schoolchildren aged 9–13 years old was examined. Anthropometric, biochemical, clinical, dietary intake and physical activity data were collected.
Results: The prevalence of ID and iron deficiency anaemia (IDA) was higher in obese boys and girls compared to their normal-weight peers ($P < 0.05$). Serum ferritin was higher in obese compared to normal-weight boys ($P = 0.024$) and higher in obese compared to normal-weight and overweight girls ($P = 0.001$). By contrast, a negative association was found between transferrin saturation and adiposity in both boys and girls ($P = 0.001$ and $P = 0.005$). Furthermore, obese girls had significantly higher fibre intake than normal-weight girls ($P = 0.048$) and also overweight and obese boys and girls recorded significantly fewer pedometer steps than their normal-weight peers ($P < 0.001$). Finally, obesity more than doubled the likelihood of ID in both boys (odds ratio = 2.83; 95% confidence inteval = 1.65–4.85) and girls (odds ratio = 2.03; 95% confidence interval = 1.08–3.81) after controlling for certain lifestyle and clinical indices as potential confounders.
Conclusions: The present study shows that obese children and adolescents were at greater risk for ID and IDA than their normal-weight peers. Low grade inflammation induced by excessive adiposity may be a reason for the observed low iron levels. This is also strengthened by the elevated serum ferritin levels, comprising an acute phase protein that is plausibly increased in inflammation.

Introduction

Wenzel *et al.* (1962) were the first to report lower serum iron levels in obese compared to non-obese adolescents. Subsequently, this association has received little attention. However, some recent studies have shown that overweight children and adolescents are at higher risk for iron deficiency (ID) compared to normal-weight counterparts (Pinhas-Hamiel *et al.*, 2003; Nead *et al.*, 2004; del Giudice *et al.*, 2009). The exact aetiology of this double burden (i.e. obesity and ID) remains uncertain. Suggested contributing factors include a poor dietary iron intake,

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

probably as a result of repeated short-term restrictive diets, in overweight individuals and genetics (Pinhas-Hamiel *et al.*, 2003; Nead *et al.*, 2004). Still, the exact aetiology of obesity-related hypofaerremia in children remains uncertain (Menzie *et al.*, 2008).

Considering that obesity represents a low-grade chronic inflammatory state, the hypofaerremia associated with obesity could be attributed to certain metabolic and molecular adaptations induced by inflammation. This hypothesis is supported by the significant inverse correlations observed in overweight and obese individuals between serum iron concentration and the concentrations of a variety of adipocytokines and pro-inflammatory cytokines after controlling for iron intake, iron needs and iron losses (Chung *et al.*, 2007; Tussing-Humphreys *et al.*, 2009). Furthermore, certain acute-phase peptides (e.g. hepcidin) that are usually elevated in obesity have also been found to regulate iron haemostasis, mainly via their iron-binding effects (Menzie *et al.*, 2008; del Giudice *et al.*, 2009).

Impaired iron status in children and adolescents could have several important clinical implications for future physical and cognitive development and maturation. Thus, identifying overweight/obese children as a high-risk group for ID could be very important from a public health perspective for designing and implementing tailored-made dietary intervention approaches to correct both excess body weight and poor iron status, even in developed countries. The present study aimed to examine the association between being overweight and iron status in children and adolescents in Greece and to identify the main risk factors for ID.

Materials and methods

Sampling

The Healthy Growth Study was a large scale cross-sectional epidemiological study, initiated in May 2007. Approval to conduct the study was granted by the Greek Ministry of National Education and the Ethical Committee of Harokopio University of Athens. The population under investigation comprised schoolchildren aged 9–13 years old, who were attending the fifth and sixth grades of primary schools located in municipalities within the prefectures of Attica, Aitoloakarnania, Thessaloniki and Iraklio. The sampling of schools was random, multistage and stratified by parents' educational level and the total population of students attending schools within these municipalities, as described in more detail elsewhere (Moschonis *et al.*, 2010). The multistage, random sampling procedure yielded 77 primary schools that responded positively when they were invited to participate in the study. Weight and height were measured in all pupils attending the fifth and sixth grades in these primary schools as a part of a school-based health and nutrition education programme. Full medical examination (i.e. anthropometric and body composition measurements, blood collection, clinical examination, etc.) and questionnaire data were obtained from a subgroup of pupils whose parents provided their written informed consent form. Signed parental consent forms were collected for 2655 out of 4145 children (a response rate of 64.1%).

Physical examination and anthropometry

Participants underwent a physical examination by two trained members of the research team. The protocol and equipment used were the same in all schools. Weight was measured to the nearest 10 g using a Seca digital scale (Seca Alpha, Model 770; SECA, Hamburg, Germany). Pupils were weighed without shoes in the minimum clothing possible. Height was measured to the nearest 0.1 cm using a commercial stadiometer (Leicester Height Measure; Invicta Plastics, Oadby, UK) with the pupil standing barefoot, keeping the shoulders in a relaxed position, arms hanging freely and head in Frankfurt horizontal plane. Weight and height were used to calculate body mass index (BMI) using Quetelet's equation [weight (kg)/height $(m)^2$]. The International Obesity Task Force cut-off points (Cole *et al.*, 2007, 2000) were used to categorise participants as 'underweight', 'normal weight', 'overweight' or 'obese' (cut-off points for 'Overweight' ranged from 19.10 to 21.91 for boys and 19.07 to 22.58 for girls; cut-off points for 'Obesity' ranged from 22.77 to 26.84 for boys and 22.81 to 27.76 for girls). Furthermore, one well-trained and experienced female paediatrician in each prefecture determined pubertal maturation (Tanner stage) after a thorough visual inspection of breast development in girls and genital development in boys (Tanner, 1955). Finally, each girl was asked by the paediatrician about her menstruation status and age of menarche.

Haematological and biochemical indices

Blood samples were obtained for biochemical and haematological screening tests between 08.30 h and 10.30 h after a 12-h overnight fast. Professional staff performed venipunctures to obtain a maximum of 10 mL of blood. A CELL-DYN haematological auto analyser (Abbott Diagnostics, Abbott Park, IL, USA) was used to assess red blood cell count (RBC), haemoglobin, haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The remaining blood was centrifuged to collect serum, divided into aliquots of 2 mL and stored at

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

−80 °C. All serum samples were transported in dry ice to the Laboratory of Nutrition and Clinical Dietetics at Harokopio University, where biochemical analyses took place. Serum iron and total iron binding capacity (TIBC) were determined by colorimetric assays (Roche Diagnostics SA, Basel, Switzerland). Transferrin saturation (TSAT) was calculated by dividing serum iron by TIBC and multiplying by 100. Finally, serum ferritin was measured using a chemiluminescence immunoassay (Siemens Healthcare Diagnostics, Tarrytown, NY, USA).

ID (with or without anaemia) and iron deficiency anaemia (IDA) were defined using the age- and sex-specific thresholds proposed by UNICEF and the World Health Organization (WHO, 2001): ID was defined as TSAT <16%; IDA was defined as ID with blood haemoglobin concentration <12 g dL^{-1}, which is the threshold value for anaemia for children aged 9–13 years. The Mentzer Index [MCV(fL)/RBC (M μL^{-1})] (Mentzer, 1973) of all the children and adolescents with anaemia was also calculated to differentiate thalassemia minor from IDA. On the basis of this index, children and adolescents with thalassemia minor (18 cases) were excluded from further analysis.

Dietary assessment

Dietary intake data were obtained for two consecutive weekdays and one weekend day, using the 24-h recall technique. Specifically, all study participants were asked to describe the type and amount of foods, as well as all beverages consumed during the previous day, provided that it was a usual day according to the participant's perception. To improve the accuracy of food description, standard household measures (cups, tablespoons, etc.) and food models were used to define amounts where appropriate. At the end of each interview, the interviewers, who were dieticians rigorously trained to minimise the interviewer's effect, reviewed the collected data with the respondent to clarify entries, servings and possibly forgotten foods. Food intake data were analysed using NUTRITIONIST V diet analysis software, version 2.1 (First Databank, San Bruno, CA, USA), which was extensively amended to include traditional Greek recipes, as described previously (Trichopoulou, 2004). Furthermore, the database was updated with nutritional information of processed foods provided by independent research institutes, food companies and fast-food chains.

Physical activity assessment

To assess physical activity, study participants were provided with and instructed to wear a waist-mounted pedometer (Yamax SW-200 Digiwalker; Yamax Corporation, Tokyo, Japan) for 1 week (i.e. from Monday to Sunday). The pedometer was positioned in accordance with the manufacturer's instructions on the right waistband, vertically aligned with the patella. The pedometer used in the present study displayed the cumulative number of steps from the time it was worn in the morning until the time it was removed at bedtime. Participants were provided with a diary template and instructed to record the total number of daily steps displayed by the pedometer at bedtime and then zero the pedometer. Participants were also instructed to take off the pedometers when bathing or swimming and to record these activities in the diary. Finally, in cases where some children or adolescents forgot to wear the pedometer for a whole day, the relevant cells in the diary were left blank and the calculation for total mean steps per day was made using the appropriate number of days.

Socio-economic and demographic variables

Data on parents' educational level (i.e. total years of education for the father and mother) were collected from the parents (mainly the mother) during scheduled interviews at school. For those parents not being able to attend (approximately 5% of the total sample), data were collected via telephone interviews. All interviews were conducted with the use of a standardised questionnaire by researchers that were rigorously trained to minimise the interviewer's effect.

Statistical analysis

Normality of the distribution of continuous variables was tested using the Kolmogorov–Smirnov test and homogeneity of variances was tested using Levene's test. Continuous variables are expressed as the mean (SD), and categorical variables are reported as frequencies (%). Comparisons between levels of the continuous variables were conducted using one-way analysis of variance for normally distributed variables and the nonparametric Kruskal–Wallis test for variables that remained non-normally distributed, even though logarithmic transformations were made. Comparisons between levels of the categorical variables were conducted using the chi-squared test or the Fisher's exact test, as appropriate. The two-sample *Z*-test was also used to make pairwise comparisons on the prevalence of ID and IDA between weight groups. To test the effect of the independent variables examined on ID and IDA, multivariate logistic regression analysis was conducted and adjusted odds ratios (OR) with 95% confidence intervals (CI) were computed. All reported *P*-values refer to two-sided tests. The level of statistical significance was set at $\alpha = 0.05$. Statistical analysis was conducted using SPSS, version 16.0 (SPSS Inc., Chicago, IL, USA).

© 2012 The Authors

Results

Voluntary participation did not affect the representativeness of the population under study because there were no significant differences observed between respondents and nonrespondents with regard to children's weight status and parental educational level (data not shown). Data were available for 2492 out of 2655 participating children and adolescents in the fifth and sixth grades of primary school. Table 1 presents the characteristics of the study population. The mean (SD) age of the study participants was 11.2 (0.7) years. No significant differences were found between boys and girls with respect to nationality and socio-economic characteristics (i.e. socio-economic level of school region, paternal and maternal educational level and family income). Similarly, no significant sex differences were found with respect to the prevalence of ID, anaemia and IDA. On the other hand, more girls than boys were found to be at Tanner stages 3–5 ($P < 0.05$), whereas the prevalence of obesity was higher in boys compared to girls (13.3% versus 9.6; $P < 0.05$).

Furthermore, regarding children and adolescents with ID and IDA, there were no significant differences either within or between groups in terms of dietary intake of iron, calcium, vitamin C and fibre, steps count, Tanner stage and prevalence of being overweight and obesity. Nevertheless, BMI was found to differ significantly between ID and IDA study participants (20.6 versus 22.7 kg m^{-2}; $P < 0.001$; data not shown).

Table 2 displays the haematological and biochemical indices of iron status across weight groups in both boys and girls. In boys, MCH, MCHC, serum iron and TSAT differed significantly among weight groups ($P < 0.05$), being lower in obese compared to normal-weight groups. By contrast, serum ferritin and TIBC were lowest in normal-weight boys ($P < 0.05$). In girls, significant differences across weight groups were found in nine haematological and biochemical indices of iron status ($P \leq 0.05$). Of those, haemoglobin, MCV, serum iron and TSAT exhibited lower values in obese than normal-weight girls; MCH and MCHC were lower in obese and overweight girls compared to their normal-weight peers. By contrast, RBC was higher in obese and overweight compared to normal-weight girls; ferritin was higher in obese compared to overweight or normal-weight girls; and TIBC exhibited higher values in overweight compared to normal-weight girls (in states of iron depletion, the iron-binding capacity of transferin, and thus TIBC levels, increases). Moreover, the prevalence of ID was higher in obese boys and girls compared to their overweight and normal-weight peers ($P < 0.001$ in boys and $P = 0.002$ in girls). Similarly, the prevalence of IDA was higher in obese compared to normal-weight boys ($P = 0.029$) and in obese compared to overweight or normal-weight girls ($P = 0.003$).

Table 1 Characteristics of the study population

	Boys % (n = 1241)	Girls % (n = 1251)	Total % (n = 2492)
Age (years)			
9–11	41.4	41.9	41.6
11–13	58.6	58.1	58.4
SEL of school			
Lower	26.7	25.1	25.9
Medium	39.2	32.6	35.9
Higher	34.1	42.3	38.2
Grade			
5th	47.6	49.2	48.5
6th	52.4	50.8	51.5
Nationality			
Greek	85.8	83.1	84.4
Other	14.2	16.9	15.6
Tanner stage			
1	44.4	21.0*	32.7
2	40.0	39.6	39.8
3	11.2	26.4*	18.8
4	3.9	10.5*	7.2
5	0.5	2.5*	1.5
Menarche			
Yes	–	77.1	–
No	–	22.9	–
Paternal education (years)			
<9	26.0	26.9	26.3
9–12	38.7	37.8	38.1
>12	35.3	35.3	35.6
Maternal education (years)			
<9	19.2	23.9	21.6
9–12	40.8	38.1	39.4
>12	40.0	38.0	39.0
Family income (€/year)			
<12 000	21.1	24.1	22.6
12 000–20 000	26.9	26.5	26.7
20 000–30 000	23.3	23.9	23.6
30 000–40 000	14.5	13.7	14.1
40 000–50 000	8.0	5.6	6.8
>50 000	6.3	6.1	6.2
Weight group			
Normal-weight	55.2	60.0	57.6
Overweight	31.5	30.4	30.9
Obese	13.3	9.6*	11.4
Iron status groups			
Normal iron status	73.6	76.4	75.0
Iron deficiency (TSAT < 16%)	16.7	14.0	15.3
Anemia (Hgb <12 g dL^{-1})	7.4	6.7	7.1
Iron deficiency anemia (TSAT < 16% and Hgb < 12 g dL^{-1})	2.4	2.9	2.6

*$P < 0.05$, significantly different from boys.
Hgb, haemoglobin; SEL, socio-economic level; TSAT, transferring saturation.

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

Table 2 Haematological and biochemical indices of iron status across weight groups in children and adolescents

	Boys				Girls			
	Normal-weight (n = 685)	Overweight (n = 391)	Obese (n = 165)	P-value	Normal-weight (n = 751)	Overweight (n = 380)	Obese (n = 120)	P-value*
Haematological indices, mean (SD)								
RBC (M μL^{-1})	4.88 (0.42)	4.93 (0.38)	4.94 (0.38)	0.219*	4.82 (0.35)^{a,b}	4.90 (0.41)^{b}	4.93 (0.45)^{a}	0.002*
Haemoglobin (g dL^{-1})	13.2 (0.87)	13.1 (0.95)	13.0 (0.74)	0.069*	13.1 (0.85)^{a}	13.1 (0.85)	12.9 (0.91)^{a}	0.028*
Haematocrit (%)	39.0 (2.3)	39.1 (2.3)	38.9 (2.0)	0.717*	39.1 (2.1)	39.3 (2.1)	39.0 (2.4)	0.646*
MCV (fL)	80.2 (6.1)	79.6 (5.4)	79.1 (5.0)	0.094*	81.5 (5.5)^{a}	80.6 (6.1)	79.6 (6.1)^{a}	0.002*
MCH (pg)	27.0 (2.5)^{a}	26.8 (2.3)	26.5 (2.1)^{a}	0.020*	27.4 (2.3)^{a,b}	26.9 (2.6)^{b}	26.3 (2.5)^{a}	0.001*
MCHC (g dL^{-1})	33.8 (1.3)^{a}	33.6 (1.4)	33.4 (1.2)^{a}	0.011*	33.6 (1.3)^{a,b}	33.3 (1.4)^{b}	33.1 (1.2)^{a}	0.001*
Biochemical serum indices, mean (SD)								
Iron (μg dL^{-1})	89.4 (35.6)^{a}	84.0 (34.1)	76.0 (30.9)^{a}	0.001*	91.4 (37.4)^{a}	90.0 (36.3)	81.6 (37.4)^{a}	0.050*
TIBC (μg dL^{-1})	339.1 (54.1)	348.0 (54.0)	351.2 (48.4)^{a}	0.013*	348.5 (55.2)^{b}	364.6 (57.1)^{b}	357.3 (81.6)	0.001*
TSAT (%)	26.7 (10.4)^{a,b}	24.5 (10.1)^{b,c}	21.6 (8.1)^{a,c}	0.001*	26.7 (12.4)^{a}	25.0 (9.9)	23.2 (10.7)^{a}	0.005*
Ferritin (ng mL^{-1})	31.1 (21.0)^{a}	33.0 (20.3)	36.5 (20.7)^{a}	0.024*	27.4 (16.1)^{a}	30.3 (19.3)^{b}	35.6 (21.5)^{a,b}	0.001*
Iron status categories (% of total)								
ID	15.5^{a,b}	20.3^{b,c}	28.6^{a,c}	<0.001†	14.3^{a}	18.2^{b}	28.9^{a,b}	0.002†
IDA	1.4^{a}	2.8	5.3^{a}	0.029†	2.0^{a}	3.3^{b}	8.2^{a,b}	0.003†

Values marked with the same superscript letters are significantly different ($P < 0.05$ after post-hoc multiple comparisons using the Bonferroni rule or after two-sample Z-test, as appropriate).

*Derived from analysis of variance.

†Derived from the Pearson chi-squared test.

ID, iron deficiency (with or without anaemia); IDA, iron deficiency anaemia; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RBC, red blood cell count; TIBC, total iron-binding capacity; TSAT, transferrin saturation.

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

Tables 3 and 4 summarise the intakes of nutrients that affect iron status, adjusted for energy intake, and the walking/running activity across weight groups in both sexes. No significant differences among weight groups in either boys or girls were found in iron, calcium or vitamin C intakes. Nevertheless, obese girls had a higher intake of fibre compared to normal-weight girls ($P = 0.048$). Overweight and obese boys and girls recorded significantly fewer steps than their normal-weight peers ($P < 0.001$).

Based on the results of the multivariate logistic regression analysis shown in Table 5, obesity was the only factor that significantly increased the likelihood of ID and IDA in both boys and girls. Furthermore, a higher maternal educational level decreased the likelihood of ID in boys, whereas boys with higher walking/running activity (fourth quartile of number of steps) were more likely to be iron deficient. Finally, a high stage of pubertal maturation in girls (Tanner stage 4) significantly increased the likelihood of ID.

Discussion

The present study showed a considerably high prevalence of ID and IDA in obese male (28.6% and 5.3%, respectively) and female (28.9% and 8.2%, respectively) children and adolescents. This prevalence was comparable to the prevalence of ID reported for overweight Swiss and Israeli children and adolescents (i.e. 20% and 12%, respectively; Pinhas-Hamiel *et al.*, 2003; Aeberli *et al.*, 2009). In the latter studies, the definition of ID was based on certain biochemical markers of iron status, such as serum transferrin receptor concentration, free erythrocyte protoporphyrin levels and TSAT, which was the sole index used in the present study to assess ID. However, the prevalence of ID reported by the present and the two studies cited above is considerably higher compared to data reported from the USA (Nead *et al.*, 2004). Specifically, the prevalence of ID reported for obese children and adolescents in the Third National Health and Nutrition Examination Survey (NHANES) was 2.4% and

Table 3 Dietary intake and physical activity levels across weight status categories in male children and adolescents

	Boys			
	Normal-weight (n = 685)	Overweight (n = 391)	Obese (n = 165)	P-value
Iron (mg/4184 kJ[§] day^{-1})	5.9 (2.5)	6.1 (4.2)	5.8 (2.6)	0.680[†]
Calcium (mg/4184 kJ[§] day^{-1})	571.8 (181.4)	594.5 (179.8)	572.1 (206.4)	0.131[†]
Vitamin C (mg/4184 kJ[§] day^{-1}) *	52.3 (48.6)	56.0 (44.3)	51.4 (42.5)	0.238[‡]
Fibre (g/4184 kJ[§] day^{-1}) *	7.8 (3.5)	8.2 (3.8)	7.4 (3.4)	0.077[‡]
Steps (number of steps day^{-1})	15745 (5202)[a,b]	13362 (4912)[b]	12842 (6591)[a]	<0.001[‡]

Values marked with the same superscript letters are significantly different ($P < 0.05$) after post-hoc multiple comparisons using the Bonferroni rule.
*Parameters were log-transformed.
[†]Derived from Kruskal–Wallis test.
[‡]Derived from analysis of variance.
[§]4184 kJ is equal to 1000 kcal.

Table 4 Dietary intake and physical activity levels across weight status categories in female children and adolescents

	Girls			
	Normal-weight (n = 751)	Overweight (n = 380)	Obese (n = 120)	P-value
Iron (mg/4184 kJ[§] day^{-1})	6.0 (3.8)	6.7 (3.8)	6.3 (3.4)	0.064[†]
Calcium (mg/4184 kJ[§] day^{-1})	576.8 (200.1)	610.8 (372.2)	576.2 (195.0)	0.577[†]
Vitamin C (mg/4184 kJ[§] day^{-1})*	59.7 (48.7)	56.2 (47.9)	55.6 (43.6)	0.228[‡]
Fibre (g/4184 kJ[§] day^{-1})*	7.5 (3.6)[a]	8.0 (3.6)	8.2 (4.3)[a]	0.048[‡]
Steps (number of steps day^{-1})	12299 (4027)[a,b]	11263 (4467)[b]	10281.4 (5335)[a]	<0.001[‡]

Values marked with the same superscript letters are significantly different ($P < 0.05$) after post-hoc multiple comparisons using the Bonferroni rule.
*Parameters were log-transformed.
[†]Derived from Kruskal–Wallis test.
[‡]Derived from analysis of variance.
[§]4184 kJ is equal to 1000 kcal.

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

Table 5 Adjusted odds ratios (95% confidence intervals) for iron deficiency and iron deficiency anaemia among children and adolescents

	Boys (n = 1241)		Girls (n = 1251)	
	ID	IDA	ID	IDA
Weight group				
Normal-weight	1.00	1.00	1.00	1.00
Overweight	1.48 (0.99–2.18)	1.90 (0.69–5.21)	1.30 (0.88–1.94)	1.29 (0.51–3.26)
Obese	**2.46 (1.52–3.96)**	**3.13 (1.01–9.71)**	**2.05 (1.19–3.51)**	**3.28 (1.15–9.33)**
Tanner stage				
Stage 1	1.00	1.00	1.00	1.00
Stage 2	1.49 (0.99–2.17)	1.20 (0.44–3.24)	1.57 (0.94–2.62)	1.10 (0.32–3.82)
Stage 3	1.39 (0.79–2.42)	2.51 (0.76–8.29)	1.55 (0.88–2.73)	1.70 (0.48–6.06)
Stage 4	1.06 (0.28–3.92)	2.29 (0.24–21.7)	**3.03 (1.51–6.09)**	3.12 (0.73–13.3)
Stage 5	–	–	0.30 (0.36–2.46)	–
Mother's education (years)				
<9	1.00	1.00	1.00	1.00
9–12	1.05 (0.65–1.70)	0.79 (0.27–2.34)	1.29 (0.81–2.05)	1.04 (0.36–3.02)
>12	**0.56 (0.36–0.88)**	0.38 (0.09–1.47)	0.93 (0.59–1.47)	1.43 (0.54–3.80)
Steps quartiles (number of steps day^{-1})				
1st Quartile	1.00	1.00	1.00	1.00
2nd Quartile	1.22 (0.74–2.02)	1.13 (0.34–3.72)	1.20 (0.73–1.97)	0.79 (0.28–2.24)
3rd Quartile	1.57 (0.96–2.57)	1.20 (0.35–4.06)	0.97 (0.58–1.61)	0.80 (0.28–2.31)
4th Quartile	**1.85 (1.13–2.04)**	1.09 (0.31–3.86)	1.41 (0.86–2.31)	0.56 (0.17–1.78)

All odds ratios were adjusted for dietary iron, calcium, vitamin C and fibre, paternal educational level and menarche (girls only).
Bold values indicate statistical significance.
ID, iron deficiency; IDA, iron deficiency anaemia.

9.1%, respectively (Nead *et al.*, 2004). The use of serum ferritin as one of the diagnostic criteria for ID in this previous study on USA children and adolescents could probably provide an explanation for this discrepancy. Ferritin is an acute-phase protein whose serum levels are plausibly elevated in states of chronic or acute inflammation (Wisse, 2004). Considering that obesity represents such a state, the increased levels of serum ferritin in overweight and obese children could have led to an underestimation of the true magnitude of ID in this cohort. This hypothesis is supported by a more recent study that also used NHANES data to examine the incidence of ID among overweight female adolescents (Tussing-Humphreys *et al.*, 2009). In that study, serum ferritin was not included in the definition of ID, leading to a prevalence rate of 30.8%. As a confirmation of the above, the present study showed that the prevalence of ID and IDA was lower when using serum ferritin as a diagnostic criterion compared to using TSAT (i.e. 15.1% for ID and 2.0% for IDA using serum ferritin versus 18.5% for ID and 2.7% for IDA using TSAT).

The prevalence of ID and IDA reported by the present study was found to be significantly higher in obese boys and girls compared to normal-weight children rather than overweight ones (Table 2). In most cases, the prevalence of ID and IDA in overweight children and adolescents did not differ significantly compared to their normal-weight counterparts. Similar to the prevalence of ID and IDA, the values of several haematological and biochemical indices of iron status differed significantly between obese and normal-weight children and adolescents (Table 2). These differences are consistent with those reported for children and adolescents in similar studies examining the association between iron and weight status (Pinhas-Hamiel *et al.*, 2003; Tussing-Humphreys *et al.*, 2009).

Differences between obese and normal-weight children and adolescents in dietary intakes of iron and nutrients that either enhance (i.e. vitamin C) or reduce (i.e. calcium and fibre) iron bioavailability could provide an explanation for the double burden of ID and obesity. However, the present study, in line with other recent studies (Menzie *et al.*, 2008; Aeberli *et al.*, 2009; Tussing-Humphreys *et al.*, 2009), did not detect such differences (Tables 3 and 4), with the exception of dietary fibre intake, which was higher in obese compared to normal-weight girls. Still, at a multivariate analysis level (Table 5), dietary fibre intake in girls was not significantly associated with either ID or IDA. Similarly, as shown by multivariate analyses, physical activity, assessed in the present study as number of steps measured by pedometers, was not associated with the prevalence of ID in girls or IDA in both sexes, in accordance with another study (Tussing-Humphreys *et al.*, 2009). On the other hand, boys with higher walking/running activity (fourth quartile

© 2012 The Authors

of number of steps) were more likely to be iron deficient than their peers in the first quartile of number of steps. Although ID is a common disorder in professional athletes (Peeling *et al.*, 2008), the exact aetiology of this association in children is hard to interpret.

Another important finding of the present study was the positive association observed between Tanner stage and ID in girls only (Table 5). Although more girls than boys were found to be at a higher level of sexual maturation, as indicated by Tanner stage, when comparing the prevalence of ID and IDA between boys and girls, no statistically significant differences were observed (Table 1). This could probably indicate that although iron losses as a result of menstruation increases the risk of ID in girls, this might not add any further risk of impaired iron status in relation to boys.

The positive associations found in the present study between obesity and either ID or IDA in both sexes (Table 5) support the hypothesis that the bioavailability of iron may be modulated by factors linked to chronic inflammation induced by excess adiposity. In particular, the increased levels of inflammatory cytokines found in obese individuals (Subramanian & Ferrante, 2009) have been inversely associated with serum iron levels (Tussing-Humphreys *et al.*, 2009). Low iron bioavailability as a result of increased sequestration by high amounts of ferritin has also been proposed as a factor contributing to the hypoferraemia of obesity (Zafon *et al.*, 2009). Furthermore, serum levels of hepcidin, which is the main regulatory hormone of iron absorption and recirculation, were higher in overweight children, adolescents (Aeberli *et al.*, 2009; del Giudice *et al.*, 2009) and adults (Tussing-Humphreys *et al.*, 2009) compared to their normal-weight peers. Adipose tissue-derived cytokines (such as interleukin-6 and leptin), produced in response to obesity related-inflammation, promote hepcidin gene transcription (Verga Falzacappa *et al.*, 2007). This results in increased serum hepcidin levels that lead to the sequestration of iron within the reticuloendothelial system and to decreased dietary iron absorption from the intestine by controlling the expression of ferroportin 1 at the basolateral membranes of the enterocytes. Consequently, there is hypoferraemia and diminished availability of iron for erythropoiesis (Munoz *et al.*, 2009).

The cross-sectional design of the present study is its main limitation because it cannot provide cause-and-effect relationships. Another limitation could be the use of BMI as a measure of adiposity because it is not a reliable index of body fat and fat-free mass, especially in children (Taylor *et al.*, 2002; Reilly *et al.*, 2010). Nevertheless, BMI remains an acceptable and easy-to-use screening tool for identifying excess adiposity in the general population (Duncan *et al.*, 2009). The use of pedometers could be an additional limitation because it is not considered as the gold standard method for estimating physical activity levels in children and adolescents (Sirard & Pate, 2001). However, the sample size (n = 2492) could not permit the use of accelerometers in terms of cost to assess participants' physical activity levels. Besides, previous studies have shown that the use of pedometers is a valid objective method for assessing physical activity levels in children and adolescents (McNamara *et al.*, 2010).

Furthermore, the use of TSAT and serum ferritin for the definition of ID instead of multiple indicators of iron status (e.g. soluble transferin receptor levels) could have possibly affected the true magnitude of ID in the present study. Finally, there were no inflammatory marker data (i.e. C-reactive protein and/or interleukin-6) available in the present study, thus making the understanding of the mechanistic relationship between iron status and body fat rather difficult.

In conclusion, the present study reports significant positive associations of obesity with ID and IDA in children and adolescents of both sexes. These associations may have important public health implications because they indicate the need to take into account children and adolescents' weight status when providing recommendations for dietary iron intake or when assessing their iron status.

Conflict of interests, source of funding and authorship

The authors declare that there are no conflicts of interest. This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program 'Education and Lifelong Learning' of the National Strategic Reference Framework (NSRF) – Research Funding Program: Heracletus II. Investing in knowledge society through the European Social Fund.

YM, GPC, CL and GM contributed to the study design. YM, VM, MK, KS, GT, AF and GM contributed to data collection. AS and AP contributed to biochemical analyses and blood management, respectively. YM, VT and GM contributed to data management and analysis. YM, GPC, CL, VM, CK and GM contributed to the interpretation of results and the writing of the paper. All authors critically reviewed the manuscript and approved the final version submitted for publication.

References

Aeberli, I., Henschen, I., Molinari, L. & Zimmermann, M.B. (2009) Stabilization of the prevalence of childhood obesity in Switzerland. *Swiss Med. Wkly.* **140**, 13046.

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.

Chung, B., Matak, P., McKie, A.T. & Sharp, P. (2007) Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. *J. Nutr.* **137**, 2366–2370.

Cole, T.J., Bellizzi, M.C., Flegal, K.M. & Dietz, W.H. (2000) Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* **320**, 1240–1243.

Cole, T.J., Flegal, K.M., Nicholls, D. & Jackson, A.A. (2007) Body mass index cut offs to define thinness in children and adolescents: international survey. *BMJ* **335**, 194.

Duncan, J.S., Duncan, E.K. & Schofield, G. (2009) Accuracy of body mass index (BMI) thresholds for predicting excess body fat in girls from five ethnicities. *Asia Pac. J. Clin. Nutr.* **18**, 404–411.

del Giudice, E.M., Santoro, N., Amato, A., Brienza, C., Calabro, P., Wiegerinck, E.T., Cirillo, G., Tartaglione, N., Grandone, A., Swinkels, D.W. & Perrone, L. (2009) Hepcidin in obese children as a potential mediator of the association between obesity and iron deficiency. *J. Clin. Endocrinol. Metab.* **94**, 5102–5107.

McNamara, E., Hudson, Z. & Taylor, S.J. (2010) Measuring activity levels of young people: the validity of pedometers. *Br. Med. Bull.* **95**, 121–137.

Mentzer, W.C. Jr (1973) Differentiation of iron deficiency from thalassaemia trait. *Lancet* **1**, 882.

Menzie, C.M., Yanoff, L.B., Denkinger, B.I., McHugh, T., Sebring, N.G., Calis, K.A. & Yanovski, J.A. (2008) Obesity-related hypoferremia is not explained by differences in reported intake of heme and nonheme iron or intake of dietary factors that can affect iron absorption. *J. Am. Diet. Assoc.* **108**, 145–148.

Moschonis, G., Tanagra, S., Vandorou, A., Kyriakou, A.E., Dede, V., Siatitsa, P.E., Koumpitski, A., Androutsos, O., Grammatikaki, E., Kantilafti, M., Naoumi, A., Farmaki, A.E., Siopi, A., Papadopoulou, E.Z., Voutsadaki, E., Chlouveraki, F., Maragkopoulou, K., Argyri, E., Giannopoulou, A. & Manios, Y. (2010) Social, economic and demographic correlates of overweight and obesity in primary-school children: preliminary data from the Healthy Growth Study. *Public Health Nutr.* **13**, 1693–1700.

Munoz, M., Villar, I. & Garcia-Erce, J.A. (2009) An update on iron physiology. *World J. Gastroenterol.* **15**, 4617–4626.

Nead, K.G., Halterman, J.S., Kaczorowski, J.M., Auinger, P. & Weitzman, M. (2004) Overweight children and adolescents: a risk group for iron deficiency. *Pediatrics* **114**, 104–108.

Peeling, P., Dawson, B., Goodman, C., Landers, G. & Trinder, D. (2008) Athletic induced iron deficiency: new insights into the role of inflammation, cytokines and hormones. *Eur. J. Appl. Physiol.* **103**, 381–391.

Pinhas-Hamiel, O., Newfield, R.S., Koren, I., Agmon, A., Lilos, P. & Phillip, M. (2003) Greater prevalence of iron deficiency in overweight and obese children and adolescents. *Int. J. Obes. Relat. Metab. Disord.* **27**, 416–418.

Reilly, J.J., Kelly, J. & Wilson, D.C. (2010) Accuracy of simple clinical and epidemiological definitions of childhood obesity: systematic review and evidence appraisal. *Obes. Rev.* **11**, 645–655.

Sirard, J.R. & Pate, R.R. (2001) Physical activity assessment in children and adolescents. *Sports Med.* **31**, 439–454.

Subramanian, V. & Ferrante, A.W. Jr (2009) Obesity, inflammation, and macrophages. *Nestle Nutr. Workshop Ser. Pediatr. Program* **63**, 151–159; discussion 159–162, 259–268.

Tanner, J.M. (1955) *Growth at Adolescence*. Oxford: Blackwell Scientific.

Taylor, R.W., Jones, I.E., Williams, S.M. & Goulding, A. (2002) Body fat percentages measured by dual-energy X-ray absorptiometry corresponding to recently recommended body mass index cutoffs for overweight and obesity in children and adolescents aged 3–18 y. *Am. J. Clin. Nutr.* **76**, 1416–1421.

Trichopoulou, A. (2004) *Composition Tables of Foods and Greek Dishes*. Athens: School of Medicine, Department of Hygiene and Epidemiology.

Tussing-Humphreys, L.M., Liang, H., Nemeth, E., Freels, S. & Braunschweig, C.A. (2009) Excess adiposity, inflammation, and iron-deficiency in female adolescents. *J. Am. Diet. Assoc.* **109**, 297–302.

Verga Falzacappa, M.V., Vujic Spasic, M., Kessler, R., Stolte, J., Hentze, M.W. & Muckenthaler, M.U. (2007) STAT3 mediates hepatic hepcidin expression and its inflammatory stimulation. *Blood* **109**, 353–358.

Wenzel, B.J., Stults, H.B. & Mayer, J. (1962) Hypoferraemia in obese adolescents. *Lancet* **2**, 327–328.

WHO (2001) *Iron Deficiency Anaemia: Assessment, Prevention and Control. A Guide for Programme Managers*. Geneva: World Health Organization.

Wisse, B.E. (2004) The inflammatory syndrome: the role of adipose tissue cytokines in metabolic disorders linked to obesity. *J. Am. Soc. Nephrol.* **15**, 2792–2800.

Zafon, C., Lecube, A. & Simo, R. (2009) Iron in obesity. An ancient micronutrient for a modern disease. *Obes. Rev.* **11**, 322–328.

© 2012 The Authors
Journal of Human Nutrition and Dietetics © 2012 The British Dietetic Association Ltd.