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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Ferroportin Expression in Adipocytes Does Not Contribute to Iron Homeostasis or Metabolic Responses to a High Calorie Diet

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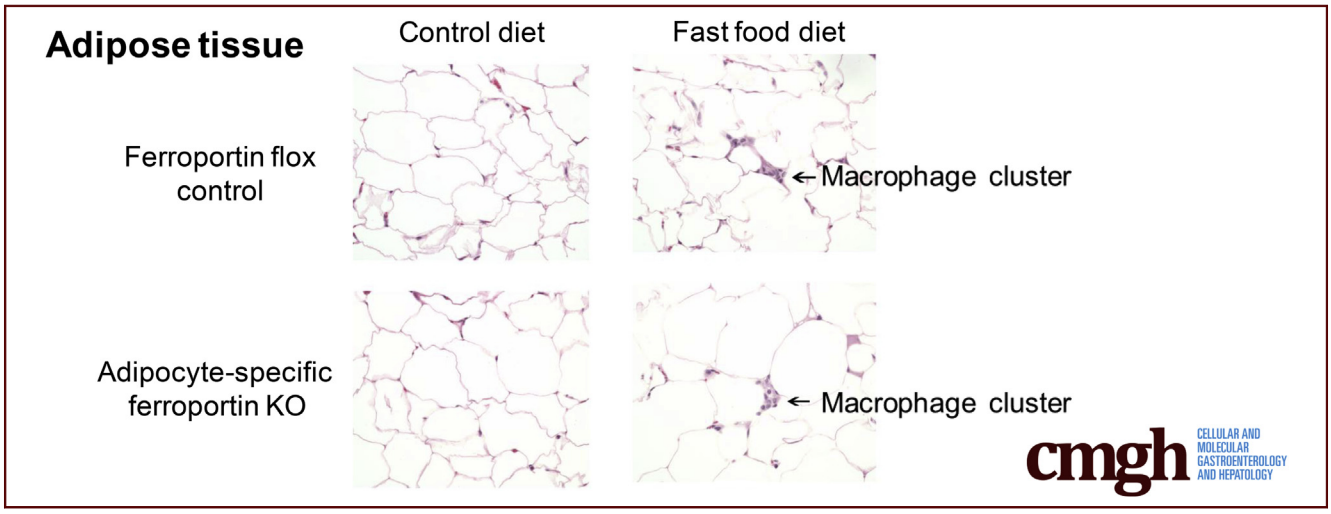

SUMMARY

The iron exporter, ferroportin, has been proposed to have a key role in adipocyte iron homeostasis. Contrary to previous reports, we show that adipocyte-specific ferroportin deletion in mice does not alter adipocyte iron loading, adipokine expression, or glucose homeostasis.

BACKGROUND & AIMS: Iron has an increasingly recognized role in the regulation of adipose tissue function, including the expression of adipokines involved in the pathogenesis of nonalcoholic fatty liver disease. The cellular iron exporter, ferroportin, has been proposed as being a key determinant of adipocyte iron homeostasis.

METHODS: We studied an adipocyte-specific ferroportin (*Fpn1*) knockout mouse model, using an *Adipoq*-Cre recombinase driven *Fpn1* deletion and fed mice according to the fast food diet model of nonalcoholic steatohepatitis.

RESULTS: We showed successful selective deletion of *Fpn1* in adipocytes, but found that this did not lead to increased adipocyte iron stores as measured by atomic absorption spectroscopy or histologically quantified iron granules after staining with 3,3'-diaminobenzidine–enhanced Perls' stain. Mice with adipocyte-specific *Fpn1* deletion did not show dysregulation of adiponectin, leptin, resistin, or retinol-binding protein-4 expression. Similarly, adipocyte-specific *Fpn1* deletion did not affect insulin sensitivity during hyperinsulinemic–euglycemic clamp studies or lead to histologic evidence of increased liver injury. We have shown, however, that the fast food diet model of nonalcoholic steatohepatitis generates an increase in adipose tissue macrophage infiltration with crown-like structures, as seen in human beings, further validating the utility of this model.

CONCLUSIONS: Ferroportin may not be a key determinant of adipocyte iron homeostasis in this knockout model.

Further studies are needed to determine the mechanisms of iron metabolism in adipocytes and adipose tissue. *(Cell Mol Gastroenterol Hepatol 2018;5:319–331; https://doi.org/10.1016/j.jcmgh.2018.01.005)*

Keywords: Iron; Ferroportin; Adipose Tissue; Nonalcoholic Fatty Liver Disease.

Nonalcoholic fatty liver disease (NAFLD) affects approximately 1 billion people worldwide.[1] Many of these individuals develop nonalcoholic steatohepatitis (NASH) and hepatic fibrosis, which can lead to liver failure and hepatocellular carcinoma.[2–4] Treatments that effectively alter the natural history of this disease are lacking and a greater understanding of its pathogenesis is essential to develop such therapies. Dysfunctional adipose tissue has been shown to be central to the pathogenesis of insulin resistance and NAFLD.[5] Adipose tissue serves as the predominant source of liver fat in NAFLD and is the source of adipokines that have significant roles in the regulation of liver injury.[6,7]

Iron is an essential element in cellular metabolism, but also has been implicated in a wide range of human disease.[8] It has been reported that adipocytes within adipose tissue use the same apparatus for iron metabolism as other cell types, such as transferrin receptor 1 (Tfr1), hepcidin, and ferroportin.[9–11] Recent data support a role for iron in the regulation of adipose tissue function. Adipose tissue iron has been proposed as having roles in the pathogenesis of NAFLD as well as type 2 diabetes mellitus.[12,13] Studies have implicated adipose tissue iron in the dysregulation of 4 key adipokines in NAFLD: adiponectin, leptin, resistin, and retinol binding protein-4 (RBP-4).[9,10,14–16] Furthermore, iron has been shown to increase lipolysis in isolated rat adipocytes.[17]

It has been proposed that the cellular iron-exporter ferroportin is a key determinant of adipocyte iron metabolism.[9] Gabrielsen et al[9] showed the down-regulation of adiponectin in response to iron across a range of in vivo and in vitro models. The investigators used an adipocyte protein 2-Cre (AP2-Cre):ferroportin (*Fpn*)$1^{fl/fl}$ model of selective adipocyte ferroportin deletion as a model of adipocyte iron loading. However, results of direct measurement of adipocyte iron were not presented and an iron-loading phenotype was inferred solely on the basis of reduced *Tfr1* messenger RNA (mRNA) quantities.[18] *Tfr1* mRNA quantification remains, at best, an indirect surrogate for iron loading that has not been well validated in adipocytes. Furthermore, the *AP2* gene has been shown to be significantly expressed in other cell types, notably macrophages.[19–21] As such, the importance of ferroportin in adipocyte iron handling requires further validation. The adiponectin (*Adipoq*)-Cre model, which uses a bacterial artificial chromosome transgene Cre recombinase in the promoter region of the adiponectin gene, has been shown to have greater adipocyte specificity than the *AP2*-Cre and is considered to be a superior model of selective adipocyte-specific gene deletion.[20,21]

In this study, we sought to determine whether ferroportin regulates adipocyte iron metabolism by selectively knocking out *Fpn1* in adipocytes using an *Adipoq*-Cre recombinase mouse model. We used the fast food diet model, as described by Charlton et al,[22] as a model for nonalcoholic steatohepatitis in these mice. This article investigates the role of ferroportin in the handling of iron by adipose tissue. In addition, we examined the effect of adipocyte-specific ferroportin deletion on glucose metabolism and liver injury using the fast food diet model of NASH. We also evaluated the utility of the fast food diet model as a model for adipose tissue dysfunction in NASH.

Methods

Experimental Animals

Mice with loxP fragments inserted in exons 6 and 7 of the mouse ferroportin gene ($Fpn1^{fl/fl}$ mice) on a 129/SvEvTac background were a kind gift from Professor Nancy Andrews (Duke University, Durham, NC).[23] $Fpn1^{fl/fl}$ mice were backcrossed for at least 8 generations onto a C57BL/6 background. Male $Fpn1^{fl/fl}$ mice then were crossed with female heterozygous C57BL/6 *Adipoq*-$Cre^{+/-}$ mice expressing Cre recombinase under the control of *Adipoq* (adiponectin gene) promotor regions on a bacterial artificial chromosome transgene (Jackson Laboratory, Bar Harbor, ME).[20] This generated both *Adipoq-Cre:*$Fpn1^{fl/fl}$, adipocyte-specific ferroportin knockout (FKO), and $Fpn1^{fl/fl}$ (Flox) littermate control mice.

After weaning, mice were housed singly. Sixteen-week-old male mice were randomly assigned, using a computerized random allocation sequence generator, to receive either control diet or fast food diet for 25 weeks until the end of the experiment.[22] Control diet mice were provided with drinking water and fast food diet mice were supplied with 42 g/L high-fructose corn syrup (23.1 g/L fructose, 18.9 g/L glucose; Chem-Supply, Gillman, Australia) in the drinking water.[24] Diets were supplied by Specialty Feeds (Glen Forrest, WA, Australia). Mice had ad libitum access to diet and water (control diet) or high-fructose corn syrup in water (fast food diet). The key constituents of the diets are outlined in Table 1.

At 41 weeks of age, mice were weighed. After a 5-hour fast, mice received an intraperitoneal injection of either 0.75 mU/g humulin R insulin (Eli-Lilly, Indianapolis, IN) in sterile 0.9% sodium chloride (0.15 mU/μL; Pfizer, New York), or 5 μL/g 0.9% sodium chloride alone. After 10 minutes, mice were sacrificed as previously described.[25]

Whole liver and epididymal fat pad weights were recorded. Liver and epididymal fat pad samples were fixed in formalin for histology. Liver samples were snap frozen in

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Abbreviations used in this paper: **AAS, atomic absorption spectroscopy; *Adipoq*, adiponectin; ANOVA, analysis of variance; AUC, area under the curve; bp, base pair; cDNA, complementary DNA; EFP, epididymal fat pad; FKO, ferroportin knockout; Ferroportin Flox, *Fpn1*$^{fl/fl}$; *Fpn1*, ferroportin; *Hamp1*, hepcidin; HIC, hepatic iron concentration; mRNA, messenger RNA; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCR, polymerase chain reaction; RBP-4, retinol binding protein-4; *Tfr1*, transferrin receptor-1.**

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