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Iron Parameters in Patients with Partial Lipodystrophy and Impact of Metreleptin Therapy

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Provocative rodent studies taken together with cross-sectional human epidemiological data have shown that there may be a cross-talk between circulating leptin levels and whole-body iron metabolism (1; 2). In this study, we aim to discover the effects of recombinant leptin (metreleptin) administration on iron parameters in patients with partial lipodystrophy.

We studied serum samples from 19 patients with partial lipodystrophy (median age: 42, interquartile range (IQR): 34-57, Male/Female: 3/16) gathered from an open-label study previously performed at the University of Michigan evaluating the efficacy of metreleptin in nonalcoholic steatohepatitis associated with partial lipodystrophy (ClinicalTrials.gov identifier: NCT01679197; accepted article). We measured iron, soluble transferrin receptor (sTfR), hepcidin, and high-sensitive C-reactive protein (hs-CRP) levels using commercially-available assays. hs-CRP levels were considered as broader changes in inflammatory pathways may potentially impact circulating ferritin levels. We integrated the results into an existing database of metabolic parameters. Repeated-measures ANOVA was used to compare multiple time points. Paired t-test was used to compare month-6 values to baseline (a prespecified endpoint). Otherwise, multiplicity correction was performed. Log transformation was used as needed. Data are presented as median, IQR.

At baseline, ferritin levels were positively correlated with fasting glucose (r = 0.533; *P* = 0.023; Fig.1A) and HbA1c (r = 0.510; *P* = 0.031; Fig 1B). After 6 months of therapy with metreleptin, hepatic fat content measured by magnetic resonance imaging Dixon method decreased (12.7%, 9.8-20.6 vs. 8.9%, 7.0-11.0; *P* = 0.031). Triglyceride levels tended to decrease at 6 months (346 mg/dL, 240-1771 vs. 295 mg/dL, 207-495; *P* = 0.091). HbA1c levels did not change significantly (9.2%, 7.3-10.3 vs. 8.5%, 6.8-9.5; *P* = 0.264).

We observed significant reductions in serum ferritin after metreleptin treatment (F = 6.436, *P* = 0.004; 83 ng/mL, 76- 179 vs. month-3: 74 ng/ml; *P* = 0.007; 68-79; and month-6: 61 ng/mL, 46-158; *P* = 0.004; Fig. 1C). Although slight reductions were observed in circulating levels of iron

(Fig. 1D) and hepcidin (Fig. 1E), these changes did not reach statistical significance. Changes in sTfR were not statistically significant either. While there were notable reductions in hs-CRP levels at 6 months compared to baseline (2.9 mg/L, 1.3-4.8 vs. 1.6 mg/L, 1.0-6.3; *P* = 0.012; Fig. 1F). the change in hs-CRP did not correlate with changes in ferritin levels. Additionally, we observed modest correlations between changes in serum iron and triglycerides (r = 0.491, *P* = 0.033) and hepatic fat (r = 0.412, *P* = 0.079).

In the NHANES study, higher serum ferritin levels were associated with newly diagnosed diabetes (3). Elevated levels of ferritin have been previously linked to altered fat distribution (4). Likewise, decreased iron stores as a result of phlebotomy have been associated with improved insulin sensitivity (5). In keeping with previous evidence from type 2 diabetes and metabolic syndrome, we found a significant relationship between ferritin and glycemic status in patients with partial lipodystrophy.

In an intriguing translational study, Gao et al. (1) reported that serum ferritin was one of the best predictors of serum leptin under physiological conditions. The study also reported that adipocyte-specific loss of the iron exporter ferroportin resulted in iron loading and decreased leptin. In *Hfe*–/– mice, a model of human hereditary hemochromatosis, low levels of hepcidin increased adipocyte ferroportin expression, resulting in decreased adipocyte iron and increased leptin. Treatment of 3T3-L1 adipocytes with iron decreased leptin mRNA in a dose-dependent manner. It has been proposed that iron may negatively regulate leptin transcription through CREB activation. Although hepcidin and iron levels tended to decrease after metreleptin in our study population, these changes were not statistically significant in our relatively small sample size. However, metreleptin therapy was associated with reductions in triglycerides and hepatic fat along with significant decreases in ferritin and hs-CRP levels. It is important to note that the changes in the latter two parameters did not appear to correlate in our population.

In conclusion, our data show that increasing leptin signal via exogenous administration may lower ferritin levels in a rare human metabolic disease associated with insulin resistance and diabetes. However, whether the decrease in ferritin directly indicates a decrease in iron stores or is mediated by an effect on inflammation remains uncertain. These results together with the previous reports suggest that the dynamic crosstalk between leptin and iron metabolism may have relevance for energy regulation in humans. It would be interesting to evaluate the other side of this crosstalk by probing the effects of leptin administration in states of iron excess and low leptinemia and determine changes in energy metabolism.

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Conflict of Interest

EAO reports the following conflicts: Grant support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Ionis Pharmaceuticals, Akcea Therapeutics, Gemphire Therapeutics, GI Dynamics (current), AstraZeneca (past two years). Consultant or Advisor: AstraZeneca, Thera Therapeutics, and BMS (past), Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Regeneron Pharmaceuticals (current). Drug support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Akcea Therapeutics, Rhythm Pharmaceuticals (all current). Other support: Aegerion Pharmaceuticals (now Amryt Pharmaceuticals), Regeneron Pharmaceuticals (current).

BA has attended Scientific Advisory Board Meetings organized by Aegerion Pharmaceuticals (now Amryt Pharmaceuticals) and Regeneron Pharmaceuticals and has received honoraria as a speaker from AstraZeneca, Lilly, MSD, Novartis, Novo Nordisk, Boehringer-Ingelheim, Servier, and Sanofi-Aventis. Other authors report no conflicts of interest.

Author contributions

E.Y.A. and S.B. performed data analyses and created the figure. E.Y.A. wrote the first draft of the manuscript. B.R. and J.H.performed measurements of the iron parameters. EAO designed the study, provided oversight in the execution of the study, data analyses, and manuscript writing. She also followed the participants clinically and performed all regulatory reporting and correspondence. All other authors (P.S., B.A., A.N., and R.H.) contributed to data collection and analysis. All authors read and approved the final version of the letter. E.A.O. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

1. Gao Y, Li Z, Gabrielsen JS, Simcox JA, Lee SH, Jones D, Cooksey B, Stoddard G, Cefalu WT, McClain DA: Adipocyte iron regulates leptin and food intake. J Clin Invest 2015;125:3681-3691

2. Andrews NC: Hungry irony. J Clin Invest 2015;125:3422-3423

3. Ford ES, Cogswell ME: Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 1999;22:1978-1983

4. Gillum RF: Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men--the Third National Health and Nutrition Examination Survey. Int J Obes Relat Metab Disord 2001;25:639-645

5. Facchini FS: Effect of phlebotomy on plasma glucose and insulin concentrations. Diabetes Care 1998;21:2190

Figure legend

Figure 1. Correlation of ferritin with fasting glucose (A) and HbA1c (B). The effect of metreleptin on ferritin (C), iron (D), hepcidin (E), and hs-CRP (F). Data are presented as median, IQR. We report the F-statistic and *P* value from a repeated-measures ANOVA. * indicates $P < 0.05$ versus baseline, paired sample t-test. These *P* values are marked if they are significant after multiplicity correction. Paired t-test was used to compare month-6 values to baseline (without multiplicity correction) as the change at 6 months vs. baseline was a prespecified endpoint. *P* values were calculated by using log-transformed ferritin, iron, hepcidin, and hs-CRP levels.

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