Hepcidin in Obese Children as a Potential Mediator of the Association between Obesity and Iron Deficiency

Emanuele Miraglia del Giudice, Nicola Santoro, Alessandra Amato, Carmine Brienza, Paolo Calabrò, Erwin T. Wiegerinck, Grazia Cirillo, Nunzia Tartaglione, Anna Grandone, Dorine W. Swinkels, and Laura Perrone

Department of Pediatrics “F. Fede” Seconda Università degli Studi di Napoli (E.M.d.G., N.S., A.A., C.B., G.C., N.T., A.G., L.P.), and Division of Cardiology (P.C.), Second University of Naples-A.O. Monaldi, 80131 Napoli, Italy; Department of Laboratory Medicine (E.T.W., D.W.S.), Radboud University Nijmegen Medical Center, 6500 HB Nijmegen, The Netherlands; and Hepcidinanalysis.com (D.W.S.), 6525 GA Nijmegen, The Netherlands

Context: Obesity and iron deficiency are two of the most common nutritional disorders worldwide. Several studies found higher rates of iron deficiency in obese than in normal-weight children. Hepcidin represents the main inhibitor of intestinal iron absorption, and its expression is increased in adipose tissue of obese patients. Leptin is able, in vitro, to raise hepcidin expression.

Objectives: Aims of this work were 1) to assess the association between poor iron status and obesity, 2) to investigate whether iron homeostasis of obese children may be modulated by serum hepcidin variations, and 3) to assess the potential correlation between leptin and serum hepcidin variations.

Methods: Iron status and absorption as well as hepcidin, leptin, and IL-6 levels were studied in 60 obese children and in 50 controls.

Results: Obese children showed lower iron and transferrin saturation (both \( P < 0.05 \)) and higher hepcidin levels (\( P = 0.004 \)) compared with controls. A direct correlation between hepcidin and obesity degree (\( P = 0.0015 \)), and inverse correlations between hepcidin and iron (\( P = 0.04 \)), hepcidin and transferrin saturation (\( P = 0.005 \)), and hepcidin and iron absorption (\( P = 0.003 \)) were observed. A correlation between leptin and hepcidin (\( P = 0.006 \)) has been found. The correlation remained significant when adjusted for body mass index, sex, pubertal stage, and IL-6 values.

Conclusions: We propose that in obese patients, increased hepcidin production, at least partly leptin mediated, represents the missing link between obesity and disrupted iron metabolism. (J Clin Endocrinol Metab 94: 5102–5107, 2009)

Nutrition in childhood has a significant impact on lifelong health. Obesity and iron deficiency are two of the most common nutritional disorders worldwide (1). The prevalence of overweight among children and adolescents has been increasing at an alarming rate (2). Iron deficiency during childhood was associated with impaired cognitive function, developmental delays, and behavioral and learning disturbances (3–5).

Several studies have consistently found higher rates of iron deficiency in obese children and adolescents. Particularly, two epidemiological studies published in the early 1960s noted an association between overweight status among children and adolescents and iron deficiency (6, 7). More recently, data from the American National Health and Nutrition Examination Survey III as well as data obtained in children from transition countries (Morocco and India) have suggested that among children, the prevalence of iron deficiency increases as body mass index (BMI) increases from normal weight to at risk for obesity to obesity (8–11).

Abbreviations: BMI, Body mass index; SDS, SD score; STAT3, signal transducer and activator of transcription 3.
A number of different factors have been typically proposed to explain the association between iron deficiency and overweight. Eating unbalanced meals (e.g. low-cost fast food) that are particularly rich in carbohydrates and fat but contain a low quantity of essential nutrient such as iron has been claimed as the most important cause for this association (7).

Radically changing this sound point of view, a recent study investigating the intake of heme and nonheme iron in a convenience sample of more than 200 obese subjects showed that differences in iron intake, or of dietary factors known to affect iron absorption, were not associated with the lower serum iron concentrations found in obese patients (12).

Adipose tissue is now recognized as an endocrine organ that can contribute to the inflammatory process by secreting proinflammatory cytokines, also named adipokines, and the resulted inflammatory state may have an important pathogenic role in some obesity-related comorbidities (13). Some studies have recently supported the idea that iron deficiency could represent one of the comorbidities associated with the typical chronic low-grade inflammation state of obese patients. In a cross-sectional analysis, examining the relationship between adiposity, serum iron, serum transferrin, and measures of inflammation in a group of obese adults, hypoferremia appeared to be explained by inflammatory-mediated functional iron deficiency (14). This link was reinforced by the observation that bariatric surgery resulting in significant and long-lasting weight loss reduces inflammation and, consequently, improves iron status in morbidly obese patients (15). Furthermore, confirming the link between adiposity and iron metabolism, a recent research in Thai women has demonstrated that, independently of iron status, greater adiposity is associated with lower fractional iron absorption (11).

The identification of hepcidin, a 25-amino-acid peptide produced prevalently in the liver, opened a new era in our understanding of iron metabolism (16). Hepcidin represents the main regulator of intestinal iron absorption and macrophage iron release and, thus, ultimately of the iron available for erythropoiesis (17, 18). Mice in which the hepcidin gene was inactivated develop iron overload, whereas transgenic mice overexpressing hepcidin show severe iron-deficiency anemia (17, 19). Hepcidin is also known to be increased in humans in generalized inflammatory disorders, causing anemia of chronic diseases (20). Evidence, in fact, has been provided for a tight direct correlation between hepcidin expression and some cytokines such as IL-6 and C-reactive protein that are increased in obesity (21). To strengthen the possible link between the poor iron status of obesity and hepcidin, there is the interesting observation that hepcidin is expressed not only in the liver but also in adipose tissue and that mRNA expression is increased in adipose tissue of obese patients (22). In the human liver cell line HuH7, IL-6-mediated hepcidin expression requires a signal transducer and activator of transcription 3 (STAT3)-binding motif located at position −64/−72 of the promoter (23). This binding motif is a key effector of hepcidin expression also during inflammatory conditions (23). Using the same kind of cells (i.e. HuH7), Chung et al. (24) have recently shown that the adipokine leptin is able to increase hepatic hepcidin expression, sharing the same signaling pathway (i.e. Janus kinase/STAT3) than IL-6.

The aims of this study were 1) to assess the association between poor iron status and obesity in a group of 60 Italian obese children and adolescents and 50 matched controls, 2) to evaluate in the same subjects the serum hepcidin levels and to investigate whether the iron status and absorption of these children may be modulated by serum hepcidin variations, and 3) to assess, in this context, the possible correlation between leptin and serum hepcidin variations.

Patients and Methods

Patients

Iron status and absorption as well as hepcidin, leptin, and IL-6 serum levels have been evaluated in a group of 60 obese children [29 girls; mean age, 11.3 ± 2.5 yr; mean BMI sd score (SDS), 2.7 ± 0.5] who have attended the Department of Pediatrics of the Second University of Naples between January 2007 and June 2008 to take part in a weight loss program and in 50 sex- and age-matched lean controls who consulted the Department for presumed diseases and were found to be normal (25). Main anthropometric features of these two groups of subjects are reported in Table 1.

Weight and height of all the subjects involved in the research were measured, and BMI was calculated as previously shown (26). BMI exceeding the 95th percentile was used for obesity diagnosis. Controls had BMI below the 85th percentile. SDS for BMI were calculated by using the Lambda-mu-sigma method (27). Pubertal status (Table 1) was evaluated according to the criteria of Tanner (28). Among the pubertal girls, none showed clinical relevant menstrual irregularity, and eight obese girls and three controls appeared to be in the follicular phase.

Blood samples for hemoglobin, iron status, hepcidin, IL-6, and leptin evaluations were drawn in the morning (at 0800 h) after overnight fasting. Hemoglobin was measured in whole blood using an automated Coulter counter, and cutoff points used to define anemia were based on the 5th percentiles for the reference groups (29).

Procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The ethical committee of the Second University of Study of Naples approved the study. Informed consent was obtained by parents and, where appropriate, by children.
Iron status evaluation

Iron status has been assessed by serum iron, transferrin, and ferritin concentrations. Transferrin saturation has been calculated. Particularly for serum iron, an iron ferrizone complex method was used with sensitivity of 5 μg/dl; serum transferrin was measured using a turbidimetric method with sensitivity of 70 mg/dl. Serum ferritin was measured by immunometric assay. Transferrin saturation was calculated by finding the molar ratio of serum iron and twice the serum transferrin (because each transferrin molecule can bind two atoms of iron) using the formula: transferrin saturation = [serum iron (micrograms per deciliter)/transferrin (milligrams per deciliter)] × 71.2.

Iron absorption study

Iron absorption was studied in the obese cohort after a 12-h overnight fast. Serum iron concentrations were measured at 0800 h (baseline) and then 120 min after administration of 1 mg/kg ferrous sulfate (at 1000 h). Change in iron concentration (Δ iron) was measured by subtracting baseline serum iron from serum iron at 120 min (30, 31).

Serum hepcidin, IL-6, and leptin evaluation

Before hepcidin analysis, samples were stored frozen at −80°C for not more than 8 months. Serum hepcidin-25 (the mature, active form of the peptide) measurements were performed by a combination of weak cation exchange chromatography and time-of-flight mass spectrometry. An internal standard (synthetic hepcidin-24; Peptide International Inc., Louisville, KY) was used for quantification (32). Peptide spectra were generated on a Microflex LT matrix-enhanced laser desorption/ionization time-of-flight mass spectrometry platform (Bruker Daltonics, Billerica, MA). Serum hepcidin-25 concentrations are expressed as nanomoles per liter. The lower limit of detection of this method was 0.5 nm; average coefficients of variation were 2.7% (intra-run) and 6.5% (inter-run). The median reference level of serum hepcidin-25 is 4.2 nm, range 0.5–13.9 nm (33).

IL-6 and leptin were measured by commercially available ELISA kits. The coefficients of variations within assays were 7% for IL-6 and 6% for leptin and between assays were 5% for IL-6 and 4% for leptin.

Statistical analysis

Differences between means relative to anthropometric measures, iron, iron saturation, ferritin, Δ iron, hepcidin, IL-6, and leptin of 60 obese children and 50 controls involved in the second step of the study were evaluated with the Student’s t test.

A simple regression was used to correlate hepcidin with BMI-SDS, serum iron, transferrin saturation, and Δ iron. A multiple regression analysis, including BMI-SDS, sex, pubertal stage, and IL-6 values as covariates was used to correlate hepcidin with serum leptin. Variables not normally distributed (hepcidin, serum iron, transferrin saturation, and leptin) were log-transformed before performing the correlations. Data are expressed as means and SD. We considered statistically significant a P value <0.05. Stat-Graphics 3.0 Centurion XV software for Windows was used for all the statistical analyses.

Results

We found lower iron and transferrin saturation (both P < 0.05) in obese children compared with lean controls and higher, although not statistically significant, serum ferritin levels (Table 1). One obese child was anemic. Differences in hemoglobin levels between obese children and controls were not statistically significant (Table 1).

Obese children showed higher serum hepcidin compared with controls (P = 0.004).

A direct correlation between serum hepcidin and BMI-SDS (r² = 0.33; P = 0.0015) (Fig. 1) was observed. Furthermore, we showed statistically significant inverse correlations between serum hepcidin and serum iron (r² = 0.16; P = 0.04), between serum hepcidin and transferrin saturation (r² = 0.22; P = 0.005), and among obese children, between hepcidin and Δ iron (r² = 0.37; P = 0.003) (Fig. 1).

Finally, we observed higher levels of both IL-6 and leptin in obese children compared with controls (Table 1) and a direct correlation between hepcidin and leptin levels (r² = 0.27; P = 0.006) (Fig. 2). This correlation remained still significant (P = 0.02) when adjusted for BMI-SDS, sex, pubertal stage, and IL-6 values.

Discussion

Iron is essential for life. It is a trace element that is critical for optimal physical and cognitive development and performance (3–5). Until recently, few studies had considered body weight as a factor related to iron deficiency. Interestingly, a recent study, using data from the American National

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**TABLE 1.** General characteristics, iron status indicators, and hepcidin, leptin, and IL-6 serum levels of 60 obese children and 50 controls

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>60 (29)</td>
<td>50 (25)</td>
<td></td>
</tr>
<tr>
<td>(girls)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>11.3 ± 2.5</td>
<td>10.7 ± 2.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>69.2 ± 17.1</td>
<td>36.5 ± 15.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>148.6 ± 13.6</td>
<td>140.1 ± 25.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Prepubertal (%)</td>
<td>50</td>
<td>68</td>
<td>0.3</td>
</tr>
<tr>
<td>BMI</td>
<td>30.9 ± 4</td>
<td>17 ± 7.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI Z-score</td>
<td>2.7 ± 0.5</td>
<td>0.5 ± 0.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.9 ± 0.8</td>
<td>13.2 ± 1.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Iron (μg/dl)</td>
<td>68.3 ± 28.8</td>
<td>79 ± 18.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Transferrin</td>
<td>18.6 ± 8.1</td>
<td>22.3 ± 6.6</td>
<td>0.01</td>
</tr>
<tr>
<td>saturation (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>37.8 ± 16</td>
<td>31.4 ± 19</td>
<td>0.06</td>
</tr>
<tr>
<td>Hepcidin</td>
<td>2.8 ± 1.6</td>
<td>1.9 ± 1.6</td>
<td>0.004</td>
</tr>
<tr>
<td>(nmol/liter)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>1.7 ± 1.3</td>
<td>1.2 ± 0.7</td>
<td>0.01</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>34.5 ± 13.8</td>
<td>12.1 ± 12.6</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD. Student’s t test has been used to evaluate differences between means.

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Health and Nutrition Examination Survey III, determined that overweight American children were twice as likely to be iron deficient than normal-weight children (8), and similar findings have since been reported in adults. Considering that both obesity and iron deficiency are diseases that continue to evolve worldwide with significant public health implications, this association deserves to be explored.

In this study, confirming the link between adiposity and serum iron levels, we have observed that obesity is associated with poor iron status. Originally, this kind of association had been attributed to erroneous dietary habits of obese patients (7), but recently it has been clarified that hypoferremia in obesity is not associated with dietary factors, and an inflammatory-mediated, functional iron deficiency was claimed (12, 14, 15).

Hepcidin is an important regulator of iron homeostasis, produced mainly in the liver. It inhibits iron absorption at the enterocyte and sequesters iron at the macrophage, leading in this way to decreased iron status and hypoferremia (17–19). When erythropoiesis needs to be increased, such as during iron deficiency, anemia, and hypoxia, hepatic production of hepcidin is rapidly halted and iron is readily absorbed from the intestine or released from macrophages to support the erythron demands (17). On the other hand, chronic hyperstimulation of hepcidin during inflammatory states, mainly mediated by the cytokine IL-6, is the leading factor producing the so-called anemia of inflammation or anemia of chronic disease, which has features of iron-restricted erythropoiesis (20, 21).

Because obesity is now considered a low-grade inflammatory disease, we have therefore explored the possibility that increased hepcidin levels may represent the mechanistic link between obesity and iron homeostasis (14, 34).

Consistent with our hypothesis, we found in a group of obese children higher serum hepcidin compared with controls. Furthermore, we observed inverse correlations between serum hepcidin and serum iron, transferrin saturation, and among obese children, between hepcidin and iron absorption.

Intestinal absorption of iron was studied using simply the iron-loading test based on the increment of iron level 2 h after administrating an iron load (30, 31), and this constitutes a methodological limitation of the study. However, the relation between hepcidin concentration and iron absorption found in this research is in agreement with two recent studies showing that in healthy humans, mature hepcidin-25 concentration was significantly correlated with iron absorption from food and supplemental sources, evaluated with the use of dual stable-iron-isotope techniques (35, 36), which is considered the gold standard technique for iron absorption studies.

These data seem to indicate that the hepcidin-inflammation connection may represent the biological framework explaining the association of poor iron status with obesity.

Nevertheless, the following experimental data reported in the literature induced us to check a parallel/alternative framework explaining the increased hepcidin production in obesity: 1) hepcidin is expressed not only in the liver but also at both the mRNA and the protein levels in the adipose tissue (22); 2) hepcidin RNA expression is increased in adipose tissue of obese patients, and there is a positive correlation between hepcidin expression in the adipocytes and BMI (22); and 3) leptin, an adipokine secreted by adipocytes in proportion to their content in triglycerides, and whose plasma levels are, therefore, particularly elevated in obesity (37, 38), is able, in vitro, to increase hepcidin production.
Hepcidin expression, sharing the same signaling pathway \textit{(i.e., Janus kinase/STAT3)} than IL-6 \textit{(24)}. We evaluated serum leptin concentrations in obese children and the relative controls and found a strong direct correlation between leptin and hepcidin levels, which remained statistically significant also when data were adjusted for obesity degree, sex, pubertal stage, and IL-6 values. We speculate that in the obese patients, leptin could play an important role in stimulating hepcidin secretion. This idea is strengthened by a study performed in 1988 \textit{(39)}, before leptin and hepcidin discovery \textit{(16, 40)}, showing that genetically obese \textit{ob/ob} mice were not iron deficient when fed a diet containing an adequate level of this micronutrient and that their iron absorption was increased compared with lean mice \textit{(39)}. Interestingly, after a few years, it has been shown that this strain of extremely obese mice is homozygous for a mutation of the leptin gene that does not allow leptin production \textit{(40)}, whereas in obese humans, concentrations of leptin are particularly elevated \textit{(37, 38)}. Measuring hepcidin in \textit{ob/ob} mice and in a strain of obese mice normally producing leptin could help to understand the specific role of this adipokine in stimulating hepcidin secretion. Furthermore, in the obesity context, considering that both liver and adipose tissue produce hepcidin, study of the relative role of these two tissues in stimulating hepcidin secretion may represent an interesting field of investigation.

In conclusion, we propose that in obese patients, increased hepcidin production, at least partly leptin mediated, can represent the missing link between obesity and disrupted iron metabolism.

Acknowledgments

Address all correspondence and requests for reprints to: Prof. Emanuele Miraglia del Giudice, Dipartimento di Pediatría, Seconda Università di Napoli, Via Luigi De Crecchio No. 2, 80138, Napoli, Italy. E-mail: emanuele.miraglia@unina2.it.

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References
12. Menzies CM, Yanoff LB, Denkinger BI, McHugh T, Sebring NG, Calis KA, Yanovski JA \textit{2008 Obesity-related hypoferrremia is not explained by differences in reported intake of heme and nonheme iron intake of dietary factors that can affect iron absorption. J Am Diet Assoc 108}:145–148
obesity is independent from diabetes and NASH. Gastroenterology 131:788–796


