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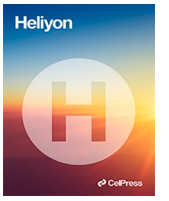

Research article

Dietary fructose regulates hepatic manganese homeostasis in female mice

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A B S T R A C T

Arginase, an enzyme dependent on manganese (Mn), plays a crucial role in the production of urea and processing of ammonia in the liver. Previous studies have shown that overconsumption of fructose disrupts Mn homeostasis in the liver of male mice. However, the potential sex-specific differences in the impact of fructose on hepatic Mn homeostasis remain uncertain. In this study, we provide evidence that heightened fructose intake disrupts liver Mn homeostasis in female mice. Elevated fructose exposure led to a reduction in liver Mn levels, resulting in decreased arginase and manganese superoxide dismutase (Mn-SOD) activity in the liver of female mice. The underlying mechanism involves the upregulation of carbohydrate-responsive element binding protein (ChREBP) expression and the Mn exporting gene Slc30a10 in the liver in response to fructose consumption. In summary, our findings support the involvement of fructose in liver Mn metabolism via the ChREBP/Slc30a10 pathway in female mice, and indicate that there is no disparity in the impact of fructose on hepatic Mn homeostasis between sexes.

1. Introduction

Manganese (Mn) is considered a vital trace element due to its indispensable role in various physiological processes, including development, digestion, reproduction, antioxidant defense, immune response, neuronal function, and energy metabolism. The beneficial impacts of Mn stem from its integration into metalloproteins such as arginase, manganese superoxide dismutase (Mn-SOD), glutamine synthetase, and pyruvate carboxylase [1]. Given the abundance of dietary sources of manganese, clinically significant manganese deficiency is uncommon in humans. However, experimental studies have reported associations between manganese deficiency and various adverse health outcomes, including impaired growth, bone defects, reduced reproductive function, birth defects, abnormal glucose tolerance, and altered lipid and carbohydrate metabolism [2,3]. On the contrary, heightened levels of exposure to manganese may result in manganese poisoning, characterized by the accumulation of excess manganese in various organs such as the liver, pancreas, bones, kidneys, and brain, with the latter being particularly susceptible. Dysregulation of intracellular manganese levels can precipitate a plethora of physiological dysfunctions and metabolic disorders [1].

The regulation of manganese homeostasis is rigorously maintained via mechanisms of intestinal absorption and hepatic biliary excretion. Excess manganese in the bloodstream is sequestered by hepatocytes in the liver and conjugated with bile, ultimately being eliminated through fecal excretion [1]. Hepatocytes exhibit a diverse array of manganese transporters on their cell membranes, such as the ferrous ion membrane transporter (DMT1), transferrin/transferrin receptor (Tf/TfR), SLC39A8, and SLC39A14, which modulate manganese influx. Additionally, manganese exporters, including SLC30A10, SLC40A1, and SPCA1 (Atp2c1), are also present in

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hepatocytes, governing the efflux of surplus manganese [1,4].

High fructose intake is thought to be a major culprit in metabolic diseases. Epidemiological studies have shown strong associations between high fructose excess intake and obesity, non-alcoholic fatty liver disease, type 2 diabetes, renal dysfunction, and cardiovascular disease [5,6]. The carbohydrate-responsive element binding protein (ChREBP, also known as Mlxipl) is a transcription factor expressed by relevant metabolic tissues and plays a key role in maintaining glycolipid metabolism. It regulates the expression of metabolic genes involved in glycolysis, lipogenesis, fructolysis and gluconeogenesis [7,8].

In the previous study, we demonstrated that excess fructose intake enhances hepatic Mn excretion by activating the ChREBP/Slc30a10 pathway, thereby reducing hepatic Mn content in male mice. As a result, Mn-dependent enzymatic activities of arginase and Mn-SOD were inhibited after excessive exposure to fructose in the male mice [9]. Considering the sex disparity in some biological effects caused by fructose, such as lipid secretion [10,11], elevated FGF21 [12], renal changes [13], neural function [14] etc., we sought to investigate whether the effect of excess fructose intake on reducing liver manganese content also exists in female mice.

In the current study, we demonstrate that excess fructose intake impairs hepatic Mn homeostasis in female mice. Fructose overconsumption increases bile Mn concentration and reduces liver Mn content as well as the activities of arginase and Mn-SOD in female mice.

2. Materials and methods

2.1. Animal study

Mice were housed in controlled conditions with a 12-h light-dark cycle, temperature set at 25 °C, and humidity maintained at 40%–60%. They were provided ad libitum access to food and water unless specified otherwise. C57BL/6J female mice aged 3–4 months were fed a purified AIN-93G diet as the control group or high fructose diet for 2 weeks (Research Diets, D11707R), and each group contained 6–8 mice.

2.2. Metabolic assays

The hepatic arginase activity was assessed using the arginase activity assay kit (Sigma, MAK112) in accordance with the manufacturer's instructions. The hepatic Mn-SOD activity and total SOD activity were determined following the protocols outlined in the Mn-SOD Assay Kit (Beyotime Biotechnology, S0103) and Total SOD Assay Kit (Beyotime Biotechnology, S0101), respectively. The metallic elements were analyzed by ICP-MS (PerkinElmer, NexION 2000B) system at Shanghai Xinhua Hospital. Liver samples were prepared by weighing and digesting them at 70 °C overnight with 6 ml of a mixture of nitric acid (HNO_3) and hydrochloric acid (HCl) in a 3:1 ratio, followed by dilution to a final volume of 15 ml for analysis. The metal elements were quantified using calibration curves generated from standards with five different concentrations of metals. Results were reported in parts per million (p.p.m.) based on volume. The metal content per gram of liver was calculated by dividing the total amount, obtained by multiplying the concentration by the volume, by the wet weight of the liver tissue. The concentration of manganese in bile was normalized using the liver Mn content.

2.3. mRNA expression analysis

Quantitative RT-PCR (qRT-PCR) was utilized to detect mRNA expression in the liver and jejunum, employing the SYBR green dye method and utilizing the *36b4* gene as an internal control. The specific sequence of quantitative RT-PCR primers can be found in Supplementary Table 1.

2.4. Protein expression analysis

Liver tissue lysates were prepared using urea lysis buffer and separated on a 10% SDS-PAGE gel. The proteins were subsequently transferred onto polyvinylidene fluoride membranes (PolyScreen) and incubated sequentially with the corresponding primary antibody and horseradish peroxidase–conjugated secondary antibodies. Visualization was achieved using an enhanced chemiluminescence reagent (Pierce Biotechnology, Rockford, IL). The Supplementary Table 2 displays information regarding antibodies.

2.5. Statistical analysis

The data were reported as mean ± SEM, unless otherwise specified. Two-tailed unpaired Student's *t*-test were utilized for statistical comparison of quantitative variables across groups. Statistical significance was defined as $^{*}P < 0.05$ and $^{**}P < 0.01$.

3. Results

3.1. Consumption of dietary fructose leads to a decrease in liver manganese content in female mice

To examine the impact of excess fructose consumption on Mn homeostasis in female mice, adult female C57BL/6J mice were subjected to a high-fructose diet (HFR, Research Diets) containing 65% fructose. Following a two-week period of exposure to the high-fructose diet, mass spectrometry analysis revealed no significant alterations in plasma Mn and calcium (Ca) levels in comparison to

those mice fed a normal chow diet (NCD) (Fig. 1A-B). Notably, the hepatic Mn content in female mice fed the HFR diet exhibited a significant decrease while Ca content remained consistent with those of mice fed the NCD (Fig. 1C-D). Significantly, after 2 weeks of HFR diet feeding, there was a 2.2-fold increase in bile Mn levels while Ca levels remained unaffected (Fig. 1E-F), suggesting an improvement in the elimination of Mn through the hepatobiliary system.

3.2. *Excessive fructose intake inhibits Mn-dependent enzyme activities in the liver of female mice*

In order to examine the physiological impacts of dietary fructose-induced Mn reduction in the liver of female mice, we assessed the activities of Mn-dependent enzymes. One such enzyme is arginase, which catalyzes the conversion of arginine to urea and ornithine, with its activity showing a positive correlation with Mn levels within a specific threshold. In comparison to female mice fed normal chow diet, those fed a high fructose diet for 2 weeks experienced a notable decrease in liver arginase activity (Fig. 2A). Superoxide dismutase 2 (SOD2), also referred to as manganese-dependent superoxide dismutase (Mn-SOD), utilizes Mn as a cofactor. The female mice fed with a high fructose diet exhibited a reduction in both Mn-SOD activity and total SOD activity in the liver compared to those fed a normal chow diet (Fig. 2B-C). Conversely, there was no significant alteration in the protein levels of arginase (ARG1), Cu/Zn-SOD, and Mn-SOD between the two groups (Fig. 2D). These findings suggest that excessive fructose consumption may hinder the activity of Mn-dependent enzymes, at least arginase and Mn-SOD, in the liver of female mice.

3.3. *The consumption of fructose leads to an increase in the expression of Slc30a10 in liver of female mice*

To investigate the molecular mechanism underlying the reduction of Mn content and Mn-dependent enzyme activity in the liver of female mice due to excessive fructose consumption, we analyzed the expression profiles of Mn transporters. Our findings align with prior studies, demonstrating a significant activation of ChREBP-β in the liver of female mice on a high-fructose diet compared to those on a normal chow diet (Fig. 3A). Notably, the mRNA levels of *Slc30a10* in the liver of female mice fed a high-fructose diet were 1.6 times higher than those in the control group (Fig. 3B). Conversely, there were no notable alterations observed in the expression of other Mn exporter genes (such as *Slc40a1* and *Atp2c1*) or importer genes (such as *Slc11a2*, *Slc39a8*, *Slc39a14*, and *Trf*) (Fig. 3B-C). We also detected the expression of intestinal ChREBP-β and *Slc30a10*, and found that fructose intake can activate intestinal ChREBP-β expression without affecting *Slc30a10* gene expression (Fig. S1). The absence of commercially available SLC30A10 antibodies designed for rodents prevented the detection of its endogenous protein expression. These findings indicate that fructose specifically increases the expression of Slc30a10 in the liver of female mice, leading to enhanced intracellular Mn efflux.

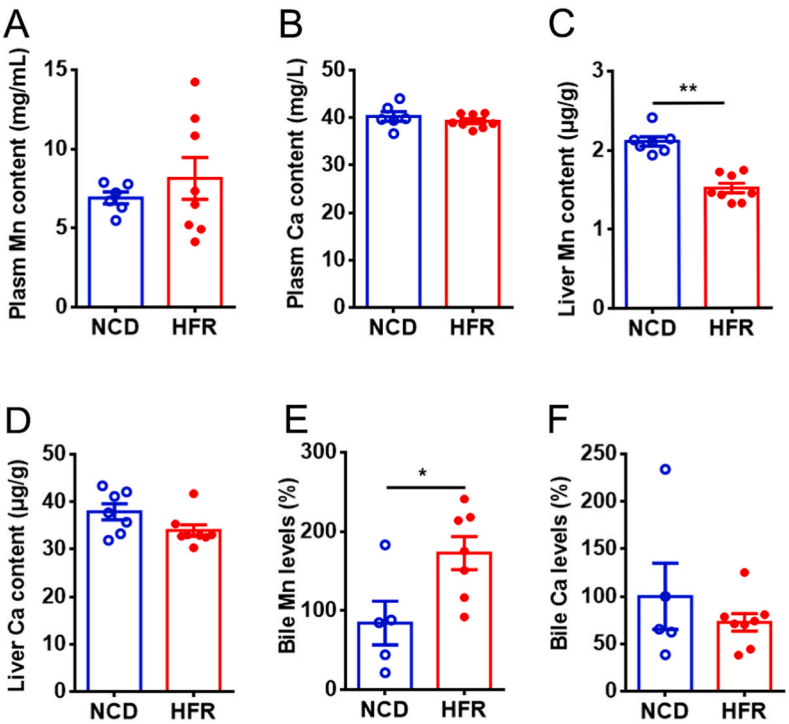

Fig. 1. Dietary fructose reduces liver manganese content in female mice. C57BL/6J female mice aged 3–4 months were fed normal chow diet (NCD) or a conventional high-fructose diet (HFR, 65% fructose, Research Diets) for 2 weeks. (A) Plasma Mn content. (B) Plasma Ca content. (C) Liver Mn content. (D) Liver Ca content. (E) Bile Mn levels. (F) Bile Ca levels. Data represent mean ± SEM. *P < 0.05, **P < 0.01.

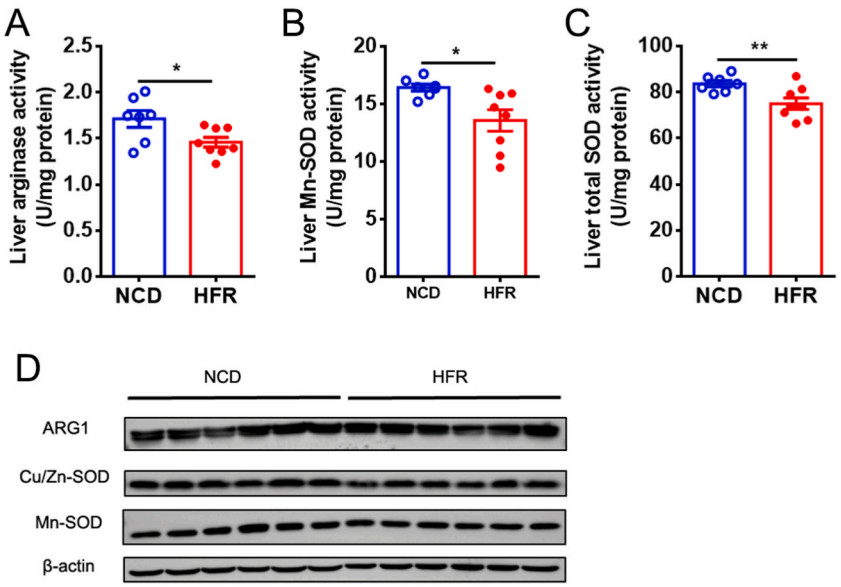

Fig. 2. Excessive fructose intake inhibits Mn-dependent enzyme activities in the liver of female mice. C57BL/6J female mice aged 3–4 months were fed normal chow diet (NCD) or a conventional high-fructose diet (HFR, 65% fructose, Research Diets) for 2 weeks. Liver arginase activity (**A**), liver Mn-SOD activity (**B**) and total SOD activity (**C**), protein expression of ARG1, Cu/Zn-SOD and Mn-SOD in the liver whole lysate analyzed by Western blot with β-actin as a loading control (**D**). Data represent as mean ± SEM. *P < 0.05, **P < 0.01.

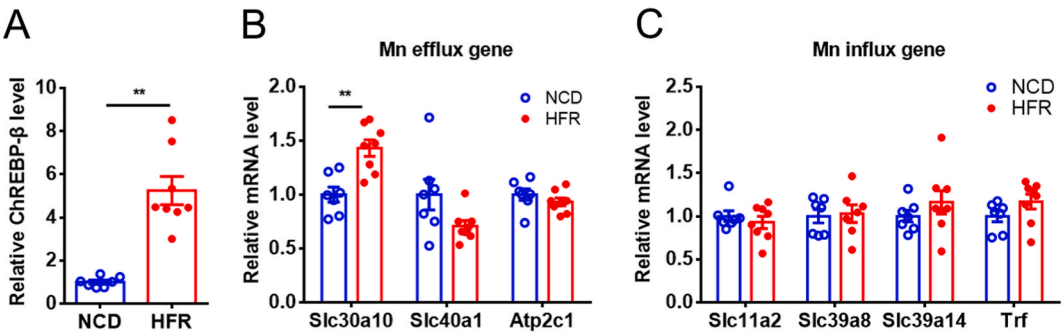

Fig. 3. Dietary fructose up-regulates the expression of Slc30a10 in liver of female mice. C57BL/6J female mice aged 3–4 months were fed normal chow diet (NCD) or a conventional high-fructose diet (HFR, 65% fructose, Research Diets) for 2 weeks. Liver mRNA levels of ChREBP-β (**A**), liver mRNA levels of Mn exporters (**B**), liver mRNA levels of Mn importers (**C**). Data represent mean ± SEM. *P < 0.05, **P < 0.01.

4. Discussion

In the preceding investigation, it was shown that increased consumption of fructose leads to heightened hepatic manganese excretion through the activation of the ChREBP/Slc30a10 pathway, consequently resulting in decreased hepatic manganese levels in male mice [9]. The impact of fructose consumption on liver manganese content in female mice remains uncertain. Our research findings indicate that excessive fructose intake disrupts manganese regulation in the liver of female mice. First, heightened exposure to fructose resulted in a reduction in hepatic manganese content in female mice. Concurrently, heightened exposure to fructose also resulted in elevated bile manganese levels in female mice, suggesting augmented excretion of manganese from hepatocytes. Conversely, Ca content in the liver of female mice remained unaffected. Second, the functionality of manganese-dependent enzymes (e.g., arginase and Mn-SOD) was diminished in the liver of female mice due to fructose consumption. Third, overconsumption of fructose led to the upregulation of Slc30a10 expression in the liver but not in the gut, while having no impact on other manganese transport genes.

This study further substantiated the pivotal function of ChREBP in the regulation of manganese homeostasis in the hepatic tissue of female mice via Slc30a10. The upregulation of ChREBP expression and activity is triggered by the consumption of carbohydrates, particularly fructose, and has been associated with the development of metabolic disorders stemming from excessive fructose consumption [5,7]. Our research results indicate that the consumption of fructose can stimulate the activation of ChREBP and its associated genes in female mice.

Our work also helps to understand the biological role of fructose intake in female mice. Previous studies have shown that sexual dimorphism is related to lipid secretion [10,11], elevated FGF21 [12], renal changes [13], and neural function [14] caused by fructose intake. In this study, we demonstrate that fructose consumption in female mice leads to a reduction in arginase activity and Mn-SOD activity through a decrease in liver Mn content. Our results, in conjunction with prior research [9], suggest that excessive fructose intake disrupts liver Mn homeostasis in both male and female mice.

Data availability statement

The data of this study will be available from the corresponding author on reasonable request.

Ethics declarations

This study was reviewed and approved by the Animal Ethical and Welfare of Tianjin Medical University Chu Hsien-I Memorial Hospital, with the approval number: DXBYY-IACUC-2022019.

CRediT authorship contribution statement

Ting Wang: Writing – original draft, Data curation. **Tie-Ning Xie:** Data curation. **Jian-Hui Shi:** Writing – original draft, Funding acquisition, Data curation, Conceptualization. **Weiping J. Zhang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33278.

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