Clinical Nutrition xxx (2016) 1–6

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

Hepatic iron content is independently associated with serum hepcidin levels in subjects with obesity

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ARTICLE INFO

Article history:
Received 30 September 2015
Accepted 23 September 2016

Keywords:
Hepcidin
Ferritin
MRI
Liver
Obesity

SUMMARY

Background & aims: Serum hepcidin concentration is known to increase in parallel to circulating markers of iron stores. We aimed to investigate whether this is reflected at the tissue level in subjects with obesity.

Methods: Serum hepcidin and ferritin levels (ELISA) and hepatic iron content (using magnetic resonance imaging) were analyzed longitudinally in 44 participants (19 without obesity and 25 with obesity). In a subgroup of 16 participants with obesity, a weight loss intervention was performed.

Results: Serum hepcidin, ferritin and hepatic iron content (HIC) were significantly increased in participants with obesity. Age- and gender-adjusted serum hepcidin was positively correlated with BMI, hsCRP, ferritin and HIC. In addition, age- and gender-adjusted serum hepcidin was positively correlated with ferritin and HIC in both non-obese and obese participants. In multivariate regression analysis, hepatic iron content ($p < 0.01$) and serum ferritin ($p < 0.001$) contributed independently to circulating hepcidin concentration variation after controlling for age, gender, BMI and hsCRP. Diet intervention-induced weight loss led to decreased serum hepcidin ($p = 0.01$), serum ferritin concentration ($p = 0.01$) and HIC ($p = 0.002$). Of note, the percent change of serum hepcidin strongly correlated with the percent change of serum ferritin ($r = 0.69$, $p = 0.01$) and HIC ($r = 0.61$, $p = 0.03$) even after controlling for age and gender.

Conclusions: Serum hepcidin is a reliable marker of the hepatic iron content in subjects with obesity.

1. Introduction

Iron is an important micronutrient required for an optimal cellular metabolism and energy status, being critical for maintaining body homeostasis. Reduced Iron (Fe^{2+}) is absorbed in the proximal duodenum by divalent metal transporter 1 and transferred through the duodenal basolateral membrane to bloodstream by the iron exporter ferroportin. In circulation, oxidized iron (Fe^{3+}) is bound to transferrin and is transported and taken up by cells through transferrin receptor. Excess of iron is stored in liver, and when iron is required it is mobilized and exported to bloodstream through ferroportin. An increasing number of epidemiologic studies have shown as iron stores predict the development of metabolic syndrome, glucose intolerance and type 2 diabetes [1–7]. The underlying mechanisms for the increased body iron stores in conditions of insulin resistance are not completely understood. Paradoxically, decreased iron availability has been described in conditions of fuel and nutrient surplus such as human obesity and high fat diet fed mice [8–12]. Thus, to achieve an optimal nutritional status in patients with obesity is important to understand the causal mechanism that leads to obesity-associated iron deficiency. Cheng et al. revised the association between adult obesity and iron homeostasis in a meta-analysis and found that obese subjects showed consistently increased blood hemoglobin and serum ferritin concentration (as markers of body iron stores) but reduced transferrin saturation (marker of iron availability) [13]. Systemic iron homeostasis is controlled by hepcidin, a 25-amino acid peptide

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http://dx.doi.org/10.1016/j.clnu.2016.09.022
0261-5614/

hormone secreted from hepatocytes in response to iron overload and inflammation. Hepcidin induces ferroportin phosphorylation and in consequence, promotes its degradation, blunting iron export from the cell and reducing serum iron availability. In fact, several studies in children and adults demonstrated increased serum hepcidin and ferritin concentration and reduced iron availability in parallel to low-grade chronic inflammation in obese subjects [14–22]. Supporting these studies, interventions of weight loss reduced hepcidin concentration in parallel to increased body iron availability [18,20]. In this context, hepcidin has been postulated to underlie the decreased iron availability of subjects with obesity [12,15,18–21]. Leptin and IL6 have been substantiated to induce liver hepcidin expression, leading to increased serum hepcidin levels, inhibition of intestinal iron absorption and iron retention in spleen, liver and macrophages [19]. In this line, a high fat diet resulted in a parallel increase in hepcidin and leptin expression in mice. The consequence was increased liver and spleen iron accumulation, increased ferritin gene expression and decreased ferroportin at the protein level [12]. However, neither weight loss nor decreased inflammatory activity was significantly linked to lower serum hepcidin after bariatric surgery-induced weight loss in humans [20].

To the best of our knowledge, the relationship among tissue iron content, serum hepcidin concentration and adiposity has not been previously examined in human obesity. Magnetic resonance Imaging (MRI) is a useful non-invasive method for evaluating liver iron [23–25], which accurately reflects body iron stores [24–27]. In the present study we aimed to investigate the relationship between serum hepcidin concentration and hepatic iron content in the context of obesity and low-grade chronic inflammation.

2. Materials and methods

Forty four subjects [control (n = 19) and obese (n = 25)] from the ongoing multicenter FLORINASH Project (www.florinash.org), were recruited. In a subgroup of 16 participants, an interventional diet weight loss was performed as detailed elsewhere [28]. Briefly, weight loss was achieved by diet prescription that provides a daily energy deficit of 500–800 kcal/d, leading to a safe and steady weight loss of 0.5–1 kg/wk when followed. The MRI was repeated 2 years after this intervention. All subjects provided informed written consent. The study protocol was revised and approved by the institutional review board.

2.1. Laboratory analysis

Serum glucose, insulin, homeostasis model assessment of insulin resistance (HOMA-IR), total cholesterol, HDL and LDL cholesterol, triglycerides, iron, glycated hemoglobin (HbA1c), ultrasensitive C- reactive protein, ferritin, transferrin, transferrin saturation, whole-blood hemoglobin and hematocrit were analyzed as detailed elsewhere [28]. Circulating hepcidin levels in serum were measured by a solid phase enzyme-linked immunosorbent assay (ELISA) (DRG® Hepcidin 25 (Bioactive) (EIA-5258, DRG International, Inc., Germany). Detection limit was 0.35 ng/mL. Intra- and inter-assay coefficients of variation were between 5 and 15%.

2.2. MR imaging

The MR study was performed on a 1.5T magnet (NT Gyroscan Intera; Philips Medical Systems, Best, The Netherlands) at baseline and 24 months thereafter. Liver FF was obtained using multiecho Dixon (FF_D) technique acquired with four echo times (repetition time [TR] 150 ms, echo time [TE] 2.3 ms, ΔTE 2.3 ms, flip angle 10°) and free-breath single-voxel spectroscopy (FF_S) (TR 2000 ms, TE 40 ms, ΔTE 40 ms). Liver iron content was assessed by means of R2* values (obtained from multiecho Dixon sequence) and by means of HIC using T2* (TR 140 ms, TE 14 ms, flip angle 10°) and proton density (TR 140 ms, TE 4 ms, flip angle 10°) sequences as described previously [23–26]. R2* was calculated as 1/T2* by fitting the monoexponential terms to T2* signal decay curve of the respective echo times. Liver FF and R2* values were obtained from the mean averages of signal intensity by drawing three regions of interest (ROI) at liver. HIC was calculated following the recommendations established by the Spanish Society of Abdominal Diagnostic Imaging (SEDIA). All image analyses were performed by trained and experienced technician, blinded to clinical information.

2.3. Statistical analysis

Statistical analyses were performed using SPSS 19.0 statistical package for Windows (IBM Corp., 188 Armonk, NY, USA). Parameters that did not fulfill normal distribution were mathematically transformed to improve symmetry for subsequent analyses. Descriptive results of continuous variables are expressed as mean and standard deviation (SD) or median and their interquartile range as appropriate for the distribution of variables. One-way analysis of variance (one-way ANOVA), student unpaired and paired T-tests were used to evaluate the effects of obesity and diet intervention-induced weight loss. Bivariate correlations (using Pearson' and Spearman' tests) and multiple linear regression analysis were performed to test the correlation between serum hepcidin and metabolic parameters.

3. Results

3.1. Cross-sectional study

The clinical and biochemical characteristics of the study subjects are presented in Table 1. Serum hepcidin, ferritin and hepatic iron content (HIC) were significantly increased in participants with obesity (Table 1). In all participants, age- and gender-adjusted serum hepcidin was associated with BMI, hsCRP, ferritin and HIC (Table 2, Fig. 1). In addition, age- and gender-adjusted serum hepcidin was positively correlated with ferritin and HIC in both non-obese and obese participants (Table 2). In multivariate regression analysis, serum ferritin and hepatic iron content contributed independently to circulating hepcidin concentration variation after controlling for age, gender, BMI, $HOMA_{IR}$ and hsCRP (Table 3).

3.2. Longitudinal study

Diet weight loss intervention led to decreased serum hepcidin, ferritin and hepatic iron content (Table 4, Fig. 2). Of note, age- and gender-adjusted percent change of serum hepcidin was strongly correlated with the percent change of serum ferritin ($r = 0.69$, $p = 0.01$) and with the percent change of HIC ($r = 0.61$, $p = 0.03$) (Table 4).

4. Discussion

To the best of our knowledge this is the first study that explores the association between circulating hepcidin concentration and hepatic iron content in subjects with obesity. Specifically, we found increased serum hepcidin concentration and body iron stores in participants with obesity in line with previous studies [18,20,21]. Most of the positive associations between serum hepcidin and obesity-associated metabolic disturbances [increased blood pressure, insulin resistance ($HOMA_{IR}$), hsCRP, liver fat content] were lost

Please cite this article in press as: Moreno-Navarrete JM, et al., Hepatic iron content is independently associated with serum hepcidin levels in subjects with obesity, Clinical Nutrition (2016), http://dx.doi.org/10.1016/j.clnu.2016.09.022